Human cytomegalovirus modulation of CCR5 expression on myeloid cells affects susceptibility to human immunodeficiency virus type 1 infection

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For some time there has been evidence suggesting an interaction between human cytomegalovirus (HCMV) and Human immunodeficiency virus (HIV) in the pathogenesis of AIDS. Here, the interaction of HCMV and HIV-1 was examined in monocyte/macrophage cells, two cell types known to be targets for both viruses in vivo. Infection experiments demonstrated that prior infection with HCMV impeded subsequent superinfection with HIV-1. In contrast, uninfected bystander cells within the population were still permissive for HIV-1 infection and were also found to express increased levels of Gag after HIV-1 superinfection. Analysis of CCR5, a co-receptor for HIV-1, on HCMV-infected and bystander cells showed a substantial loss of surface CCR5 expression on infected cells due to HCMV-induced reduction of total cellular CCR5. In contrast, uninfected bystander cells displayed increased surface CCR5 expression. Furthermore, the data suggested that soluble factor(s) secreted from HCMV-infected cells were responsible for the observed upregulation of CCR5 on uninfected bystander cells. Taken together, these results suggest that, whilst HCMV-infected monocytes/macrophages are refractory to infection with HIV-1, HCMV-uninfected bystander cells within a population are more susceptible to HIV-1 infection. On this basis, HCMV infection may contribute to the pathogenesis of HIV-1.

INTRODUCTION

Circumstantial evidence has, for some time, implicated human cytomegalovirus (HCMV) infection as a co-factor for progression to AIDS in human immunodeficiency virus (HIV) infection (Curran et al., 1985; Nelson et al., 1988; Webster, 1991). There is a well-established interaction between HCMV and HIV using transfection assays in vitro (Duclos et al., 1989; Ho et al., 1990; Jault et al., 1994; Koval et al., 1991). Generally, it has been shown that infection of cell lines with HCMV upregulates expression from the HIV-1 long terminal repeat (LTR) in transfection/superinfection assays (Duclos et al., 1989; Ho et al., 1990). Such studies have emphasized the role of the HCMV immediate early (IE) gene products in the activation of the HIV-1 LTR (Davis et al., 1987; Rando et al., 1990; Walker et al., 1992). However, many of these analyses have used cell lines that are permissive for HCMV but not normally permissive for HIV-1 infection. Some transfection/superinfection assays have also shown specific differences in the effects of HCMV on HIV-1 infection depending on the cell type used and the relative levels of HIV-1 Tat and HCMV IE gene products in the co-infected cells (Jault et al., 1994; Moreno et al., 1997) and have also suggested that activation of the HIV-1 LTR by HCMV depends on whether the LTR reporter construct used is integrated into host genomic DNA or not (Koval et al., 1995). However, such assays were carried out using HIV-1 pseudotypes in cell lines that are not normally permissive for HIV-1 infection (Koval et al., 1995).

Cells of the myeloid lineage are one cell type that can be co-infected with HCMV and HIV in vitro (Nelson et al., 1988) and good evidence now exists as to the importance of myeloid cells in the carriage of both latent HIV (Meltzer et al., 1990; Donaghy et al., 2003) and HCMV in vivo (Reeves et al., 2005). It has been shown that HCMV infection of peripheral blood monocyte-derived macrophages (MDMs) increases HIV-1 production (Lathey et al., 1994). However, it is not clear why HIV-1 production is increased in MDMs following co-infection with HCMV and particularly why this appears to conflict with reports of inhibition of HIV-1 production by HCMV in fibroblasts using pseudotyped HIV-1 (Koval et al., 1991). Also, it is known that only a small proportion of MDMs can generally be infected with HCMV; hence, it is unclear whether the increase in HIV-1 production occurs specifically in the HCMV-infected cells.

Consequently, we analysed the effect that HCMV infection has on subsequent HIV-1 infection in myeloid cells, which are accepted to be important cell types for the carriage of both viruses in vivo. In particular, we assessed whether
co-infection of the same cell with HCMV and HIV-MN, a
myelotropic strain of HIV-1, had any effect on HIV-1
production. Our data demonstrated that HCMV-infected
primary MDMs and differentiated THP1 cells displayed
reduced permissiveness for superinfection with HIV-MN.
In contrast, uninfected bystander cells were permissive to
HIV-MN infection, displaying increased levels of HIV-1
Gag. Analysis of HIV-1 co-receptor expression levels on
HCMV-infected and uninfected bystander MDM and THP1
cells demonstrated decreased CCR5 expression on HCMV-
infected cells reflecting a significant decrease in total CCR5
protein levels. In contrast, uninfected bystander cells dis-
played increased cell-surface expression of CCR5 that was,
in part, mediated by soluble factor(s) produced from
HCMV-infected cultures.

METHODS

Cell lines. HFFs (primary human foreskin fibroblasts; ECACC)
were maintained in Eagle’s minimal essential medium (Gibco-BRL)
supplemented with 10% fetal calf serum (FCS; Gibco-BRL) at 37 °C,
5% CO₂. THP1 cells, a myelomonocytic cell line, were maintained
in RPMI 1640 (Gibco-BRL) supplemented with 10% FCS, 100 U
penicillin ml⁻¹ and 100 µg streptomycin (Life Technologies) ml⁻¹
and passed twice a week. THP1 cells were differentiated overnight
in RPMI 1640 supplemented with 10 ng phorbol myristate acetate
(PMA; Sigma) ml⁻¹ to a macrophage-like phenotype that was
fully permissive for HCMV infection, as described previously
(Weinshenker et al., 1988; Turtin & Seufzer, 1994).

Primary human MDMs. Primary human MDMs were generated as
described previously (Lathey & Spector, 1991). Briefly, peripheral
blood mononuclear cells (PBMCs) were isolated by density-gradient
centrifugation using Ficoll-Hypaque (Nycomed Pharma AS).
Primary monocytes were obtained by adherence for 1–5 h at 37 °C,
5% CO₂. Following adherence, monocytes were cultured in Iscove’s
modified Dulbecco’s media (Gibco-BRL) supplemented with 15% horse
serum (Sera-Lab), 15% FCS, 2 mM l-glutamine (Gibco-BRL), 100 U
penicillin ml⁻¹, 100 µg streptomycin ml⁻¹ and 5 × 10⁻⁵ M
hydrocortisone sodium succinate (Sigma) for 5 days at 37 °C, 5% CO₂.
After 5 days, cultures were stimulated with 10 ng PMA ml⁻¹
overnight to induce differentiation to HCMV-permissive macrophages.

Viruses. HCMV stocks were generated and infectious units assessed
in HFFs. Green fluorescent protein (GFP)-tagged HCMV was a
generous gift from Richard Greaves (Imperial College Faculty of
Medicine, London, UK) and has been described previously (Murphy
et al., 2002). Briefly, CR[IE1–GFP] is a recombinant Towne strain
of HCMV expressing enhanced GFP fused to the C terminus of IE1
p72 protein. The laboratory-adapted strain AD169, the endothelial-
cell-tropic clinical isolate TB40/E and the low-passage laboratory
strain Toledo were used where stated and have been described previously (Sinclair et al., 2000; Sinzger et al., 2000). HCMV viral
titres were determined by plaque assay on HFFs. The macrophage-
tropic strain of HIV-1 (HIV-MN) (MRC AIDS directed programme)
was propagated in H9 lymphoblastoid cells (CFAR/NIBSC).

Co-infection of HCMV and HIV-1. Differentiated THP1 and MDM
cells were inoculated with HCMV (m.o.i. of 5–10) or mock
inoculated with medium alone and cultured in appropriate medium
for 48 h. HCMV-infected cultures were subsequently co-infected
with the macrophage-tropic HIV-MN strain for 72 h.

Flow cytometric analysis and immunofluorescence. For FACS
analysis, differentiated THP1 and MDM cells inoculated with
HCMV (m.o.i. of 5–10) or mock inoculated were harvested at 48 h
post-infection (p.i.). Analysis of CCR5 and major histocompatibility
complex (MHC) class I surface expression on HCMV-infected or
uninfected cells was evaluated on live cells with phycoerythrin (PE)-
conjugated mouse anti-human CCR5 (clone 45531, subclass IgG2b,
1:10 dilution; R&D Systems) and fluorescein isothiocyanate (FITC)-
conjugated mouse anti-MHC class I (clone G46-2.6, subclass IgG1,
1:10 dilution; Pharmingen) or isotype-matched IgG control antibi-
odies. Cells were analysed using a FACS flow cytometer (Becton
Dickinson) and FCS Express software (DeNovo Software). For some
experiments, cells were fixed in 1% paraformaldehyde for 10 min
and permeabilized with 0.1% Triton X-100 for 4 min at room
temperature prior to staining.

For immunofluorescence analysis, differentiated THP1 and MDM
cells were inoculated with HCMV (m.o.i. of 5–10) or mock inoculated.
At 48 h p.i., cultures were inoculated or mock inoculated with the
macrophage-tropic HIV-MN. HCMV and/or HIV-1 infection was
visualized by indirect immunofluorescence as described previously
(Baillie et al., 2003) at 72 h after HIV-1-infection. HCMV infection
was assessed by IE gene expression (if infected with AD169, Toledo or
TB40/E) using an FITC-conjugated mouse anti-IE72/IE86 antibody
(subclass IgG2a, 1:50 dilution; Chemicon). HIV-1 infection
was assessed using a mouse monoclonal anti-HIV-1 p24 antibody (clone
38187.47, subclass IgG1, 2 µg ml⁻¹; Abcam) and visualized with a
goat anti-mouse tetramethylrhodamine B isothiocyanate (TRITC)-
labelled secondary antibody (1:40 dilution; Sigma). All staining was
carried out in parallel with appropriate mouse IgG isotype-matched
control antibodies.

For some experiments, differentiated THP1 cells were inoculated with
CR[IE1–GFP] or Toledo and cell-free supernatants were harvested at
48 h p.i. Supernatants were centrifuged to remove cell debris, UV inactivated and filtered through a 0.2 µm sterile filter.
Differentiated and uninfected THP1 cells were then cultured in the
presence of these supernatants (HCMV-conditioned media) for 48 h
or in supernatants from mock-infected cells. Both HCMV-infected
THP1 cells and THP1 cells incubated with cell-free supernatants
were surface stained to detect CCR5 as described above.

Western blot analysis. THP1 cells differentiated with PMA were
inoculated with HCMV AD169 (m.o.i. of 5–10). At 48 h after
HCMV infection, cultures were inoculated or mock inoculated with the
macrophage-tropic HIV-MN. At 72 h p.i., cultures were washed in
PBS and resuspended at a concentration of 1 × 10⁵ cells in 20 µl
SDS lysis buffer. Cells were sonicated for 2 min and resolved by
10% SDS-PAGE. Proteins were then transferred to nitrocellulose
membranes overnight. Membranes were blocked for 30 min with
5% non-fat dried milk in PBS/0.1% Tween 20. Western blot
analysis was carried out using a mouse anti-Gag antibody (ADP313,
1:200 dilution) or a mouse anti-IE72/IE86 antibody detected with the
appropriate horseradish peroxidase (HRP)-conjugated antibody
(1:10000 dilution; Santa Cruz Biotechnology). Analysis of CCR5
protein levels in HCMV-infected and bystander populations was
carried out at 48 h p.i. Differentiated THP1 cells were infected with
CR[IE1–GFP] and sorted using a Becton Dickinson FACSort into
GFP-positive and -negative (bystander) populations. CCR5 protein
levels were detected using a rabbit anti-CCR5 antibody (1:1000
dilution; AnaSpec). Confirmation of equal protein loading was
carried out using a rabbit anti-glyceraldehyde-3-phosphate dehydro-
genase (GAPDH) antibody conjugated to HRP (1:2000 dilution;
Abcam). The chemiluminescence reaction was visualized by enhanced
chemiluminescence (ECL) according to the manufacturer’s instruc-
tions (Pharmacia).

Transient transfection assays. To generate a CAT reporter con-
struct based on the HIV-MN LTR, a PCR product encompassing the
HIV-MN LTR was subcloned into pSVOCAT (Clontech), THP1
cells \((1 \times 10^7)\) in PBS were transfected by electroporation in pre-chilled 4 mm cuvettes \((960 \mu F, 250 V)\) with 5 µg LTR–CAT reporter plasmid. Transfected cells were incubated on ice for 15 min and then transferred to warm medium. Cells were harvested at 48 h post-transfection and lysed by three cycles of freezing and thawing. CAT activity was determined using equivalent amounts of protein. Acetylated and non-acetylated CAT species were separated by thin-layer chromatography and spots were quantified using InstantImager (Packard).

**Statistical analysis.** The statistical significance of changes in total CCR5 protein levels in response to HCMV was assessed by a non-parametric approach. These data were initially analysed with Friedman's test and followed by examination of specific groups using the Dunn’s multiple comparisons test.

## RESULTS

### HCMV infection of differentiated THP1 cells results in increased HIV-1 infection

To determine the exact effects of HCMV infection on subsequent infection with HIV-1 in myeloid cells, we used a myelomonocytic cell line (THP1), which is fully permissive for infection with HCMV when differentiated with PMA (Weinshenker *et al.*, 1988; Turtinen & Seufzer, 1994) and which is also permissive for infection with HIV-1 (Asin *et al.*, 2001). Control differentiated THP1 cells or cells infected with HCMV Toledo for 48 h were superinfected with HIV-MN. Fig. 1 shows that infection of differentiated THP1 cells with HIV-1 resulted in expression of Gag protein, as detected by indirect immunofluorescence (Fig. 1b). Interestingly, HCMV-infected cells expressing IE gene products were rarely found to express HIV-1 Gag (Fig. 1c). In contrast, HCMV-uninfected bystander cells showed good levels of Gag expression and the amount of Gag expression appeared to be higher than levels of Gag expression in HIV-MN-infected cells that had not been exposed previously to HCMV (compare Fig. 1b and d). Confirmation of increased Gag expression in cultures previously infected with HCMV was carried out by Western blot analysis (Fig. 1e). The failure of THP1 cells to express HIV-1 Gag whilst expressing IE gene products and the apparently increased Gag levels in HCMV-uninfected bystander cells suggested that HCMV infection not only repressed subsequent infection with HIV-1 but also stimulated HIV-1 superinfection of HCMV-uninfected bystander cells.

### HCMV activates the HIV-1 LTR in monocytic cells

One explanation for the observation that prior HCMV infection decreases HIV-1 Gag expression in HCMV-infected cells could be that, in this system, HCMV represses transcription from the HIV-1 LTR. To date, the ability of HCMV to activate the HIV-1 LTR has only been shown for LTR constructs based on T-cell-tropic strains of HIV. Therefore, we demonstrated that HCMV was also able to activate the HIV-1 LTR from a myelotropic strain of HIV-1 in myeloid cells. PCR amplification of an LTR from the myelotropic MN strain of HIV-1 was carried out and cloned upstream of a CAT reporter gene. Fig. 2 clearly shows that, as expected, HCMV superinfection of the monocytic THP1 cell line also resulted in LTR activation. These data suggested that the decrease in HIV-1 Gag expression in cultures previously infected with HCMV was not the result of inhibition of the HIV-1 LTR.
Modulation of CCR5 surface expression by HCMV reflects changes in intracellular protein levels in infected cells but not uninfected bystander cells

Our findings suggested that HCMV infection resulted in a decrease in cell-surface CCR5 on infected cells and an increase on uninfected bystander cells. We next determined whether these changes were also reflected in total levels of CCR5. First, we analysed CCR5 protein levels on fixed and permeabilized differentiated THP1 cells infected with HCMV or uninfected cells by FACS analysis. THP1 cells were differentiated overnight with PMA and then infected with CR[IE1–GFP] (m.o.i. of 5–10) or mock infected. At 48 h p.i., cells were harvested, fixed, permeabilized and stained for CCR5 expression. FACS analysis demonstrated that changes in surface CCR5 levels on HCMV-infected cells were concomitant with changes in intracellular protein levels. HCMV infection of monocytic cells resulted in a significant (n = 3, P < 0.03) downregulation of CCR5 protein levels. In contrast, the increase in the level of CCR5 cell-surface expression on uninfected bystander cells in an HCMV-infected population, which we observed in Fig. 3, was not reflected by changes in levels of total CCR5 protein (Fig. 4a).

We also confirmed this FACS analysis of total CCR5 protein in fixed and permeabilized cells by Western blot analysis. THP1 cells infected with CR[IE1–GFP] were sorted into IE1–GFP-positive and -negative populations at 48 h p.i. and analysed for CCR5 protein levels (Fig. 4b). This clearly showed that, in IE1-positive cells (Fig. 4b, lane 3), total and steady-state levels of CCR5 were substantially reduced. Once again, uninfected bystander cells (Fig. 4b, lane 2) showed little change in overall levels of CCR5 compared with uninfected control cells (Fig. 4b, lane 1).

HCMV increases CCR5 expression on primary macrophages

Although the THP1 cell line is of myelomonocytic lineage and does differentiate with phorbol esters to macrophage-like cells, we wanted to confirm the change in CCR5 levels using primary macrophages. MDMs, generated by in vitro differentiation of PBMCs using long-term culture in the presence of hydrocortisone and PMA, were infected with HCMV TB40/E (Fig. 5). FACS analysis of control and
HCMV-infected cultures for CCR5 and class I expression confirmed the HCMV-induced increase in CCR5 expression on uninfected bystander cells. MDMs were found to express good levels of CCR5 (Fig. 5a) and class I (Fig. 5b), which were reduced following infection with HCMV TB40/E (Fig. 5c and d). Furthermore, HCMV-infected MDMs expressing reduced class I displayed a total loss of CCR5 surface expression (Fig. 5e), whilst uninfected bystander cells, expressing normal levels of class I, displayed increased CCR5 expression (Fig. 5f) compared with uninfected controls (Fig. 5a).

**Soluble factor(s) produced during HCMV infection of monocytic cells induce increased CCR5 and Gag expression on uninfected bystander cells**

To assess whether soluble factor(s) released by the HCMV-infected cells within the cell population could be responsible for the induction of CCR5 expression on uninfected bystander cells, differentiated THP1 cells were mock inoculated or inoculated with HCMV CR[IE1–GFP] and cell-free supernatants were harvested at 48 h p.i. Differentiated THP1 cells were then cultured in the presence of these supernatants for 48 h and surface stained to detect CCR5. FACS analysis clearly demonstrated that supernatant harvested at 48 h after HCMV infection induced a similar increase in CCR5 levels on differentiated THP1 cells (Fig. 6c) to that seen on bystander cells during HCMV infection (Fig. 6e), whilst supernatants from mock-infected cells did not modulate CCR5 expression (Fig. 6c) compared with uninfected controls (Fig. 6a).

We also determined whether this increase in CCR5 expression mediated by soluble factor(s) released into HCMV-infected supernatants had any effect on their ability to be superinfected with HIV-1. Interestingly, immunofluorescence microscopy indicated increased intensity of Gag expression after HIV-1 superinfection of THP1 cells treated with HCMV-conditioned supernatants (Fig. 7a). Differentiated THP-1 cells were pre-treated with control media or with HCMV-conditioned media or infected with HCMV Toledo and superinfected with HIV-MN 48 h later. At 72 h p.i., cells were stained for Gag expression. Increased Gag staining was observed in cells pre-treated with supernatants from HCMV-infected cells [Fig. 7a, panel (v)] compared with control cells (Fig. 7a, panel (iv)).

**Fig. 3.** HCMV-infected monocytic cells display decreased levels of surface CCR5, whilst uninfected bystander cells display increased CCR5 levels. THP1 cells were differentiated with PMA overnight as described in Methods and infected with HCMV Toledo or mock infected. At 48 h p.i., cells were stained with IgG isotype-matched control antibody (a, b), mouse anti-CCR5 PE-conjugated antibody (c, d) or mouse anti-class I FITC-conjugated antibody (e, f), or were double stained to detect both class I and CCR5 (g, h). Note that the population of cells expressing class I were HCMV-negative (population y); the population expressing a low level of class I were HCMV-positive (population x). Cells were analysed using a Becton Dickinson FACSort. Data are representative of five separate experiments.

HCMV modulates permissiveness to HIV-1 infection
compared with medium controls [Fig. 7a, panels (i) and (ii)]. We confirmed these immunofluorescence assays quantitatively by FACS analysis (Fig. 7b). Consistent with the immunofluorescence analysis, HIV infection of control differentiated THP1 cells led to readily detectable levels of Gag expression (Fig. 7b, 29 % of cells, histogram 2), which was increased by pre-treatment of cells with HCMV-conditioned medium (Fig. 7b, 56 % of cells, histogram 3) and also increased by HCMV infection (Fig. 7b, 52 % of cells, histogram 4). We also quantified the level of HCMV infection in these samples by IE1 detection, which showed no IE expression in untreated cells or in cells treated with HCMV-conditioned medium and 12 % infection with HCMV in HCMV-infected cells. Similarly, cells that were not inoculated with HIV-MN showed no Gag staining (data not shown). These data suggest that soluble factor(s) produced during HCMV infection of monocytic cells mediate the increase in surface levels of CCR5 on bystander cells, which results in these cells showing higher levels of Gag expression on co-infection with HIV-MN.

DISCUSSION

In order to investigate further the well-documented interplay between HIV-1 and HCMV, we analysed the effect of HCMV infection of myeloid cells on their subsequent infection with HIV-1. Our data demonstrated that infection of myeloid cells with HCMV increased the levels of HIV-1 Gag...
expression after superinfection with HIV-1. This increase was found to be restricted to the HCMV-uninfected bystander cell population. In contrast, cells expressing the IE gene products IE72/IE86 showed little or no HIV-1 gene expression. Furthermore, the lack of Gag expression in these cells was not due to transcriptional repression of the HIV-MN LTR by HCMV. FACS analysis of CCR5 co-receptor levels on live cells indicated that HCMV-infected cells had reduced levels of surface CCR5, whilst uninfected bystander cells in the culture had increased levels of surface CCR5 compared with uninfected controls.

Our observation that HCMV infection of macrophages downregulated levels of surface CCR5 is consistent with previous work by Lecointe et al. (2002) who showed that primary MDMs infected with HCMV AD169 exhibited downregulation of plasma membrane expression of CCR5 with no change in surface CD4 expression. Similarly, HCMV infection of dendritic cells (Varani et al., 2005), astrocytes and microglia (Lecointe et al., 2002) also results in a decrease in surface CCR5 levels. The role of CCR5 in virus infections...
is unclear. Downregulation of CCR5 on the cell surface has been demonstrated to protect cells from HIV envelope-mediated apoptosis (Algeciras-Schimnich et al., 2002), whilst CCR5–CCL5 interactions have been shown to protect macrophages from respiratory syncytial virus- and influenza virus-induced cell death (Tyner et al., 2005). Modulation of CCR5 by multiple HCMV strains and in cell lines and primary cells is intriguing and suggests that CCR5 might have an as yet unknown biological significance during HCMV infection.

HCMV-infected cells express low levels of CCR5 – either HCMV only infects low-CCR5-expressing cells or it represses CCR5 expression. To address the possibility that HCMV preferentially infected cells with low levels of CCR5 expression, we aseptically sorted live differentiated THP1 cells based on high or low CCR5 expression by FACS. Cultures of low and high CCR5-expressing THP1 cells were then infected with HCMV CR[IE1–GFP] and the number of IE1-expressing cells was assessed at 48 h p.i. Both populations of THP1 cells were permissive to HCMV (data not shown). In addition, analysis of total (surface and intracellular) CCR5 protein in permeabilized cells by FACS analysis demonstrated that HCMV infection resulted in a significant decrease in CCR5 protein in infected cells. CCR5 has been shown to exist in two forms, a 42 kDa form suggested to be present in the cytoplasm of cells and a 62 kDa form that resides mainly on the cell surface (Suzuki et al., 2000). Western blot analysis of CCR5 in IE1-expressing versus bystander cells clearly demonstrated that the 42 kDa form is decreased in IE1-positive cells compared with uninfected controls. Interestingly, increases in cell-surface expression of CCR5 on uninfected bystander cells was not reflected by changes in the total amount of CCR5 protein. The mechanism by which total CCR5 versus cell-surface expression is differentially affected by HCMV depending on whether the cell is itself infected or is an uninfected bystander is at present unclear. We were unable to detect the 62 kDa form of CCR5, consistent with the observed low levels of CCR5 present on the surface of THP1 cells and the indication that the 62 kDa form is more readily detectable under non-reducing conditions (Suzuki et al., 2002). The promoter region of CCR5 contains multiple binding sites for a variety of transcription factors including YY1 (Moriuchi & Moriuchi, 2003; Lei et al., 2005), Oct-1, Oct-2 (Moriuchi & Moriuchi, 2001), T-cell factor 1α and GATA1 (Moriuchi et al., 1997). The observed reduction in CCR5 protein levels following HCMV infection could be the result of upregulation of negative-regulating transcription factors, a decrease in the stability of CCR5 mRNA or an increase in protein degradation. Current studies in our laboratory are under way to investigate the mechanism(s) of HCMV-induced CCR5 downregulation. In contrast to HCMV-infected cells, uninfected bystander cells displayed increased levels of surface expression but had unchanged total levels of CCR5. To extend the relevance of our findings, we confirmed the HCMV-induced changes in CCR5 expression using HCMV-infected primary MDMs. MDMs exhibit similar changes in CCR5 expression on infected and uninfected cells.

We tested whether soluble factor(s) released by the HCMV-infected cells within the population could be responsible for the induction of CCR5 expression on uninfected bystander cells. Differentiated THP1 cells exhibited increased Gag expression after HIV-1 superinfection if HCMV-conditioned medium was used in place of virus. This suggests that a factor or factors produced during HCMV infection of monocytes cells is, at least in part, responsible for the observed increase in bystander CCR5 expression. Furthermore, indirect immunofluorescence experiments and quantitative FACS analysis demonstrated that the upregulation in CCR5 surface expression on bystander cells by conditioned medium also resulted in an increase in HIV-1 Gag expression after HIV-1 superinfection. The exact nature of the soluble factor(s) responsible is currently under investigation, but several soluble mediators have been shown to influence HIV-1 CCR5 co-receptor expression. For instance, both mRNA and protein expression of CCR5 were increased in monocytes/macrophages in response to interleukin 2 (IL-2) (Weissman et al., 2000) and IL-10 (Percherancier et al., 2001) and are augmented by gamma interferon (Hariharan et al., 1999) and tumour necrosis factor alpha (TNF-α) (Croitoru-Lamoury et al., 2003). Furthermore, HCMV encodes a biologically active viral IL-10 homologue (Spencer et al., 2002) that has been demonstrated to bind the IL-10 receptor (Kotenko et al., 2000). Similarly, TNF-α produced by HCMV-infected PBMCs obtained from HCMV-infected donors has been shown to induce HIV-1 replication (Peterson et al., 1992).

Our observations suggest that HCMV infection results in the release of soluble factor(s) that are able to mediate increased CCR5 surface expression on uninfected bystander cells. However, the HCMV-infected cells themselves are refractory to such stimuli. From preliminary experiments, we favour the hypothesis that HCMV infection inhibits expression of CCR5 in the infected cell, thereby protecting or ‘isolating’ the infected cell from any external signals, ensuring an optimal environment for virus replication and dissemination. Downregulation of CCR5 also appears to protect the cells from subsequent HIV-1 infection, as determined by Gag expression. We note that this occurs even in the presence of viral US28 expression, which has been shown to act as a co-receptor for HIV-1 in transduced U373 cells (Pleskoff et al., 1997).

Modulation of cell-surface receptors by HCMV is not uncommon and our laboratory has previously shown downregulation of surface TNFRI and EGFR by HCMV (Fairley et al., 2002; Baillie et al., 2003). It is clear that HCMV specifically targets multiple host surface receptors and such modulation most likely confers a survival advantage.

Taken together, our data suggest that prior infection with HCMV and subsequent reactivation during the
immunocompromised state of HIV-positive patients may contribute to the pathogenesis of AIDS by modulating CCR5 co-receptor levels on neighbouring cells and thus promoting HIV dissemination and replication. We speculate that CCR5 modulation by HCMV in vitro in both mononuclear cells and primary MDMs leads to a direct increase in HIV-MN gene expression following super-infection. Due to conflicting studies, it remains unclear how HCMV influences HIV-1 infection in vivo. Much of the work to date was carried out using pseudotyped HIV-1 and cell lines that cannot normally be infected with HIV-1. The present study employed primary HCMV and HIV target cells, as well as a fully permissive cell line, coupled with naturally infectious virus strains, to investigate the interplay between these two important viral pathogens.

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