Human cytomegalovirus latent genes facilitating human immunodeficiency virus type 1 coinfection in CD34+ cells: abridged secondary publication

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KEY MESSAGES

1. Latent infection of human cytomegalovirus (HCMV) in CD34+ progenitor cells modulates the innate STING immune pathway.
2. Although the STING pathway remains active, the critical step of p-IRF3 translocation required to initiate type-I IFN expression is impaired.
3. This impairment inhibits the differentiation of CD34+ progenitor cells into immune-activating myeloid cells, thus promoting viral persistence.
4. HCMV infection of early CD34+ progenitor cells facilitates HIV-1 coinfection.
5. Detection of HCMV in urine enables early prognosis and prediction of end-organ diseases in HIV-1 patients.

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Introduction

Human cytomegalovirus (HCMV) is a ubiquitous betaherpesvirus that infects 60% to 90% of the global population. The virus establishes lifelong latent infection in CD34+ haematopoietic stem and progenitor cells (HSPCs) and myeloid lineage cells in the human host. It remains asymptomatic unless immune surveillance is impaired, as observed in immunocompromised HIV-1 patients or immunosuppressed transplant recipients. However, the persistence of latent HCMV is an underlying factor in the development of post-transplant organ pathogenesis (eg, hepatitis and pneumonia), congenital conditions (eg, biliary atresia), autoimmune diseases, and the phenomenon of immune memory inflation.

A critical aspect of HCMV latency involves the impairment of differentiation in latently infected CD34+ HSPCs. These progenitor cells can generate innate immune responses or differentiate into antigen-presenting cells such as dendritic cells, which can induce antiviral adaptive immune responses. HCMV latency inhibits the differentiation of HSPCs through various mechanisms. For instance, the latent gene *LAcmvIL-10* suppresses pro-inflammatory cytokines and prevents latently infected myeloid progenitor cells from differentiating into dendritic cells.[1] Latently expressed miRNAs, such as miR-US5-2, target the transcriptional repressor NGFI-A binding protein 1 and upregulate transforming growth factor-β expression, resulting in myelosuppression. HCMV also modulates signal transducer and activator of transcription 3 activity to induce HSPC differentiation into an immunosuppressive monocyte subset, which produces high levels of nitric oxide to facilitate latency.[2] Acute and chronic exposure to type-I interferons (IFNs) can influence the maintenance of haematopoiesis. Innate immune activation regulates haematopoiesis in CD34+ cells through the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway and autocrine type-I IFNs.[3] Moreover, the bacterial second messenger c-di-GMP binds and activates the STING pathway to modulate HSPC homeostasis through type-I IFNs. However, the effect of HCMV latent infection on type-I IFN expression and its influence on CD34+ HSPC differentiation remain poorly understood.

Among immunocompromised HIV-1-infected individuals, HCMV can be reactivated in patients with advanced, stage 4 HIV/AIDS (ie, when the CD4 count is <200 cells/mm^3), leading to multiple end-organ diseases (EODs) and mortality, particularly in the absence of antiretroviral therapy. HCMV-seropositive individuals infected with HIV-1 progress to AIDS approximately 30 months faster and exhibit a more aggressive disease course than HCMV-seronegative patients.[4] There is an association between HCMV DNA and HIV RNA concentrations in peripheral blood and manifestation of serious EODs including retinitis, colitis, hepatitis, and pneumonitis. This illustrates the implications of latent HCMV pathogenesis in HIV-1-infected patients. HCMV and HIV-1 may interact synergistically within CD34+ progenitor stem cells through unknown mechanisms.[5]

HCMV can increase HIV-1 pathogenicity by post-transcriptionally activating proviral HIV-1 DNA or by inducing inflammatory responses that trigger virulent HIV-1 gene expression. The detection of HCMV serves as a predictor of EODs and is associated with a 40% to 60% increased risk of developing EODs in HIV-1-infected individuals. The reactivation of latent HCMV in HIV-1-infected patients may attribute to the onset of EODs. Although associations between plasma HCMV DNA and EODs in stage 4 HIV-1 infection have been reported, there are limited data regarding HCMV in urine and its prognostic value during the early stages of HIV-1 infection.

This study aimed to determine how HCMV latent infection increases its persistence in CD34$^+$ cells and to evaluate the potential of HCMV as a prognostic marker for EOD risk in HIV-1-infected patients.

Results

Latent HCMV impairs type-I IFN-induced CD34$^+$ cell differentiation

We first examined the effect of type-I IFNs (ie, IFN-β) on CD34$^+$ cell differentiation. Flow cytometric analysis revealed that IFN-β at concentrations of 1000 or 3000 U/mL increased the frequencies of CD38$^+$ cells among CD34$^+$ cells, accompanied by an increase in myeloid progenitor subsets including common dendritic cell progenitors, common myeloid progenitors, and granulocyte-monocyte progenitors (Fig 1). In contrast, the frequencies of early progenitor subsets such as haematopoietic stem cells and multipotent progenitors decreased. Stimulation of the cells with 2′3′-cGAMP activated the STING pathway and downstream IFN-β production, modestly increasing the myeloid subsets by day 7 post-treatment. These findings indicate that IFN-β can induce myelopoiesis in the CD34$^+$ cell model and suggest that cell differentiation is suppressed in the presence of latent HCMV infection.

To determine whether differentiation is inhibited in HCMV^{GFP-} cells due to viral modulation of type-I IFNs during latency, we measured expression and secretion levels of IFN-α and IFN-β in both mock-infected and HCMV^{GFP-} cells. HCMV^{GFP-} cells showed significantly decreased expression of *ifna1*, *ifna2*, and *ifnb*, as well as decreased secretion of IFN-β relative to mock-infected controls. To examine whether IFN-β could initiate differentiation in latently infected cells, we added exogenous IFN-β to the culture. In HCMV^{GFP-} cells, treatment with IFN-β increased the proportion of CD38$^+$ cells relative to untreated controls, indicating induction of myelopoiesis. The myeloid subsets were elevated,

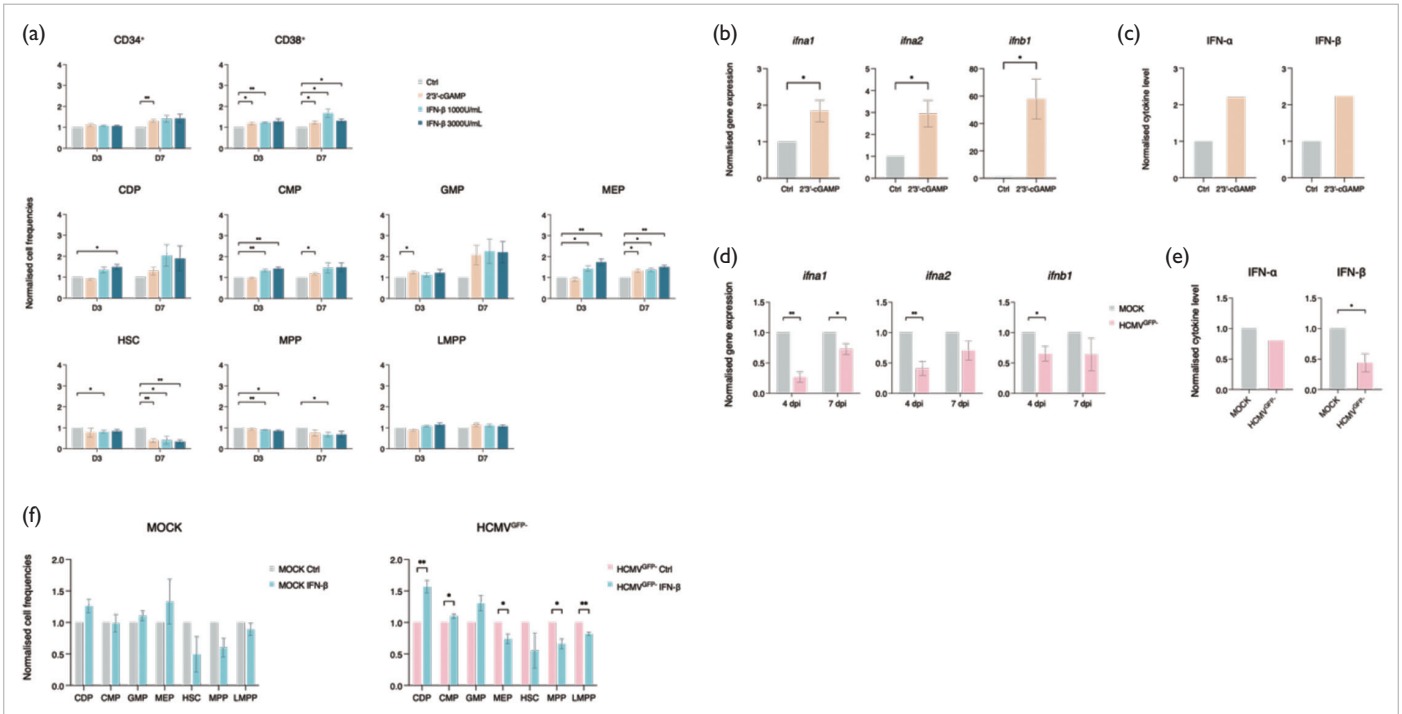

FIG 1. Evaluation of IFN-β-induced myeloid differentiation in CD34$^+$ cells regulated by latent HCMV infection: (a) flow cytometric analysis of CD34$^+$ cell subsets after 2′3′-cGAMP or IFN-β treatment, (b to e) effects of 2′3′-cGAMP or HCMV infection on type-I IFN expression according to qRT-PCR or ELISA, and (f) effect of IFN-β on CD34$^+$ cell differentiation.

Abbreviations: CDP=common dendritic cell progenitor, CMP=common myeloid progenitor, dpi=days post-infection, ELISA=enzyme-linked immunosorbent assay, GMP=granulocyte-macrophage progenitor, HCMV= human cytomegalovirus, HSC=haematopoietic stem cell, IFN-α/β=interferon alpha/beta, LMPP=lymphoid-primed multipotent progenitor, MEP=megakaryocyte-erythroid progenitor, MPP=multipotent progenitor, qRT-PCR= quantitative reverse transcription polymerase chain reaction

whereas the non-myeloid subsets decreased in relative abundance. Similar differentiation patterns were observed in mock-infected cells treated with IFN-β. These results demonstrate that type-I IFNs attribute to myeloid differentiation in CD34+ cells and are likely inhibited by latent HCMV to prevent the development of immune-competent cells.

The STING pathway is activated in CD34+ cells with latent HCMV infection

STING is a key adaptor protein involved in innate immune activation, responding to DNA sensors to recognise viral DNA in HCMV-infected cells. It initiates a type-I IFN response by activating TANK-binding kinase 1 (TBK1), which subsequently drives the phosphorylation of interferon regulatory factor 3 (IRF3). To investigate how latent HCMV suppresses type-I IFN expression, we examined STING pathway activation in CD34+ cells. We also assessed the DNA sensor cGAS, which functions upstream of STING. Unexpectedly, HCMV^{GFP-} cells exhibited elevated expression of *cgas* and *sting1* genes compared with mock-infected controls (Fig 2). This observation was confirmed by Western blot analysis, where protein levels of cGAS and STING, along with downstream signalling molecules TBK1 and IRF3 and their phosphorylated forms, were substantially increased after HCMV latent infection relative to mock-infected controls.

Translocation of p-IRF3 is inhibited by latent HCMV to prevent type-I IFN expression

We next sought to determine whether latent HCMV infection inhibits p-IRF3 nuclear translocation. Confocal microscopy was used to examine mock-infected and HCMV^{GFP-} cells with or without stimulation by 2′3′-cGAMP. In the absence of stimulation, mock-infected cells showed minimal p-IRF3 signal, whereas HCMV^{GFP-} cells displayed elevated cytoplasmic p-IRF3 signal without distinct nuclear localisation. This finding is consistent with the increased p-TBK1 and p-IRF3 levels detected by Western blot analysis. In contrast, HCMV^{GFP+} cells showed elevated p-IRF3 signals in both the cytoplasm and nucleus, indicating active translocation in productively infected CD34+ cells. After 2′3′-cGAMP stimulation, p-IRF3 signal intensity increased across mock, HCMV^{GFP-}, and HCMV^{GFP+} cells. However, nuclear p-IRF3 was observed only in mock and HCMV^{GFP+} cells, not in HCMV^{GFP-} cells, where p-IRF3 was predominantly retained in the cytoplasm. Signal intensity analysis revealed that both HCMV^{GFP-} and HCMV^{GFP+} cells had increased p-IRF3 levels compared with mock-infected controls prior to stimulation. After stimulation, only mock-infected cells exhibited further increases in p-IRF3 intensity. These findings suggest that latent HCMV activates the STING/p-TBK1/p-IRF3 pathway but inhibits p-IRF3 nuclear translocation, thereby reducing type-I IFN expression and preventing CD34+ cell differentiation.

Urine HCMV-DNA presence is correlated with EODs among patients with stage 2/3 HIV-1 infection

Using a Cox regression model, we evaluated correlation between the presence of HCMV DNA and EOD onset. Analysis parameters included

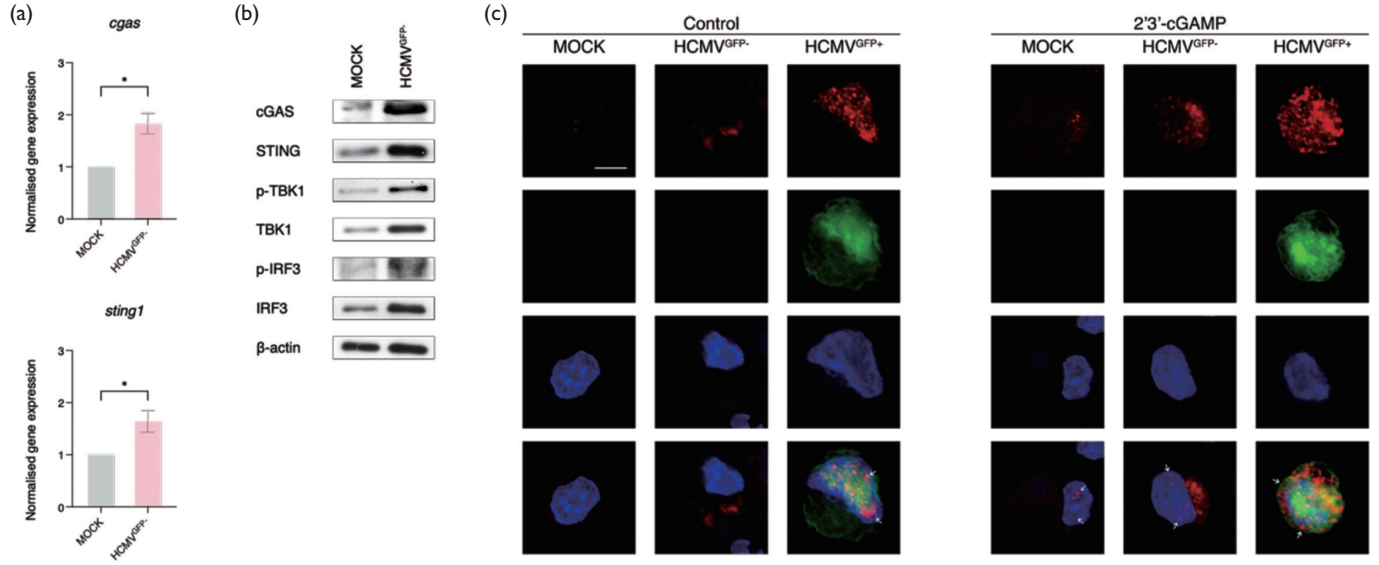

FIG 2. Latent human cytomegalovirus (HCMV) promotes STING pathway activation but prevents p-IRF3 translocation in CD34+ cells: (a) qRT-PCR of *cgas* and *sting1*, (b) Western blot analysis of STING pathway proteins, and (c) confocal microscopy of p-IRF3 signals during HCMV infection (red=p-IRF3, green=HCMV-GFP, blue=DAPI).

HCMV-DNA copy numbers in blood and urine, CD4 counts, HIV-1 RNA copy numbers, and EOD incidence across patient subgroups. In the $HCMV^{blood-}$ subgroup, only one of 26 patients exhibited no EODs; in the $HCMV^{blood+}$ subgroup, only one of 38 patients exhibited no EODs. In the latter group, significance was evident only for skin disease (adjusted hazard ratio [HR]=1.960, 95% confidence interval [CI]=0.922-3.600, P=0.045, Fig 3). In the $HCMV^{urine-}$ and $HCMV^{urine+}$ subgroups, four of 51 and zero of 118 patients, respectively, had no EODs. Significant HRs were observed in the $HCMV^{urine+}$ subgroup for cardiovascular diseases (adjusted HR=0.696, 95% CI=0.492-0.953, P=0.030) and lung diseases (adjusted HR=1.939, 95% CI=1.326-2.761, P<0.001). No significant associations were identified in the $HCMV^{urine-}$ subgroup. Incidence rates of other EOD categories did not differ significantly between groups.

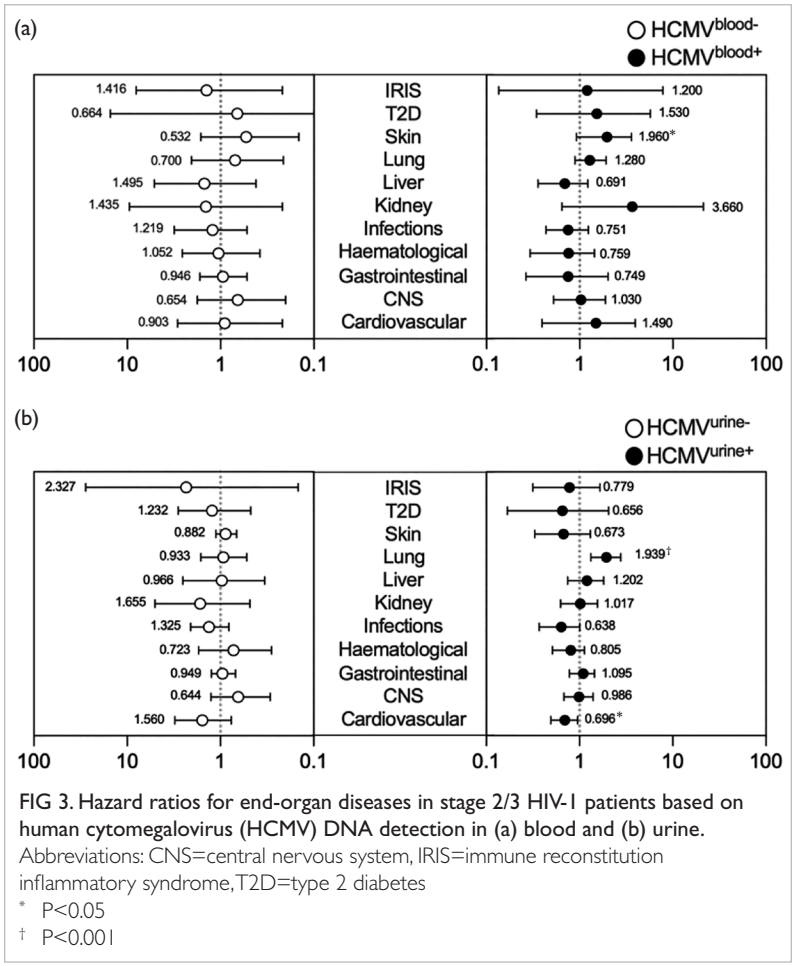

FIG 3. Hazard ratios for end-organ diseases in stage 2/3 HIV-1 patients based on human cytomegalovirus (HCMV) DNA detection in (a) blood and (b) urine.
Abbreviations: CNS=central nervous system, IRIS=immune reconstitution inflammatory syndrome, T2D=type 2 diabetes
* P<0.05
† P<0.001

Discussion

During latency in $CD34^+$ cells, HCMV modulates the STING pathway by inhibiting downstream p-IRF3 nuclear translocation, thus suppressing type-I IFN expression. This suppression prevents $CD34^+$ cells from differentiating into functional antigen-presenting cells, ensuring viral persistence within latent reservoirs. Identification of the HCMV protein responsible for this inhibition is essential for developing therapeutic strategies that restore p-IRF3 activity. The persistence of HCMV as a latent reservoir represents a substantial threat for individuals living with HIV-1, given its association with EODs. Accordingly, we recommend routine testing for HCMV DNA in patients with early-stage HIV-1 infection. For those exhibiting HCMV DNA positivity, treatment with anti-HCMV agents should be considered to delay or manage the onset of EODs, particularly lung and cardiovascular complications.

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Disclosure

The results of this research have been previously published in:
1. Zhao F, Fung TY, Chen Z, Wang H, Cheung AKL. Association of human cytomegalovirus in urine with end-organ diseases in stage 2/3 HIV-1-infected individuals. J Clin Virol 2023;158:105351.

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