

Selective activation of inflammation factors by human parvovirus B19 and human bocavirus VP1 unique region on H9c2 cardiomyocyte

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Abstract. Human parvovirus B19 (B19) and human bocavirus 1 (HBoV) are the only known pathogenic parvoviruses, and are responsible for a variety of diseases in human beings. Mounting evidence indicates a strong association between B19 infection and cardiac disorders including myocarditis, dilated cardiomyopathy and heart failure. However, very limited information about the role of HBoV in cardiac disorders is known. To elucidate the effects of B19 and HBoV on cardiac disorders, we expressed EGFP-conjugate constructs of B19-VP1 unique region (VP1u) and HBoV-VP1u, along with the mutants EGFP-B19-VP1uD175A and EGFP-HBoV-VP1uV12A, in H9c2 cells by stable transfection. The protein expression levels of EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A in H9c2 cells were observed under a fluorescence microscope and confirmed by western blotting. Secreted phospholipase A2 (sPLA2) activity was detected in B19-VP1u and HBoV-VP1u but not B19-VP1uD175A and HBoV-VP1uV12A recombinant proteins. Significantly higher expression levels of MCP2 and IP-10 mRNA were detected in H9c2 cells that were transfected with pEGFP-B19-VP1u, compared with in those cells transfected with pEGFP-HBoV-VP1u, pEGFP-B19-VP1uD175A or

pEGFP-HBoV-VP1uV12A. Significantly higher protein levels of IL-1 β and IL-6 were detected in H9c2 cells transfected with pEGFP-B19-VP1u or pEGFP-HBoV-VP1u, compared with in those cells transfected with pEGFP-B19-VP1uD175A or pEGFP-HBoV-VP1uV12A. Notably, significantly higher expression of both TNF- α and NF- κ B was observed only in H9c2 cells transfected with pEGFP-B19-VP1u, but not in those cells transfected with pEGFP-HBoV-VP1u, pEGFP-B19-VP1uD175A or pEGFP-HBoV-VP1uV12A. These findings, to our knowledge for the first time, reveal the difference between B19-VP1u and HBoV-VP1u in H9c2 cells and provide insight into the roles of B19-VP1u and HBoV-VP1u in the pathogenesis of cardiac inflammation.

Introduction

Human parvovirus B19 (B19) and human bocavirus 1 (HBoV) are the only known pathogenic parvoviruses in human beings (1,2). B19, discovered in 1975 by Cossart during screening for hepatitis B virus (3), is highly infectious and causes various pathological conditions including fifth disease in children, persistent anemia in immunocompromised patients, transient aplastic crises, hydrops fetalis in pregnant women, arthropathy, and autoimmune diseases (4-7). HBoV, discovered in 2005 by Allender *et al* (8), was first identified in the respiratory nasopharyngeal aspirates of children with lower respiratory tract infections. HBoV is known to be a significant causative agent in acute respiratory tract infections, in which wheezing is the most common symptom (9).

In the literature, intra-nuclear viral particles typical of B19 were first documented in an electron microscopic examination of fetal cardiac tissue (10); however, B19 is not regarded as a cardiotropic virus (11). Notably, a previous investigation identified B19 as a pathogenic agent in cases of myocarditis in children and adolescents (12). More recently, an increasing body of evidence has shown that B19 is strongly associated with cardiovascular disorders. In the last decade, B19 has emerged as a potential pathogenic agent in adult patients with

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inflammatory heart disease (13). Indeed, the B19 genome has been detected in endomyocardial biopsies of patients with acute myocardial infarction (14). Another investigation found that the persistence of B19 may be associated with progression of left ventricular dysfunction (15). In a study of 208 patients, dominantly higher prevalence of the B19 genome was found in endomyocardial biopsies of inpatients with inflammatory cardiomyopathy or myocarditis compared with controls (16). These findings strongly indicate a connection between B19 and heart disorders.

Phospholipase A2 (PLA2)-like activity of B19-VP1 unique region (VP1u) has been identified (17) and associated with its infectivity and the pathogenesis of many disorders (18–21). Recently, B19-VP1u has been associated with cardiac disorders (22,23). Abnormal ultrastructural changes in the myocardia and elevated levels of myocardial functional enzymes, including aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase isoenzyme (CK-MB) and alpha-hydroxybutyric acid dehydrogenase (alpha-HBDH), have been detected in mice receiving recombinant B19-VP1u proteins (22). Similarly, dilated cardiomyopathy has been observed in BALB/c mice immunized with VP1u; an observation clinically relevant to B19-associated cardiac damage (24). Although a recent investigation indicated that HBoV-VP1u also has a PLA2 motif, and could exhibit sPLA2 activity (25), little is known about the role of HBoV-VP1u in cardiac injury. Therefore, this study compared B19-VP1u and HBoV-VP1u with respect to their potential roles in inducing injury in H9c2 cardiomyocytes.

Materials and methods

Plasmids. Plasmid pEGFP-C1 was purchased from CLONTECH (Clontech Laboratories, Inc., Mountainview, CA, USA). The B19-VP1u and B19-VP1uD175A genes described in our previous study (26) were ligated into the pEGFP-C1 expression vector, which are known as pET32a-B19-VP1u and pET32a-B19-VP1uD175A. A 387-bp DNA fragment encompassing nucleotides 3056–3442 of the Taiwan HBoV strain (TW125_07: GeneBank accession no. EU984241.1) provided by Centers for Disease Control, Taipei, Taiwan (27) was amplified by the polymerase chain reaction (PCR) using the following primers, including 5'-GCA GATCTATGCCTCCAATTAAG-3' (forward primer) and 5'-GCGTCGACTGAGGTTTCCTGG-3' (reverse primer). The HBoV-VP1u and HBoV-VP1uV12A, the mutant form of HBoV-VP1u without sPLA2 activity (25,28), were constructed into pEGFP-C1, which are known as pET32a-HBoV-VP1u and pET32a-HBoV-VP1uV12A. All constructants were verified by DNA sequencing analysis forwardly and reversely.

Secreted sPLA2 catalytic activity. The secreted sPLA2 activity was detected as cribbed elsewhere (27). The recombinant protein samples, including B19-VP1u, B19-VP1uD175A, HBoV-VP1u and HBoV-VP1uV12A, were assayed for sPLA2 activity by using a colorimetric assay (cat. no. 765001, sPLA2 Activity kit; Cayman Chemical), in accordance with the manufacturer's instructions, with dynamic colorimetric measurements at the optical density of 414 nm determined

every minute for 10 min. Results are revealed as micromoles per minute per milliliter.

Cell culture, transfection and stable clones. H9c2 cardiac myoblast cells were purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C in a 5% CO₂ incubator. A total of 1x10⁶ cells were grown to 70–80% confluence in a 100 mm² dish before transfection. The transfection reaction was performed using Lipofectamine reagent with PLUS reagent (Invitrogen; Thermo Fisher Scientific, Inc.) with 2 µg of the plasmids, pEGFP, pEGFP-B19-VP1u, pEGFP-B19-VP1uD175A (a mutant of the B19-VP1u region), pEGFP-HBoV-VP1u and pEGFP-HBoV-VP1uV12A (a mutant of HBoV-VP1u region), respectively. To determine the transfection efficiency, pCMV-SPORT-β-gal plasmid (0.5 µg/transfection, Invitrogen; Thermo Fisher Scientific, Inc.) and co-transfected with the constructs mentioned above, respectively. After X-gal staining (29), cells were fixed with chilled methanol and the extent of β-gal expression was measured by determining the ratio of the X-gal-stained area to the area of each observation field under a light microscope. No significant variation was observed among all groups (data not shown). The cells were then cultured in serum-free DMEM for 12 h at 37°C in a 5% CO₂ incubator and subsequently in DMEM with 10% FBS. The stable clones were obtained by G418 selection at a concentration of 600 mg/ml (Promega Corporation, Madison, WI, USA) in DMEM containing 10% FBS for 8 weeks. The expression levels of EGFP and the EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A fusion proteins were examined by using a Zeiss Axioplan-2 epifluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) and by western blot analysis.

Fluorescence microscopy. Expression of recombinant EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A in H9c2 cells was observed with a Zeiss Axioplan-2 fluorescence microscope (Carl Zeiss AG). Transfected H9c2 cells were fixed by 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, and blocked with 1% BSA in phosphate-buffered saline (PBS) for 10 min. The cell nuclei were stained with DAPI (blue). Excitation filters/emission filters were set at 480/535 and 358/460 nm for green fluorescent protein (GFP) and DAPI, respectively. Digital images of the cells were recorded by using a spot camera system.

Reverse transcription-semi quantitative (RT-sq)PCR. All procedures were carried out in a designated PCR clean area. RNA was extracted from infected cells using Trizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was isolated from the cells transfected with EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A, respectively. The RNA samples were suspended in diethyl pyrocarbonate (DEPC)-treated water, quantified, and then stored at -80°C until use. RNA concentration and purity were determined by a spectrophotometer by calculating the ratio of optical density at wavelengths of 260 and 280 nm. The first-strand cDNA for PCR was synthe-

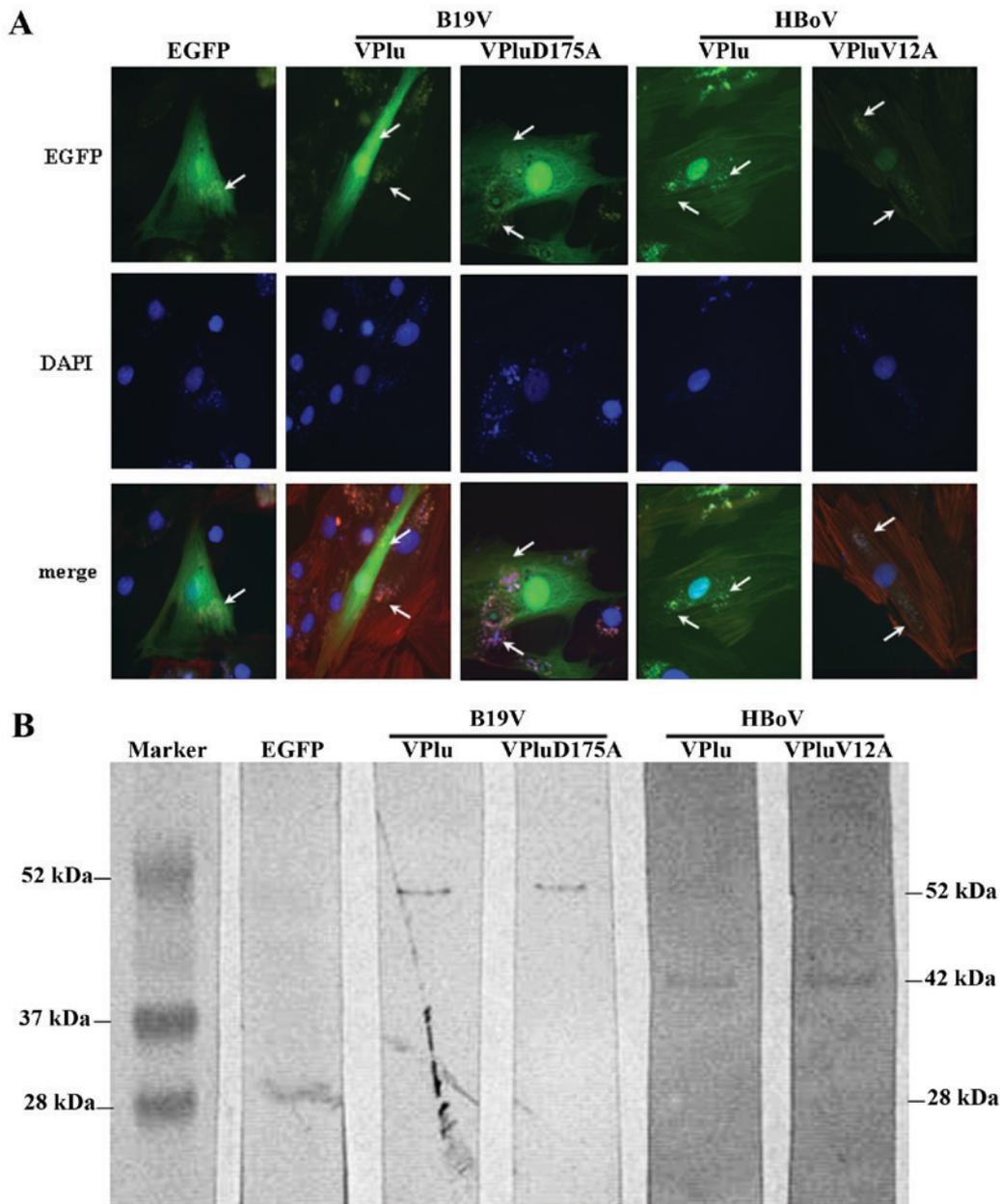


Figure 1. Expression of EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A in H9c2 cells. (A) Representative images of H9c2 cells transfected with pEGFP, pEGFP-B19-VP1u, pEGFP-B19-VP1uD175A, pEGFP-HBoV-VP1u or pEGFP-HBoV-VP1uV12A, observed under a fluorescence microscope. EGFP expression is indicated with white arrows (magnification, x200). (B) Expression of EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A recombinant proteins was detected using antibodies against EGFP. Similar results were observed in triplicate experiments.

sized from total RNA (2 μ g) using the ImProm-II Reverse Transcription System (Promega Corporation). The cDNAs encoding monocyte chemoattractant protein 2 [MCP2, also known as chemokine ligand 8 (CCL-8)], IFN- γ -inducible protein 10 [IP-10, also known as CSC motif chemokine 10 (CXCL10)] and GAPDH were amplified by using a multiplex PCR kit (cat. no. MP-70070; Maxim Biotech, Inc., Rockville, MD, USA). The intensity of MPC2, IP-10 and GAPDH were then quantified using densitometric apparatus (Alpha-Imager 2200; ProteinSimple, San Jose, CA, USA).

Protein extraction. The cells were centrifuged at 800 g for 5 min and washed twice with ice-cold PBS twice. The cell pellets were then suspended in 600 μ l of PRO-PREP™ buffer

(iNtRON Biotech, Gyeonggi-do, Korea) and chilled on ice for 1 h. The supernatant containing protein extracts were then collected by centrifugation at 17,982 g for 5 min at 4°C. Protein concentration of the samples was determined by a modified Bradford assay using a spectrophotometer (Hitachi U3000; Hitachi, Ltd., Tokyo, Japan) at 595 nm with BSA as the standard.

Immunoblotting. Protein samples were separated by 10 or 12% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes (GE Healthcare, Chicago, IL, USA). After blocking with 5% non-fat dry milk in PBS, antibodies against GFP (cat. no. 460092; Invitrogen; Thermo Fisher Scientific, Inc.), TNF- α (cat. no. sc-8301), IL-6 (cat. no. sc-1265), IL-1 β

(cat. no. sc-7884), NF- κ B p65 (cat. no. sc-109) (1:500-1:1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and β -actin (1:5,000; MAB1501, Chemicon; EMD Millipore, Billerica, MA, USA) were diluted in PBS with 2.5% BSA and incubated with the membranes for 1.5 h with gentle agitation at room temperature. The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (cat. nos. sc-2004 or sc-2005; Santa Cruz Biotechnology, Inc.). Immobilon Western Chemiluminescent HRP Substrate (EMD Millipore) and a chemiluminescence imaging analyzer (GE ImageQuant TL 8.1; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) were used to detect the antigen-antibody complexes. The blotting results were then quantified using densitometric apparatus (Alpha-Imager 2200; ProteinSimple).

Statistical analysis. All of the statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA) by one-way analysis of variance (One-way ANOVA) followed by Tukey's multiple-comparisons test. Data were represented as mean \pm SEM and verified at least three independent experiments. $P < 0.05$ was considered to indicate a statistically significant difference. The significant differences were stressed with symbols as shown in figures.

Results

Expression of recombinant B19-VP1u and HBoV-VP1u. To detect the expression of the recombinant EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A proteins in H9c2 cells, an epifluorescence microscope and western blot analysis were employed. The upper panel of Fig. 1A shows photographs of H9c2 cells that were transfected with pEGFP, pEGFP-B19-VP1u, pEGFP-B19-VP1uD175A, pEGFP-HBoV-VP1u and pEGFP-HBoV-VP1uV12A. The expressions of EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A was observed as indicated by the arrow in the figure. The nuclei of H9c2 cells were stained with DAPI. The expression of the EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A recombinant proteins was further verified using antibodies against EGFP (Fig. 1B).

Secreted sPLA2 activity of recombinant B19-VP1u and HBoV-VP1u. To determine the sPLA2 catalytic activity of the purified recombinant proteins, an sPLA2 activity assay was performed. Table I summarizes the sPLA2 activities in B19-VP1u, B19-VP1uD175A, HBoV-VP1u and HBoV-VP1uV12A recombinant proteins. As a positive control, bvPLA2 exhibited an sPLA2 activity of 0.368 ± 0.009 μ mol/min/ml, whereas B19-VP1uD175A and HBoV-VP1uV12A exhibited no detectable sPLA2 activities. Accordingly, sPLA2 activities were detected for B19-VP1u (400 ng) and HBoV-VP1u (400 ng) at the values of 0.098 ± 0.012 and 0.046 ± 0.007 μ mol/min/ml, respectively (Table I).

Effects of recombinant B19-VP1u and HBoV-VP1u on chemokine expression in H9c2 cells. To study the effects of EGFP, EGFP-B19-VP1u, EGFP-B19-VP1uD175A, EGFP-HBoV-VP1u and EGFP-HBoV-VP1uV12A on chemo-

Table I. Determination of sPLA2 activity.

Proteins	sPLA2 activity (mmol/min/ml)
bvPLA2 (10 ng)	0.368 ± 0.009
B19-VP1u (400 ng)	0.098 ± 0.012
B19-VP1uD175A (400 ng)	ND
HBoV-VP1u (400 ng)	0.046 ± 0.007
HBoV-VP1uV12A (400 ng)	ND

Similar results were observed in triplicate experiments (N=3) and the data was expressed with mean \pm SEM. bvPLA2, sPLA2 from bee venom PLA2 control; ND, no detected; sPLA2, secreted phospholipase A2.

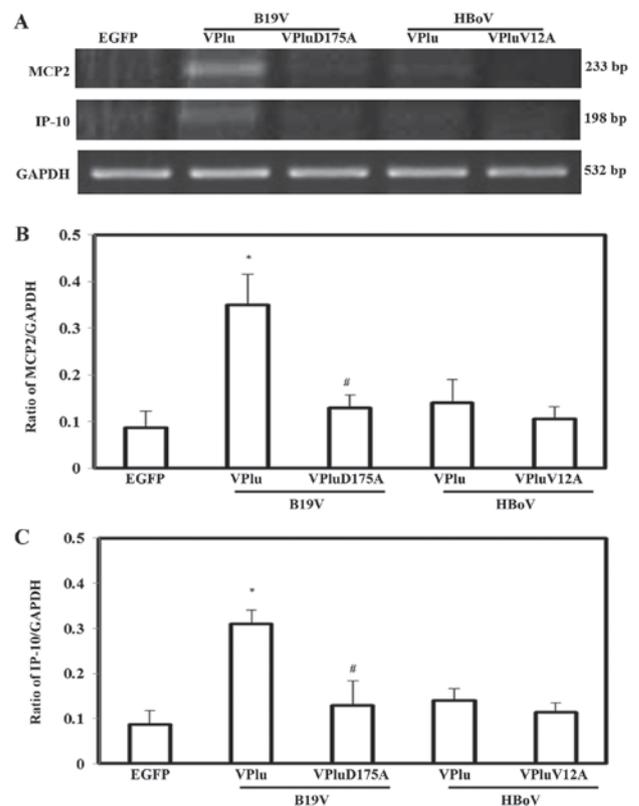


Figure 2. Expression of MCP2 and IP-10 mRNA. (A) Total mRNA of MCP2 and IP-10 in H9c2 cells transfected with pEGFP, pEGFP-B19-VP1u, pEGFP-B19-VP1uD175A, pEGFP-HBoV-VP1u or pEGFP-HBoV-VP1uV12A, detected by reverse transcription-semi quantitative polymerase chain reaction. Relative levels of (B) MCP2 and (C) IP-10 are shown based on GAPDH level. Similar results were observed in triplicate experiments. * $P < 0.05$, relative to the EGFP transfection group and # $P < 0.05$, relative to the B19V-VP1u transfection group.

kine expression, the mRNA expressions of MCP2 and IP-10 were measured (Fig. 2). Significantly higher levels of MCP2 and IP-10 mRNA were detected in H9c2 cells transfected with pEGFP-B19-VP1u, but not in those cells transfected with pEGFP-HBoV-VP1u, pEGFP-B19-VP1uD175A or pEGFP-HBoV-VP1uV12A, relative to expression in cells transfected with pEGFP. Quantified results of MCP2 and IP-10 mRNA levels based on GAPDH level are shown in Fig. 2B and C.

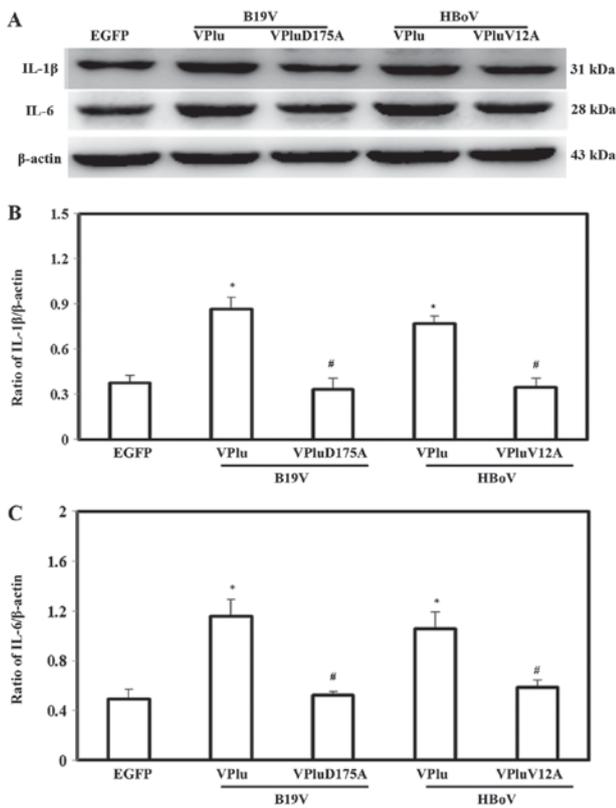


Figure 3. Expression of IL-1β and IL-6 proteins. (A) IL-1β and IL-6 expression in H9c2 cells transfected with pEGFP, pEGFP-B19-VPlu, pEGFP-B19-VPluD175A, pEGFP-HBoV-VPlu or pEGFP-HBoV-VPluV12A, detected by immunoblotting. Relative levels of (B) IL-1β and (C) IL-6 are presented based on β-actin expression. Similar results were observed in triplicate experiments. *P<0.05, relative to the EGFP transfection group and #P<0.05, relative to the B19V-VPlu transfection group.

Effects of recombinant B19-VPlu and HBoV-VPlu on inflammatory cytokines expression in H9c2 cells. To examine the effects of EGFP-B19-VPlu, EGFP-B19-VPluD175A, EGFP-HBoV-VPlu and EGFP-HBoV-VPluV12A on the expressions of proinflammatory cytokines, IL-1β, IL-6 and TNF-α levels were detected by western blotting. Significantly higher protein expression of IL-1β and IL-6 was detected in H9c2 cells transfected with pEGFP-B19-VPlu and pEGFP-HBoV-VPlu, respectively, compared with in those cells transfected with pEGFP. Conversely, no significant difference in the expression of either IL-1β or IL-6 was detected in H9c2 cells transfected with pEGFP-B19-VPluD175A and pEGFP-HBoV-VPluV12A, respectively, from that in cells transfected with pEGFP. The lower panel in Fig. 3 shows quantitative results concerning the expression levels of IL-1β and IL-6 normalized to β-actin. Notably, significantly higher expression of TNF-α was observed only in H9c2 cells transfected with pEGFP-B19-VPlu, and not in those cells transfected with pEGFP-HBoV-VPlu, pEGFP-B19-VPluD175A or pEGFP-HBoV-VPluV12A, relative to that in cells transfected with pEGFP. The lower panel in Fig. 4 shows quantitative results concerning TNF-α levels normalized to β-actin. To identify the potential signaling involved in B19-VPlu-mediated cytokine induction, the expression of NF-κB was detected. Significantly higher expression of NF-κB protein was detected in H9c2 cells transfected with pEGFP-B19-VPlu, compared with in

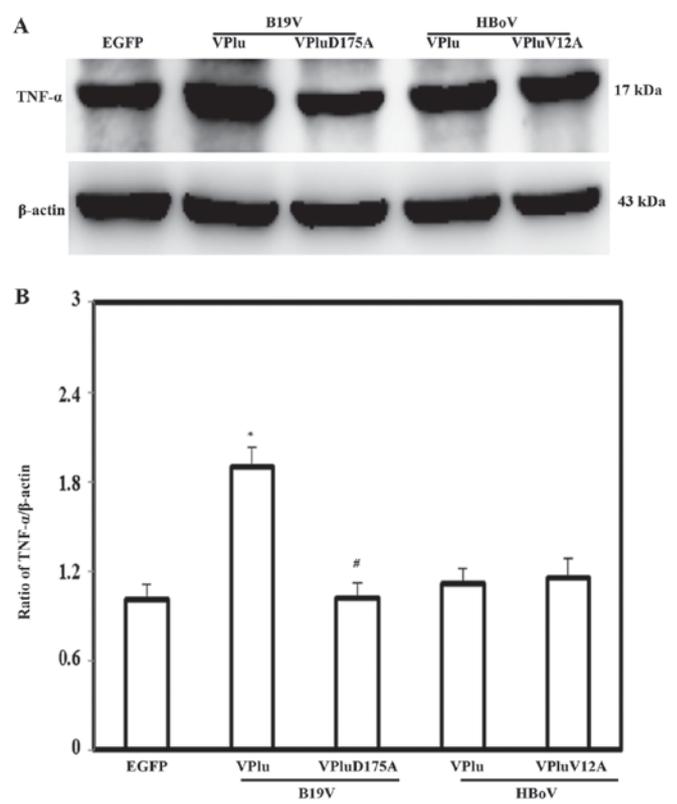


Figure 4. Expression of TNF-α protein. (A) TNF-α expression in H9c2 cells transfected with pEGFP, pEGFP-B19-VPlu, pEGFP-B19-VPluD175A, pEGFP-HBoV-VPlu or pEGFP-HBoV-VPluV12A, detected by immunoblotting. (B) The relative level of TNF-α is presented based on β-actin expression. Similar results were observed in triplicate experiments. *P<0.05, relative to the EGFP transfection group and #P<0.05, relative to the B19V-VPlu transfection group.

those cells transfected with pEGFP. No significant difference in the expression of NF-κB protein was detected between H9c2 cells that were transfected with pEGFP-HBoV-VPlu, pEGFP-B19-VPluD175A or pEGFP-HBoV-VPluV12A, and those cells transfected with pEGFP. The lower panel in Fig. 5 shows quantitative results concerning NF-κB levels normalized to β-actin.

Discussion

B19 and HBoV are the only established pathogenic parvoviruses in the literature, and are responsible for many diseases in human beings and thought to circulate globally. Although both B19-VPlu and HBoV-VPlu exhibit sPLA2-like activity (27), only B19-VPlu has been associated with cardiac diseases (22,23). The present investigation, to the best of our knowledge, is the first to evaluate the involvement of HBoV-VPlu in inducing cardiac inflammatory cytokines, and the difference between B19-VPlu and HBoV-VPlu with respect to induced cytokine profiles. B19-VPlu was found herein to induce significant expression of inflammatory chemokines and cytokines, including MCP2, IP-10, IL-1β, IL-6 and TNF-α, in H9c2 cells, whereas HBoV-VPlu only induced significant expression of IL-1β and IL-6 in the H9c2 cells. These findings demonstrate for the first time the effect of HBoV-VPlu on cardiac inflammation and the different

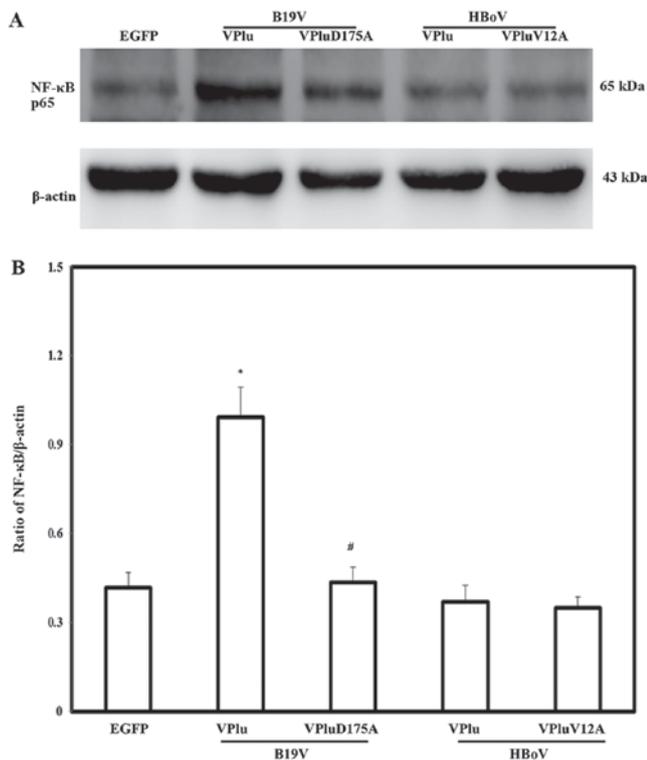


Figure 5. Expression of NF- κ B protein. (A) NF- κ B expression in H9c2 cells transfected with pEGFP, pEGFP-B19-VP1u, pEGFP-B19-VP1uD175A, pEGFP-HBoV-VP1u or pEGFP-HBoV-VP1uV12A, detected by immunoblotting. (B) The relative amount of NF- κ B is shown based on β -actin expression. Similar results were observed in triplicate experiments. * $P < 0.05$, relative to the EGFP transfection group and # $P < 0.05$, relative to the B19V-VP1u transfection group.

cytokine profiles induced by B19-VP1u and HBoV-VP1u in H9c2 cells.

The acute response to heart injury involves the production of inflammatory cytokines; however, sustained and long-term inflammation is a primary cause of further damage that can manifest as cardiac hypertrophy and chronic heart failure (30). Both experimental and clinical studies have shown that increased levels of inflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6, are important in the pathogenesis of chronic heart injuries, contributing to cardiac remodeling by influencing hypertrophy, fibrosis and apoptosis (31). Although all of these cytokines are involved in cardiac disorders, various results indicate that the healthy heart does not express TNF, whereas the failing heart generates substantial levels (32), suggesting a role of TNF- α in more severe cardiac disorder. Therefore, various chemokines, such as CXCL10 (IP-10) and CCL8 (MCP2), may also be associated with various heart disorders, including atherosclerosis, ischemia of the myocardium and cardiac fibrosis (33-35). Over recent decades, mounting evidence has indicated a pathogenic role of B19 in various cardiac disorders, including myocarditis, acute myocardial infarction, left ventricular dysfunction, inflammatory cardiomyopathy and heart failure (10,12-16). In this study, B19-VP1u induced significant expression of MCP2, IP-10, TNF- α , IL-1 β and IL-6 in H9c2 cells and HBoV-VP1u induced significant expression of IL-1 β and IL-6. Therefore, although B19-VP1u and HBoV-VP1u may differ in

their induction of proinflammatory factors in H9c2 cells, the above results imply that both B19-VP1u and HBoV-VP1u are involved in cardiac disorders. However, further investigations must be performed to verify the precise role of HBoV-VP1u in cardiac injuries.

B19 infection has been strongly associated with sPLA2 activity, mediated by its VP1u region. Meanwhile, a previous study reported that VP1u in HBoV exhibited sPLA2-like enzymatic activity (25). The sPLA2-like motif of the VP1-unique (VP1u) region of B19 and HBoV has been demonstrated to be critical to B19 neutralization and infectivity (2,17-19,27,36). However, the role of sPLA2-like activity in the potential pathogenic function of HBoV in cardiac injury remains unclear. In this study, different profiles of inflammatory indicators were induced in H9c2 cells by B19-VP1u and HBoV-VP1u, respectively. Notably, no induction of the inflammatory indicators was observed in H9c2 cells that were transfected with the mutant forms of B19-VP1u and HBoV-VP1u. These findings demonstrate, to the best of our knowledge for the first time, the critical role of the sPLA2 activity of B19-VP1u and HBoV-VP1u in inducing cardiac inflammatory cytokines.

Interestingly, the chemokine MCP-2 and IP-10 were not significantly induced in H9c2 cells that were transfected with pEGFP-HBoV-VP1u, indicating the possibility that the VP1u region of HBoV has a different specificity and only mildly affects the induction of chemokines in cardiac cells. However, further work is required to clarify the precise mechanism of HBoV-VP1u in cardiac cells. Overall, B19-VP1u probably has a more prominent role than HBoV-VP1u in the inflammatory responses that are associated with cardiac injury.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HPH was involved in the study conception and design, drafting and revising of the manuscript, and analysis of data. CCC was involved in drafting of the manuscript, study conception and design, and performing experiments. DWL and YFS performed experiments. TCH and BST was involved in the study conception and design, drafting and revising of the manuscript, analysis of data and study supervision.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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