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Evidence of Inability of Human Cytomegalovirus to Reactivate Kaposi's Sarcoma-Associated Herpes Virus from Latency in Body Cavity-based Lymphocytes

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Abstract

Background—Kaposi's sarcoma-associated herpesvirus (KSHV; also known as *Human herpesvirus 8*) has been determined to be the most frequent cause of malignancies in AIDS patients. It is associated primarily with Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL), as well as with multicentric Castleman's disease (2). The switch from the latent to the lytic stage is important both in maintenance of malignancy and viral infection. So far, the mechanism of its reactivation has not been fully understood.

Objectives—HCMV and KSHV might infect the same cells and it was found by other groups that several viruses could reactivate KSHV from latency. We wonder whether HCMV infection could reactivate KSHV from latency in BCBL-1 cells.

Study Design and Results—laboratory strains of HCMV can not infect B cells. In this paper, we demonstrate that the UL131-repaired HCMV (vDW215-BADrUL131) derived from AD169 strain are able to infect B lymphocytes. We directly infected KSHV latent cells including body cavity-based lymphocytes (BCBL-1) with vDW215-BADrUL131 to evaluate the ability of HCMV to reactivate KSHV. Inconsistent with previous reports in human fibroblast cells, our results provide direct evidence that HCMV is unable to reactivate KSHV from latency to lytic infection in BCBL-1 cell lines. As a control, HSV-1 was shown to be able to reactivate KSHV.

Conclusions—Different observations of ours from others suggest that reactivation mechanisms for KSHV might vary in different cells.

Keywords

Kaposi's Sarcoma Herpes Virus; Human Cytomegalovirus; Reactivation; body cavity-based lymphocytes (BCBL-1); Herpes Simplex type 1

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Conflicts of Interest Statements

1 We don't have any conflict interests regarding this paper.

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BACKGROUND AND OBJECTIVES

Kaposi's sarcoma-associated herpesvirus (KSHV or human herpesvirus 8, also known as HHV-8) is the most frequent cause of malignancy in AIDS patients. This newly identified virus is associated with Kaposi's sarcoma and several B-cell malignancies such as primary effusion lymphoma as well as multicentric Castleman's disease (MCD) (2,6,7,19, and 25). KSHV infection in permissive cells including endothelial and fibroblast cells experiences a burst of lytic gene expression at very early stage and subsequently causes a latent infection that can switch to a lytic infection upon reactivation(4,13, and 26). Latent infection persists in a majority of cells, and only a small percentage of latent infection can be reactivated to the lytic cycle (8). KSHV can successfully infect monocytes, endothelial/spindle cells, B cells, and epithelial cells. The virus replicates primarily in KSHV-infected B cells and spindle cells (5). Accumulating studies have revealed that KSHV latency to lytic switch is important in viral pathogenesis, secondary infection to maintain the amount of infected cells and tumorigenesis (17). Information regarding reactivation of KSHV latency is mostly from *in vitro* reactivation by using chemicals or changing environment (such as hypoxia) or transfect activating genes including RTA and so on.

Herpesviruses including HCMV and KSHV are ubiquitous, and HCMV infect large populations worldwide, and exist latently in the infected host. Indeed, KSHV and HCMV are often detected simultaneously in the same patient, more importantly both viruses can be isolated from blood cells (3,9,12,15,16,22,29). HCMV IE1 can inhibit histone deacetylase (HDAC) activity (21,27), which mimics the function of trichostatin A (TSA) or sodium butyrate (NaB); it is reasonable to speculate that HCMV can cause the lytic switch of KSHV infection if HCMV infected the cells that harbor the KSHV genome. Clinical data so far are still ambiguous regarding whether the mixed infection could result in KSHV reactivation. Recent studies showed that Tat of HIV could activate KSHV lytic infection through JAK/STAT signaling and that co-culture of HHV-6-infected T cells with KSHV latent B cells resulted in KSHV reactivation (14,34). Vieira et al. clearly showed that an HCMV laboratory strain (AD169) of infection in established KSHV harbored a human fibroblast (HFF) cell line that can reactivate KSHV to produce viral particles (30), and more recently the group mapped that UL112/113 is the viral component to be responsible for the reactivation of KSHV in HFF (33). It is necessary to know whether HCMV can reactivate KSHV in BCBL-1 cells that is our objectives in current studies.

STUDY DESIGN AND RESULTS

HCMV laboratory strains (AD169 and Towne) lost their infectivity other than in human fibroblast cells due to mutation in gene locus of UL131–128 (10). AD169 has one nucleotide insertion in UL131 that causes amino acid (aa) frame shift and functional defect of UL131. Towne strain has aa frame shift in UL130 and also causes defective tropism to other cells (1, 20). Clinical (wild) strains of HCMV might infect a wide range of cells. Fibroblast, endothelial, epithelial, and blood cells are all susceptible to HCMV infection; it was reported that B cells isolated from 40% patients with active HCMV infection have viral DNA (11). Remarkably, clinical (wild) strains of HCMV isolated from patients and then propagated in fibroblasts for just a few passages lose their ability to infect any cells other than the fibroblast cells. That laboratory and clinical (wild) strains of HCMV are not equally infectious also serves to emphasize the difficulties inherent in the study of HCMV pathogenesis. The hypothesis that UL131–128 should be the determinant of cell tropism and that mutation of the gene locus is the mechanism by which HCMV loses its tropism to many cells in the laboratory was supported by recent molecular studies of the HCMV tropisms (31,32). In the studies, the investigator removed the one nt insertion of UL131 based on AD169. Due to the recovery of the mutation, the repaired AD169, namely vDW215-BADrUL131, was found to be able to infect not only

fibroblast cells, but also endothelial cells, epithelial cells. Therefore, after repair of the mutations, the viral tropism can be recovered. However, it is still unknown whether the repaired HCMV could infect blood cells like B lymphocytes.

First, we infected B lymphocytes (BJAB—B lymphocyte without KSHV latency and BCBL-1—B lymphocyte with KSHV latency) and human fibroblast cells (Mrc-5 and HFF) with a laboratory strain of HCMV (AD169) at an MOI (multiplicity of infection) of 5 for 72 hours, then whole cell lysates were applied in order to run PAGE, and we performed Western blot to detect HCMV proteins. Compared with the infection of HCMV in human fibroblast cells (in which HCMV can express viral proteins as seen in IE1/2, MCP, and pp28), no viral protein could be detected in BJAB or BCBL-1 cells (Fig. 1A). We subsequently infected BCBL-1 and BJAB cells with vDW215-BADrUL131, a repaired HCMV, at an MOI of 5 for different time. As can be seen in Fig. 1B, viral proteins from different stages can be detected. Both MCP and pp28 (UL99) are late proteins, pp28 (also called true late gene) production was proved to be DNA-replication dependent; such production suggests, as well, that DNA replication also occurred. Therefore, the production of pp28 implies a successful infection of vDW215-BADrUL131 in B lymphocytes. Finally, we performed the plaque formation unit (PFU) assay. First, we infected BCBL-1 with HCMV AD169 or vDW215-BADrUL131 at MOI of 1 for different day as indicated in Fig. 1C. The medium and cells were collected and viral particles were released from cells by thaw and freeze for 3 cycles. After centrifugation, the supernatant were used to infect human fibroblast cells for counting viral plaques. The results in Fig. 1C show that vDW215-BADrUL131 can productively infect BCBL-1 cells while AD169 failed to produce any viral particles. Taken together, the data here provided evidence that HCMV is able to infect B lymphocyte after the tropism is recovered from AD169, and the existence of KSHV has no effect on the infection.

Although most of those with latently infected KSHV never suffer from any KSHV-associated diseases, such individuals run a risk of developing KS, PEL, or MCD, with the greatest risk factor for these conditions being immunodeficiency, which occurs in AIDS patients, transplant recipients, and patients with viral infections. The HCMV genome encodes some products that have immunomodulatory effects that can interfere with the host's immune system. In addition, the above-presented observation demonstrates the fact that HCMV can infect B lymphocytes, which cells can also be infected by KSHV. Several recent studies on the pathogenesis of KSHV have shown that a number of viruses (including HHV-6, HSV-1, HIV, and HCMV) can induce KSHV lytic infection (14,23,30,34). HCMV has been the focus of our interest not only because it can infect the same type of cells as KSHV—as has been shown by many laboratories—but also because it infects a large population (about 50–90%). An observation by Vieira et al. that HCMV can reactivate KSHV from latency might explain the virus-virus interactions (30).

It is important to study on the KSHV latency disruption by HCMV in B lymphocytes because HCMV and KSHV can be detected in the blood samples from the same patient, which suggests a co-infection. We infected BCBL-1 cells with vDW215-BADrUL131 HCMV at an MOI of 5 for different time as indicated in Fig. 2A. TPA-treated BCBL-1 was used as positive reactivation control. The whole cell lysates were collected at the times indicated; Western blot was performed to show HCMV and KSHV proteins. An increase in IE1/2 production is evidence of active HCMV infection in BCBL-1 cells; faint bands above the major bands indicate Small Ubiquitin-Like modifier (SUMO)-modified IE1/2. KSHV reactivation is indicated by the presence of RTA, a gene essential for KSVH reactivation; RTA can only be produced (and is the first gene expressed) after reactivation, as shown in TPA-treated BCBL-1 cells. The absence of RTA in HCMV-infected BCBL-1, then, suggests that HCMV failed to reactivate KSHV. LANA, a protein produced during KSHV latency on BCBL-1 cells and cellular protein (tubulin), was used as sample loading control. As a control, we infected BCBL-1 cells with a wild-type strain of HSV-1, as shown in Fig. 2B, and ICP8 was used to

indicate HSV-1 infection. We demonstrated that HSV-1 infection in BCBL-1 cells resulted in the reactivation of KSHV as indicated by the presence of RTA, which is consistent with the report from the other group (24). Question about the clinical significance of the observation of interaction of HSV-1 with KSHV still remains. An animal model might be needed to determine whether HSV-1 can reactivate KSHV.

Furthermore, we visualized the HCMV infection in BCBL-1 cells by immunofluorescence. First, when the BCBL-1 cells were infected with vDW215-BADrUL131 at an MOI of 5 for 72 hours, the positive infection was shown by the production of IE1 (Figs. 3A1 and B1) and major capsid protein (MCP) in the nuclei (C1 and D1) in green. KSHV protein production was shown in red using anti-RTA polyclonal antibody (Figs. 3A2 and C2) and anti-K8 monoclonal antibody (Figs. 3B2 and D2), which signal the reactivation of KSHV because they are both lytic-stage proteins. Total cells in the microscope field were shown by 4', 6-diamidino-2-phenylindole (DAPI) staining in blue (A3 through D3). The immunofluorescence studies showed that neither RTA nor K8 was detected in most of the HCMV positively infected cells. Three single colors were merged (A4 through D4) in order to be able to visualize the relationship between HCMV infection and KSHV reactivation. It has been noticed that there is a positive cell in Fig. B2 (positive K8, in red) in which HCMV IE1/2 is much less than other cells in the same microscope field (Fig. 3B1). In BCBL-1 cell cultures that have not been stimulated, KSHV was reactivated (called "leaking") in less than 1% of the cells; figure 3B1 shows one of them. This immunofluorescence study further demonstrated that HCMV infection failed to reactivate KSHV in BCBL-1 cells.

We next wondered whether HCMV could affect KSHV reactivation by TPA in BCBL-1 cell. After we infected BCBL-1 cells with vDW215-BADrUL131 at an MOI of 5 for 48 hours, we added TPA in the cell culture for 24 hours. The cells were cytopun to slides for detection with immunofluorescence with anti-HCMV MCP antibody (Figs. 3C and D) or with anti-IE1/2 (table 1) and KSHV RTA or K8 antibody. As shown in Fig. 3C, HCMV infected cells (MCP positive: C1, left) can be still reactivated by TPA (RTA in red, C2). We also randomly counted cells in two groups: HCMV infected (shown by IE1/2 in green) and non-infected to see whether there is any difference between the KSHV reactivation rates. As shown in table 1, we counted 300 HCMV-infected cells in which 35 cells were reactivated (as marked with RTA staining). In 500 non-infected cells, 59 cells were reactivated. No significant difference was found between the two groups using student's *t*-test.

DISCUSSION

Human herpes viruses mostly cause latent infection in immune-competent populations and results in disease when reactivated by many different environmental conditions (28). Several herpes viruses might infect the same person because many of them have high infection incidence. KSHV infection in general population is not as high as other herpes viruses, but it is the first important pathogen causing cancer in AIDS patients. HCMV infects most population and can be occasionally reactivated (18). Mixed infection of KSHV and HCMV intrigued us to investigate the interaction of the two viruses. The finding that HSV-1 infection can switch KSHV infection to lytic is further demonstrated in this study, however, interactions between HCMV, KSHV and HIV might be of more importance because those viruses could infect the same kind of cells.

The facts that HCMV IE1 and IE2 can interact with HDAC and inhibit HDAC activities and that HDAC inhibitors can reactivate KSHV in cell culture suggest that HCMV infection might be able to reactivate KSHV infection. As a matter of fact, in human fibroblast cell system, it has been reported recently that HCMV UL112/113 molecule can leads KSHV infection from latency to lytic stage in human fibroblast cells (33). It is necessary to know whether HCMV

could also reactivate KSHV in other cell line. We infected BCBL-1 harboring KSHV genome with vDW215-BADrUL131 HCMV and detected the state of KSHV; it is clear that HCMV infection failed to reactivate KSHV in BCBL-1 cells. We also found that KSHV reactivation by TPA was not affected by HCMV infection. We also found that the two viruses can exist in the same cells and continue to express their own gene products. The differences of the results between ours and the others about the effect of HCMV infection on KSHV reactivation imply a different mechanism of reactivation of KSHV in different type of cells.

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Fig. 1.

Infectivity of laboratory strain and UL131-repaired HCMV in B cells. A. after infection of laboratory strains of HCMV in cells for the time as indicated, cells were collected and lysed in Lamile buffer and applied in 7.5% PAGE; the viral proteins were detected with Western blot assay. B. UL131-repaired HCMV, vDW215-BADrUL131 infection in B cells were detected by Western blot assay. C. BCBL-1 cells in 12-well plates were infected with HCMV AD169 or vDW215-BADrUL131 at MOI of 1 and the medium was changed at 12 hours after infection. The cells and medium was collected as the days post infection as indicated and the viral particles were released by thaw-and freeze for 3 cycles. After centrifugation, the

supernatant were applied for PFU assay. Data from triplicates were obtained and statistically analyzed; the error bars stand for the standard error from the three independent experiments.

Fig. 2.

KSHV reactivation in BCBL-1 cells by HCMV, HSV-1, and TPA. BCBL-1 cells were infected with vDW215-BADrUL131 (left) or HSV-1 (right) for the time indicated, and the cells were treated with TPA for 48 hours and were collected and lysed in Lamile buffer; cellular proteins and viral proteins were detected with Western blot assay.

Fig. 3.

Immunofluorescence to detect HCMV infection in BCBL-1 cells. BCBL-1 cells were infected with vDW215-BADrUL131, untreated (A and B) or treated (C and D) with TPA, and were washed with PBS and fixed with 1% paraformaldehyde and cytopun to slides for immunofluorescence assay using different antibodies, as indicated, to detect viral proteins: IE1/2 (A1 and B1) and MCP (C1 and D1) of HCMV in green; RTA (A2 and C2) and K8 (B2 and D2) of KSHV in red. Total cells were shown with DAPI blue (A3 to D3). Three different colors were merged and shown in A4 through D4.

Table 1

KSHV reactivation rate in HCMV infected cells versus non-infected cells

Relationship between HCMV infection and KSHV reactivation. 48 hours after infection of BCBL-1 cells with vDW215-BADrUL131 at an MOI of 5, we added TPA in the cell culture for 24 hours. The cells were cytopun to slides for detection with immunofluorescence with anti-HCMV IE1/2 and KSHV RTA antibody. Cells were chosen at random and counted.

HCMV infection state	Total no. of cells counted	no. of RTA positive cells	reactivation rate (%)
IE1/2 positive	300	35	11.7
IE1/2 negative	500	59	11.8

Student *t* test: $P>0.05$