Tech Science Press

Association of TRIM22 with the type 1 interferon response during primary human cytomegalovirus infection in THP-1 macrophages

Wei LI; Huihui GAO; Ran TAO; Lifang LIU; Shiqiang SHANG*

Department of Clinical Laboratory, the Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center For Child Health, Hangzhou, 310052, China

Abstract: As a response factor of interferon, tripartite motif (TRIM) 22 was reported to exert antiviral activity against viruses. In this study, THP-1 macrophages were infected with human cytomegalovirus (HCMV) to establish the HCMV lytic infection model. The mRNA levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interferon-beta (IFN-β) were significantly up-regulated in THP-1 macrophages at different infection time and titers. Moreover, for the first time, upregulation of TRIM22 expression was found during HCMV infection at both mRNA and protein levels in THP-1 macrophages. Furthermore, IFN-β could induce TRIM22 expression in THP-1 macrophages or HCMV infected THP-1 macrophages. Depletion of TRIM22 increased replication activity of HCMV with increasing of HCMV titers and HCMV proteins. In conclusion, it is the first report that HCMV can induce TRIM22 activation through IFN-β signaling and TRIM22 can suppress replication of HCMV in THP-1 macrophages.

Introuction

Human cytomegalovirus (HCMV) is a beta-herpesvirus with the genome of double-stranded DNA, which is widespread in human populations (Cheng *et al.*, 2017). HCMV infection is generally asymptomatic in healthy adults; however, infections are often much more severe in immunocompromised patients or congenitally infected infants (Aiello *et al.*, 2019). HCMV has a wide cell tropism within its human host (Sinzger *et al.*, 2008). In different cells or organs, HCMV infection may activate cytokines that are directly or indirectly involved in virus replication (Clement and Humphreys, 2019).

The tripartite motif (TRIM) family contains more than 70 human proteins that are involved in many biological processes such as cell proliferation, differentiation, apoptosis, oncogenesis and immunity (Nisole *et al.*, 2005; Ozato *et al.*, 2008). TRIM members are wide and well-conserved proteins characterized by a tripartite structure comprising a RING domain (Lian *et al.*, 2017). Many TRIM family members are inducible by interferon (IFN) and have emerged as antiviral molecules involved in both innate and adaptive immunity (Hattlmann *et al.*, 2012). TRIM5α is a potent restriction factor of retroviruses, including N-tropic murine leukemia virus (NMuLV), and human immunodeficiency virus-1 (HIV-1) (Stremlau *et al.*, 2004; Yap *et al.*, 2004). TRIM19 has activity against vesicular stomatitis virus, HIV-1, and influenza A virus (Nisole *et al.*, 2005). TRIM79α can against tick-borne encephalitis virus replication through the flavivirus NS5 protein degradation (Taylor *et al.*, 2011).

The human TRIM22 gene lies on chromosome 11, which is adjacent to TRIM5, TRIM6, and TRIM34 genes (Vicenzi and Poli, 2018). Like other TRIM proteins, TRIM22 has a RING domain that has homology with E3 ligases. E3 ligase activity is important for TRIM22-mediated antiviral activity through the degradation of key factors of the virus cycle (Barr *et al.*, 2008; Di *et al.*, 2013; Uchil *et al.*, 2008). TRIM22 has been reported to be involved in against of encephalomyocarditis virus (ECMV), HBV, HIV-1, and influenza A virus (Barr *et al.*, 2008; Di *et al.*, 2013; Hattlmann *et al.*, 2012). In this study, we would investigate whether HCMV could regulate TRIM22 expression during HCMV primary infection.

Materials and Methods

Cell culture

THP-1 monocytic cells were used in this study and cells were maintained in RPMI Medium 1640 (Gibco, USA) supplemented with 10% fetal calf serum (Gibco, USA), penicillin G (100 U/mL), streptomycin sulfate (100 μg/mL), l-glutamine (1%), and nonessential amino acids (1%). Cells were cultured at 37°C under 5% CO_2. THP-1 monocytic cells were treated with 100 ng/mL phorbol myristate acetate (PMA) (Solarbio, Beijing, China) for 24 h to make sure that THP-1 monocytic cells had differentiated into THP-1 macrophages.

Real-time PCR

THP-1 macrophages were collected by using 100 μL lysis buffer (Da'an Gene Co., Ltd, China) and repeated freezing and thawing for 3 times. The mixture was boiled with 100°C for 10 min. Then the mixture was centrifuged at 13000 g for 5 min.

*Address correspondence to: Shiqiang Shang,
shangsq@zju.edu.cn

Further, a volume of 5 μL of the supernatant and 45 μL of PCR mix (Da'an Gene Co., Ltd., China) was utilized to perform TaqMan real-time PCR using the Applied Biosystems 7500 real-time PCR system (Applied Biosystems, USA) according to the following protocol: 93℃ for 2 min, 10 cycles of 93℃ for 45 s, and 55℃ for 60 s, followed by 30 cycles of 93℃ for 30 s and 55℃ for 45 s. For each assay, a negative quality control, a critical quality control, a positive quality control, and four positive quantity controls (10^4 copies/mL, 10^5 copies/mL, 10^6 copies/mL, and 10^7 copies/mL) were used.

qRT-PCR

Total RNA was extracted from THP-1 macrophages by using the UNIQ-10 RNA extraction kit (Sangon Biotech, China) according to the manufacturer's instructions. One microgram of total RNA from each sample was reverse transcribed by using the PrimeScript™ RT reagent Kit with gDNA Eraser kit (Takara, Japan). For quantify the mRNA level of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IFN-alpha (IFN-α), IFN-beta (IFN-β), IFN-gamma (IFN-γ), TRIM22 and GAPDH, cDNA was subjected to real-time PCR in a 20 μL sybgreen (Takara, Japan) reaction mixture, and cDNA was also subjected to Taqman (Vazyme, China) real-time PCR in a 20 μL reaction mixture for quantifying the mRNA level of UL122 and UL138. The primers and probes were shown in Tab. 1. The real-time PCR was performed in ABI7500 with the following conditions: 94℃ for 5 min and 40 cycles of 94℃ for 15 s, 55℃ for 30 s, and 72℃ for 35 s. All genes expression was calculated following normalization to GAPDH levels by the comparative DD threshold cycle method. The specificity of the amplification reactions was confirmed by melt curve analysis.

Short interfering RNA transfection

Short interference RNA (siRNA) targeting TRIM22 and normal control siRNA were synthesized by Hanbio (Shanghai, China). Transfection of siRNA was performed using Lipofectamine® 3000 (Thermo Fisher Scientific, USA) with a final concentration of siRNA of 100 nM according to the manufacturer's instructions. The sequence of siRNA targeting TRIM22 as follows: GCAUCACUGCAAAGAUCAADTDT. NC as control: UUCUCCGAACGUGUCACGUDTDT. After 6 h of transfection, HCMV was seeded into THP-1 macrophages.

Western blotting

THP-1 macrophages after desired treatment were scraped, spun down, washed by PBS and resuspended in RIPA buffer with standard protease and phosphatase inhibitors (Beyotime Biotechnology, China). After quantification of protein concentration by BCA method (Beyotime Biotechnology, China), cell lysates were mixed with loading buffer and subjected to SDS-PAGE with 20 μg protein per lane. The proteins were later transferred to PVDF membranes (Millipore, USA), which were incubated by primary and secondary antibodies. The primary antibodies used in this study were rabbit polyclonal anti-TRIM22 (Sigma, USA), mouse monoclonal anti-IE (Abcam, England), mouse polyclonal anti-GAPDH (Proteintech, USA), or rabbit polyclonal anti-UL138 (Huabio, China). The membranes were developed with an enhanced chemiluminescence kit (Lianke Bio, China). Each experimental condition was repeated at least three times.

Statistical analysis

Comparison between two groups was performing a two-tailed Student' t-test by GraphPad Prism 6.0 (GraphPad Software, La Jolla California USA). $p < 0.05$ was considered as significantly different.

Results

HCMV infection in THP-1 macrophages

THP-1 cells were differentiated into macrophages by PMA (Fig. 1(A)). THP-1 macrophages were infected with 2 multiplies of infection (MOI) HCMV Towne to establish the HCMV primary infection model. As shown in Fig. 1(B), the copies of the HCMV genome increased with prolonged time. The mRNA levels of GAPDH, UL122, and UL138 were determined by qRT-PCR. HCMV infection factors UL122 and UL138 mRNAs were detected after HCMV infected for 6 h, and the expression of both factors was significantly increased after HCMV infected for 24 h and 48 h comparing to 6 h ($p < 0.01$; Figs. 1(C) and 1(D)). The protein levels of IE1/2 and UL138 also increased with HCMV persistent infection (Fig. 1(E)).

TABLE 1

PCR Primer Sequences

Gene		
IL-6	FP:CTGCGCAGCTTTAAGGAGTT	RP:TAAGTTCTGTGCCCAGTGGA
TNF-alpha	FP:TTCCTCACTCACACCATCAGCC	RP:TGCCCAGATTCAGCAAAGTCC
IFN-alpha	FP:CAGAGGACCATGCTGACTGA	RP:GTGCAAAGGTGCACATGACG
IFN-beta	FP:CATTACCTGAAGGCCAAGGA	RP:AGCAATTGTCCAGTCCCAGA
IFN-gamma	FP:TCCAGTTACTGCCGGTTTGA	RP:TGGAAGCACCAGGCATGAAA
TRIM22	FP:ACCAAACATTCCGCATAAAC	RP:GTCCAGCACATTCACCTCAC
GAPDH	FP:ATCCCATCACCATCTTCCAG	RP:GAGTCCTTCCACGATACCAA
UL122	FP:CATACTGGGAATCGTGAAGG	RP:TTGGACAACGAGAAGGTGC
	Probe: FAM-5'CGGCTCACCTCGTCAATCTTGACGCGAC-3'BHQ1	
UL138	FP:TATCGTCTGTCCGACTCCCG	RP:TGGCACGACACCTTCAAACTGG
	Probe: FAM-5'CGTACAGCTCGCAACAGCGGATCAGCC3'-BHQ1	

FP = Forward Primer; RP = Reverse Primer

A.

PMA   −   +
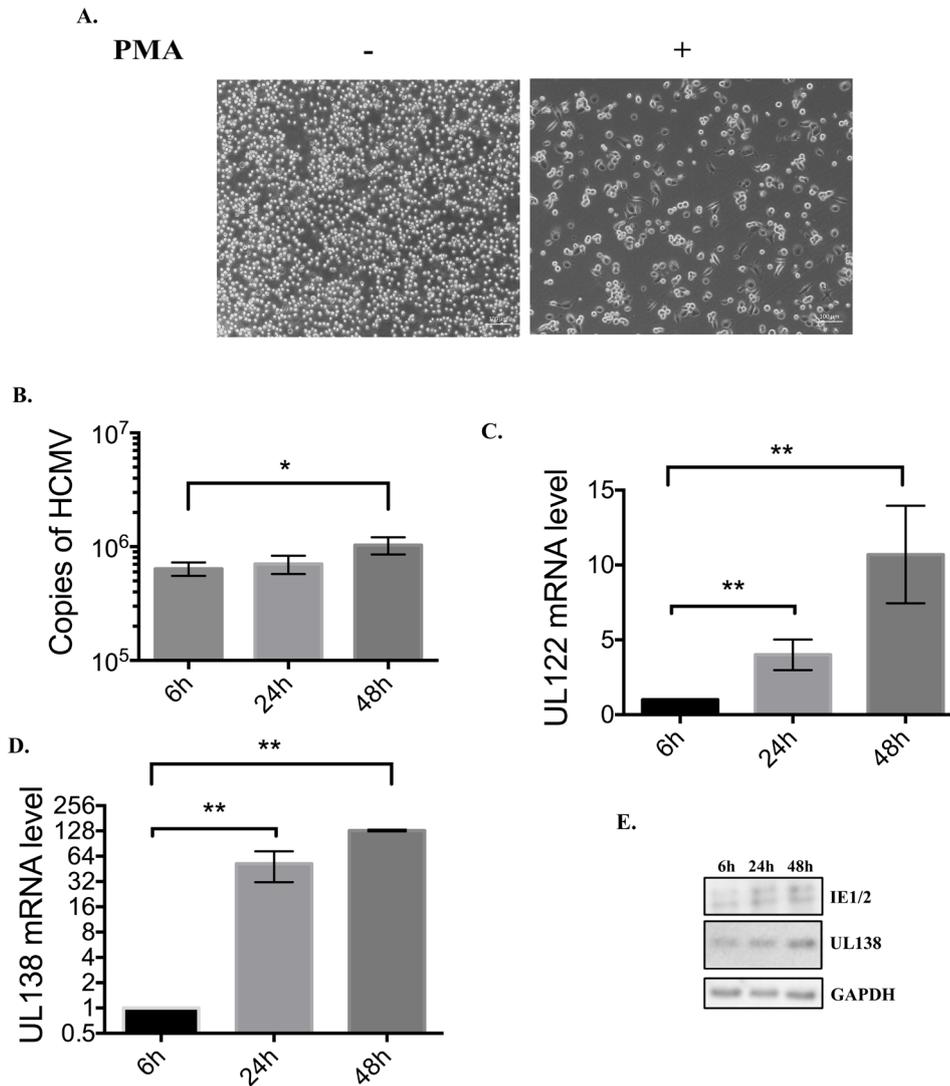
FIGURE 1. Lytic infection of HCMV in THP-1 macrophages. (A) THP-1 cells were differentiated into macrophages by PMA. Then THP-1 macrophages were infected with 2 MOI HCMV Towne strain within different time. (B) The copies of HCMV genome in different time of HCMV infection. (C) The expression level UL122 mRNA. (D) The expression level UL138 mRNA. (E) The expression level IE1/2 and UL138 protein. All data are mean ± SD of experiments performed in triplicate (* $p < 0.05$; ** $p < 0.01$ by Student's t-test).

HCMV induced cytokines change in THP-1 macrophages

To detect cytokines levels after HCMV primary infection in THP-1 macrophages, THP-1 macrophages were infected with HCMV at 2 MOI for 6, 24, and 48 h, respectively. Total RNA was extracted for the qRT-PCR test. Higher levels of IL-6 and TNF-α mRNA were observed in HCMV infected cells at different infected time points compared with that in normal cells ($p < 0.01$). IL-6 and TNF-α mRNA levels were at a peak in point of HCMV infected for 6 h (Figs. 2(A) and 2(B)). As shown in Fig. 2(C), the level of IFN-β was also significantly higher in the HCMV infected group with a peak in 24 h after infection, as compared with that in normal cells ($p < 0.001$). The expression of IFN-α and IFN-γ had no change after infection of HCMV (Data not showed). We then addressed whether different titers of HCMV (0.1MOI, 0.5MOI, 2.5MOI, 12.5MOI) could affect IL-6, TNF-α, and IFN-β expression. With increasing of HCMV titers, the level of UL122, UL138, IL-6, TNF-α, and IFN-β mRNAs were significantly upregulated in a dose-dependent manner in THP-1 macrophages (Figs. 3(A), 3(B), 3(C), 3(D), and 3(E); $p < 0.05$).

HCMV induced TRIM22 change in THP-1 macrophages

After HCMV infection, the mRNA expression of TRIM22 was up-regulated at 6 h with an expression peak at 24 h (Fig. 2(D)). With increased MOIs of HCMV, TRIM22 mRNA expression was upregulated in a dose-dependent manner in THP-1 macrophages (Fig. 3(F)). As shown in Figs. 4(A) and 4(B), the results of the western blot were consistent with qRT-PCR results in different HCMV infected time points and MOIs.

To investigate whether HCMV would induce TRIM22 expression through the IFN-β signaling pathway, THP-1 macrophages were treated with 1000 U/mL IFN-β, HCMV, or IFN-β plus HCMV. After the treatment of 24 h, total proteins were collected and detected by western blot. As shown in Fig. 4(C), IFN-β significantly raised TRIM22 protein levels in THP-1 macrophages or HCMV infected THP-1 macrophages.
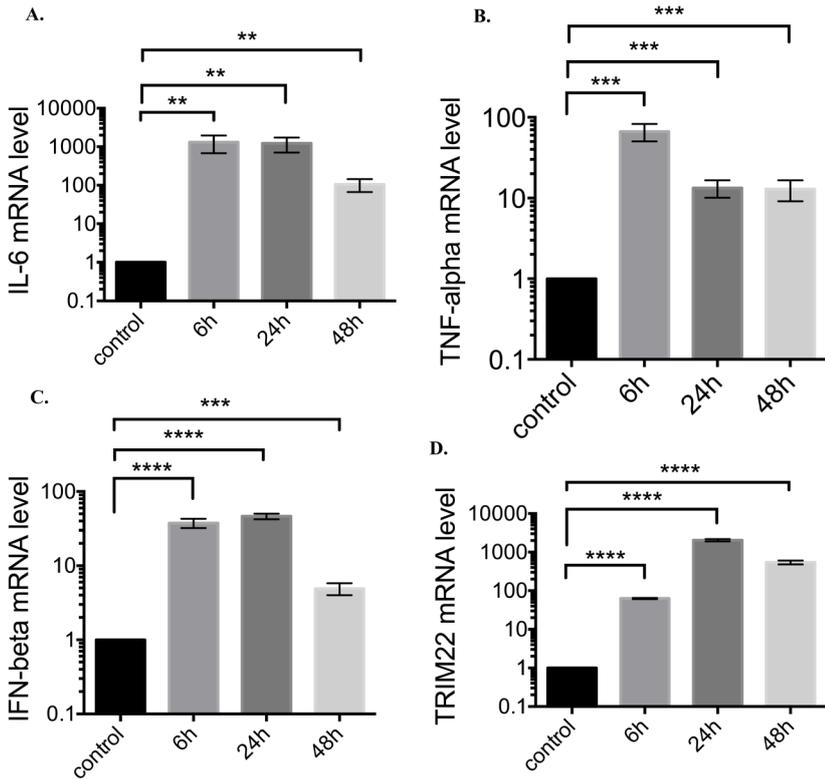
FIGURE 2. Quantitative analysis of cytokine transcriptions in THP-1 macrophages induced by HCMV in different infection time. THP-1 cells were differentiated into macrophages by PMA, and then THP-1 macrophages were infected at 2 MOI Towne within different time. (A) The expression level IL-6 mRNA. (B) The expression level TNF-α mRNA. (C) The expression level IFN-β mRNA. (D) The expression level TRIM22 mRNA. All data are mean ± SD of experiments performed in triplicate (** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ by Student's t-test).
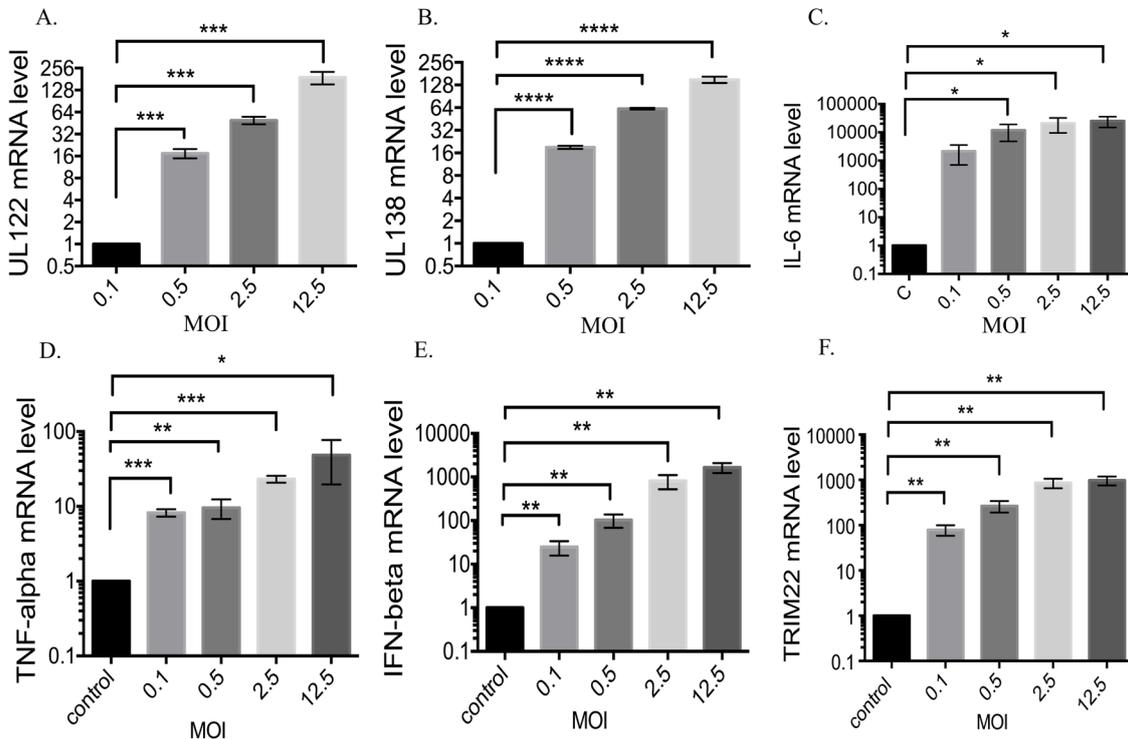
FIGURE 3. Quantitative analysis of cytokine transcriptions in THP-1 macrophages induced by HCMV in different MOI. THP-1 cells were differentiated by PMA, then THP-1 macrophages were infected at 2 MOI Towne for 24 h. (A) The expression level UL122 mRNA (B) The expression level UL138 mRNA. (C) The expression level IL-6 mRNA. (D) The expression level TNF-α mRNA. (E) The expression level IFN-β mRNA. (F) The expression level TRIM22 mRNA. All data are mean ± SD of experiments performed in triplicate (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ by Student's t-test).
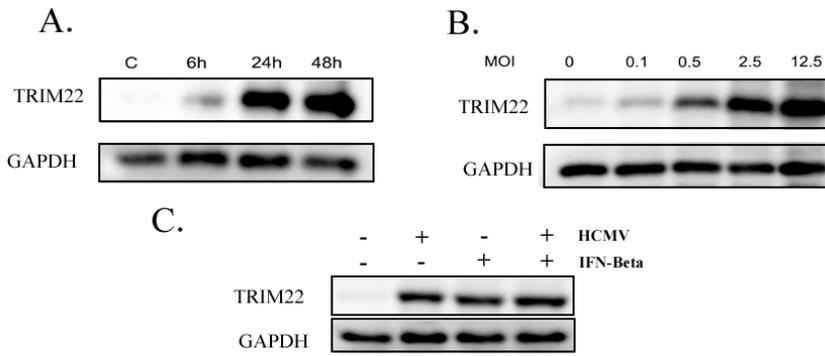
FIGURE 4. Levels of TRIM22 protein in THP-1 macrophages induced by HCMV. (A) The expression level TRIM22 protein in THP-1 macrophages induced by 2MOI HCMV in different infection time. (B) After treated with Towne 24 h, the expression level TRIM22 protein in THP-1 macrophages induced by HCMV in different MOI. (C) After treated with Towne or IFN-β 24 h, the expression level TRIM22 protein in THP-1 macrophages induced by HCMV.
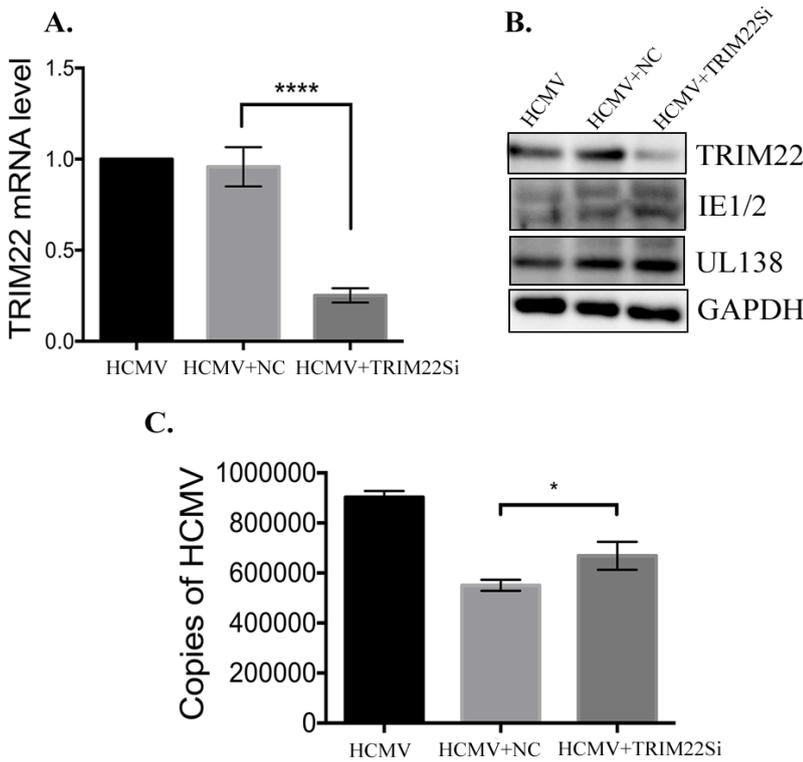
FIGURE 5. Influence of HCMV replication by silencing of TRIM22. THP-1 cells were differentiated by PMA, and then THP-1 macrophages were transfected with TRIM22si and NC. After transfection of 6 h, then THP-1 macrophages were infected with 2 MOI Towne 24 h. (A) The expression level TRIM22 mRNA. (B) The level of TRIM22, IE1/2 and UL138 protein. (C) The copies of HCMV genome. Quantitative data are mean ± SD of experiments performed in triplicate (* $p < 0.05$; **** $p < 0.0001$ by Student's t-test).

TRIM22 restricted HCMV replication

To confirm the functional role of TRIM22 in HCMV infection, THP-1 macrophages were treated with siRNA targeting TRIM22. Firstly, we investigate the inhibitory efficiency of TRIM22 siRNA. As shown in Fig. 5(A), TRIM22 siRNA significantly inhibited the expression of TRIM22 mRNA by about 80% degree ($p < 0.0001$). Similar results were obtained from detecting the protein expression of TRIM22 by western blot (Fig. 5(B)). As shown in Fig. 5(C), silencing of TRIM22 affected HCMV replication. The knockdown of TRIM22 by siRNA significantly raised IE1/2 and UL138 protein levels. Consistent with HCMV proteins result, TRIM22 silence led to an increase of HCMV copies at 24 h post-infection.

Discussion

HCMV can establish lytic or latent infection in different cell lines (Schwartz and Stern-Ginossar, 2019). THP-1 macrophages are usually used as an HCMV experimental lytic infection model (Sanchez *et al.*, 2012). After infection of HCMV Towne strain in THP-1 macrophages, we found that HCMV copies and replication-related factors were increased in a time-dependent manner within 48 h infection. This is the evidence that Towne can establish an HCMV lytic infection in THP-1 macrophages. Previous reports suggested that HCMV induced IFN-β and other cytokines expression, which increased in THP-1 macrophages through the TLR4 signaling pathway (Yew *et al.*, 2012). In THP-1 macrophages incubated with HCMV, levels of IL-6 and TNF-α mRNAs were peaked early at 6 h and remained elevated throughout the 48 h in incubation. We also detected the level of IFN-β mRNA increasing and the expression peak of IFN-β was at 24 h after HCMV infection. Furthermore, the up-regulation of IL-6, TNF-alpha, and IFN-β were in an HCMV dose-dependent manner in THP-1 macrophages. The expression of IFN-α and IFN-γ were not changed in our HCMV lytic infection model.

Many proteins of the TRIM family are regulated in response to IFN stimulation and are involved in antiviral and immune activity. TRIM22 is highly up-regulated in

response to Type I and II interferons, including interferon alpha, beta and gamma (Gao *et al.*, 2013; Singh *et al.*, 2011). After increasing of IFN-β level in HCMV infected cells, TRIM22 was up-regulated with an expression peak at 24 h after HCMV infection. The rising trend of TRIM22 was consistent with that of IFN-β. This upregulation was also detected in the HCMV dose-dependent manner. To validate the role of IFN-β in TRIM22 induction, we used IFN-β treated with THP-1 macrophages. TRIM22 protein was successfully induced by IFN-β in THP-1 macrophages with or without infection of HCMV. This evidence suggested that HCMV can activate TRIM22 expression via IFN-β signaling in macrophages.

TRIM22 inhibits the replication of several kinds of retrovirus (Barr *et al.*, 2008; Di *et al.*, 2013; Singh *et al.*, 2011). Little research has been done on the relationship between TRIM22 and DNA viruses. In this study, we found that TRIM22 was activated by HCMV infection in THP-1 macrophages. It was found that the HCMV copies and protein levels of IE1/2 and UL138 were up-regulated in THP-1 macrophages after knockdown TRIM22. These results preliminarily provided evidence that TRIM22 was a significant component of the IFN-β response against HCMV infection. Previous studies suggested that TRIM22, as a Ring-finger E3 ubiquitin ligase protein, could restrict virus replication by inhibiting virus protein expression (Lian *et al.*, 2017; Turrini *et al.*, 2015). We would identify which proteins could interact with TRIM22 during affecting HCMV replication in further studies.

Thus, we have provided evidence that HCMV can induce TRIM22 expression via IFN-β mediating in macrophages. More importantly, TRIM22 can restrict the replication of HCMV in THP-1 macrophages. These results revealed the possible mechanism of IFN anti-virus and provide a potential treating target through TRIM22.

Acknowledgement

Financial Disclosure

No competing financial interests exist.

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