Biochemical and Biophysical Research Communications 448 (2014) 448–453

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Interleukin-10 blocks in vitro replication of human cytomegalovirus by inhibiting the virus-induced autophagy in MRC5 cells

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ARTICLE INFO

Article history:
Received 21 April 2014
Available online 2 May 2014

Keywords:
IL-10
Cytomegalovirus infection
Autophagy
PI3K pathway

ABSTRACT

Background: Interleukin-10 is an important cytokine that regulates immune response. Previous studies have shown that human cytomegalovirus can trigger cell autophagy during the early stages of infection. To our knowledge, whether IL-10 inhibits HCMV-induced autophagy and virus replication has not been studied previously.
Objectives: We investigated whether IL-10 affects cell viability and autophagy under the conditions of starvation and HCMV infection by using the MRC5 cell line. We also explored the role of IL-10-mediated autophagy on HCMV replication.
Results: Our data showed that IL-10 inhibited the autophagic flux of the MRC5 cells irrespective of starvation or HCMV infection, and suppressed HCMV replication. The promotion of autophagy with either a pharmacological inducer (rapamycin), or a technique to over-express the BECN1 gene reversed the effect of IL-10 on virus replication. Furthermore, the PI3K/Akt signal pathway was activated when the cells were pretreated with IL-10.
Conclusions: Our results indicated that IL-10 can suppress HCMV replication by inhibiting autophagy in host cells during the early stages of infection.

1. Introduction

Autophagy plays a crucial role in many cellular activities including development, differentiation, survival, and homoeostasis. In addition to being a response to internal and external stress, autophagy also shapes cellular immunity. It serves as an essential component of host defense against viral infection, by orchestrating pathogen degradation (xenophagy), innate immune signaling and certain aspects of adaptive immunity [1].

Viruses have evolved a variety of strategies to evade autophagic attack and manipulate the autophagic machinery for their own benefit. Human cytomegalovirus (HCMV) modulates autophagy in two opposing ways. In the early stages of infection, virions of HCMV promotes the conversion of endogenous microtubule-associated protein 1 light chain 3 (LC3-I to LC3-II) independent of the de novo viral protein, suggesting the dependence on the components of the nucleocapsid or the viral genome [2]. In the later stages of infection, HCMV blocks autophagy through promoting de novo viral protein synthesis [3].

IL-10 expression was upregulated during acute MCMV infection, especially in the lung and spleen. When IL-10 receptor (IL-10R) was blocked, the viral genome load increased in lung and spleen at 4 days post-infection [4]. Additionally, in IL-10$^{-/-}$ mice, acute MCMV infection caused amplification of proinflammatory cytokine production and increased the numbers of mononuclear cells and lymphocytes in the liver as compared with the wild mice. The above data suggested that IL-10 plays protective roles in the early stages of CMV infection [5].

A previous study found that IL-10 inhibited starvation-induced autophagy in murine macrophages through activating the PI3K/Akt signaling pathway [6]. However, it is still unclear whether and how IL-10 modulates autophagy in human fetal lung fibroblast (MRC5) cells during HCMV infection. In this study, we hypothesized that IL-10 suppresses viral replication in the early stages of infection through inhibition of HCMV-induced autophagy in MRC5 cells. We examined the autophagy in MRC5 cells insulted by serum deprivation and HCMV infection. Viral replication was evaluated when cells were treated with IL-10 and the signaling pathways necessary for IL-10 were investigated.

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[1] The first two authors contributed to this work equally. This work was supported by Scientific Research Grant from Shanghai Education Commision (Number 14ZZ10B).

http://dx.doi.org/10.1016/j.bbrc.2014.04.120
0006-291X/

2. Materials and methods

2.1. Cells and viruses

MRC5 cells were used between passages 20 and 25 post-isolation and allowed to proliferate in Dulbecco's modified Eagle's medium (DMEM) (Gibco®), supplemented with 10% (vol./vol.) fetal calf serum (FCS) (Gibco®), penicillin G (100 U/mL), and streptomycin sulfate (100 μg/mL). Recombinant HCMV virus and adenovirus encoding green fluorescent protein (Ad-GFP) (cite Dr. Qian's paper [7]) was provided by Dr. Zhikang Qian at the Institut Pasteur of Shanghai Chinese academy of sciences.

2.2. Reagents and antibodies

IL-10 was purchased from Pepro Tech, while the Earles' balanced salt solution (EBSS), bovine serum albumin (BSA), and Rapamycin (Rap) were obtained from Sigma–Aldrich (USA). The following antibodies were purchased form Cell Signaling Technology: anti-β-actin, anti-beclin1, anti-LC3-II/I, anti-p62, anti-Akt, anti-phosphorylation of Akt (p-Akt), and anti-PI3Kp85α. The antibodies of anti-HCMV IE1/2 and pp65 were purchased from Abcam and anti-IL10RA was purchased from BBI Solutions.

2.3. Transfection

The (cyan fluorescent protein-LC3) CFP-LC3 expression vector was a kind gift from Professor Dexi Chen (Beijing institute of hepatology, Beijing, China). Cells were seeded at a density of 9×10^5 cells/mL on glass coverslips placed in 24-well tissue culture plates (Corning Glass Works, Corning, NY). The following day, cells were transfected at 50–80% confluence with the CFP-LC3 plasmid using Fugene HD transfection reagent (Roche). After 4 h, the medium was replaced by DMEM containing 10% FCS, and the cells were left for another 24–48 h. Different treatments such as starvation by Earle's balanced salt solution (EBSS) or infection (processed as above) were carried out for different durations before fixation. The cells were then fixed in 4% paraformaldehyde for 20 min at room temperature, and were washed three times with Dulbecco's Phosphate-Buffered Saline (PBS). Data were statistically compared by using a global Student's *t*-test ($P < 0.01$) as indicated in the figure legends.

2.4. Overexpression of Beclin-1 (BECN1)

The lentiviral vector containing the BECN1-expressing gene (LV-BECN1) was synthesized by Genechem (Shanghai, China) and lentiviral infection was carried out according to the manufacturer's instructions. Briefly, MRC5 cells were incubated overnight in growth media with the LV-BECN1 at MOI of 100 and in the presence of 2 mg/mL polybrene (Gibco, USA). Following transfection for 48 h, the cells were grown in a culture medium with 2 mg/mL puromycin (Sigma, USA) to select stably transfected cells for subsequent experiments.

2.5. Western blot analysis

Cells were collected, washed, and lysed in the sodium dodecyl sulfate (SDS)-containing sample buffer. Proteins was transferred to nitrocellulose membranes, hybridized with primary antibodies, reacted with horseradish peroxidase-conjugated secondary antibodies and visualized by using Image Lab, Bio-Rad.

2.6. Quantitative RT-PCR analysis (qRT-PCR)

The mRNA was extracted from the cells using Trizol reagent (Invitrogen, USA). The expression of IL-10 receptor (IL-10R) genes was determined by real-time PCR using SYBR Premix Ex Taq (Takara, Japan) and an ABI Prism 7500 sequence detection system (Applied Biosystems, USA). The reaction mixture was amplified at 50 °C for two minutes, 95 °C for 30 s, and then 40 cycles of 95 °C for 5 s followed by 60 °C for 34 s. The qRT-PCR reaction was carried out using the following primers: IL-10R (Forward, 5′-ACACCCA TCCCAAATCAGTC-3′; Reverse, 5′-GGTCACTGCGGTAAGGTCAT-3′) and β-actin (Forward, 5′-AGCGCGGCTACAGCTTAC-3′; Reverse, 5′-G GCGACGTAGCACAGCTTCT-3′). The fold-change in gene expression relative to the control was calculated by $2^{-\Delta\Delta CT}$.

2.7. Analysis of viral growth kinetics

Normal MRC5 cells were seeded in 12-well dishes overnight to produce a subconfluent monolayer. Cells were then inoculated with HCMV viruses for 2 h at 37 °C at a multiplicity of infection (MOI) of 3 for single-step growth analysis. The inoculum was removed, the infected monolayers were rinsed with DPBS, and fresh medium was added. At various time points post-infection, cell-free virus was collected from cells by harvesting medium from infected cultures and was titred by 50% tissue culture infectious dose ($TCID_{50}$) assay in MRC5.

2.8. Immunofluorescence (IF)

The cells were seeded in 24-well plates at a density of 5000 per well with sterile glass cover slips. After treated according to protocol, the cells were then fixed with 4% paraformaldehyde in PBS for 15 min, blocked with 5% BSA for 15 min at room temperature, and then incubated with the appropriate primary antibodies at required dilution. Subsequently, the treated cells were washed in PBS three times and incubated with secondary antibodies. The slides were finally stained with DAPI (4′,6-diamidino-2-phenylindole) (Invitrogen) and then examined by fluorescence microscopy (Olympus BX-FLA, Japan) and confocal microscopy (Olympus FluoView™ FV1000).

2.9. Autophagolysosome detection by transmission electronic microscopy

At the indicated times post treatment with HCMV, the cells were fixed in 0.2% glutaraldehyde (pH = 7.4) for 2 h at room temperature, post-fixed in osmium tetroxide (1% in water milliQ) for 1 h, and then stained in uranyl acetate (2% in water) for 1 h in the dark. After dehydration in a series of increasing concentration of ethanol, the samples were embedded in Durcupan ACM for 6 h, and cut into 80 nm sections. These sections were stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope (TEM, Philips CM, Netherlands).

2.10. WST-1 assay for cell viability

The MRC5 cells were plated in 96-well culture plates (5×10^3 cells/well). At 1d, 3d, 6d post treatment with IL-10 at 1 ng/mL, 10 ng/m, 100 ng/mL, viable cell numbers were measured by a cell proliferation assay using Cell Proliferation Reagent WST-1 (Roche). The absorbance or optical density at each time point was detected by a microplate spectrophotometer at 450 nm.

2.11. Statistical analysis

Data are expressed as means ± standard errors of the means (SEM) of at least three experiments. The statistical significance was assessed by a Student's *t* test.

3. Results

3.1. IL-10 receptor expression in MRC5 cells

To determine whether the IL-10 receptor was expressed on the surface of the MRC5 cell, RT-PCR were performed to detect the IL-10 receptor mRNA expression, while the IF analysis were used to detect IL-10 receptor protein in MRC5 cell with IL-10 or HCMV stimulation. Interestingly, slightly increase in expression of IL-10 receptor was observed in the MRC5 cell treated with 100 ng/mL of IL-10 (Fig. 1A). When treated with EBSS or HCMV, the expression of IL-10 receptor decreased, but the subsequent treatment with IL-10 (100 ng/mL) could increase the expression of IL-10 receptor to approximately normal levels. Additionally, IF analysis was also performed to identify the expression of the IL-10 receptor on the MRC5 cell. Consistent with the RT-PCR analysis, higher level of expression of the IL-10 receptor was observed in MRC5 cell treated with IL-10 (Fig. 1B).

3.2. The effect of IL-10 on autophagy induced by HCMV infection

Because most of the initial studies demonstrated that autophagy was triggered under certain stimuli, such as starvation, infection, and growth factor withdrawal [8], our intention was to examine the effects of these stimuli on autophagy in MRC5 cells. We first investigated the expression of the autophagic markers, LC3-I, LC3-II, and SQSTM1/p62 (hereafter referred as p62) and detected the conversion of LC3I to LC3II (referred as LC3II/I conversion) in the MRC5 cells at the indicated time points after starvation (2, 6, 12, and 24 h) or HCMV infection (2, 6, 12, and 24 h post infection, hpi) (Fig. 1C). Western blot analysis demonstrated that the expression of LC3II/I conversion in the MRC5 cells increased as starvation time increased. However, the expression of protein marker p62 gradually decreased. The LC3II/I conversion proteins in the MRC5 cells increased after infection with HCMV at MOI 3, reaching maximal expression levels at 12 hpi, and maintaining the same levels at 24 hpi. However, starting from 2 to 16 hpi, the expression of p62 showed a gradual decrease. Further evidence for the autophagy induced by HCMV, was detected in MRC5 cells infected with HCMV at 6 hpi under TEM (Fig. 1D). The images showed that the presence of autophagosomes with double membranes, and an increase in number of autolysosomes containing cargo.

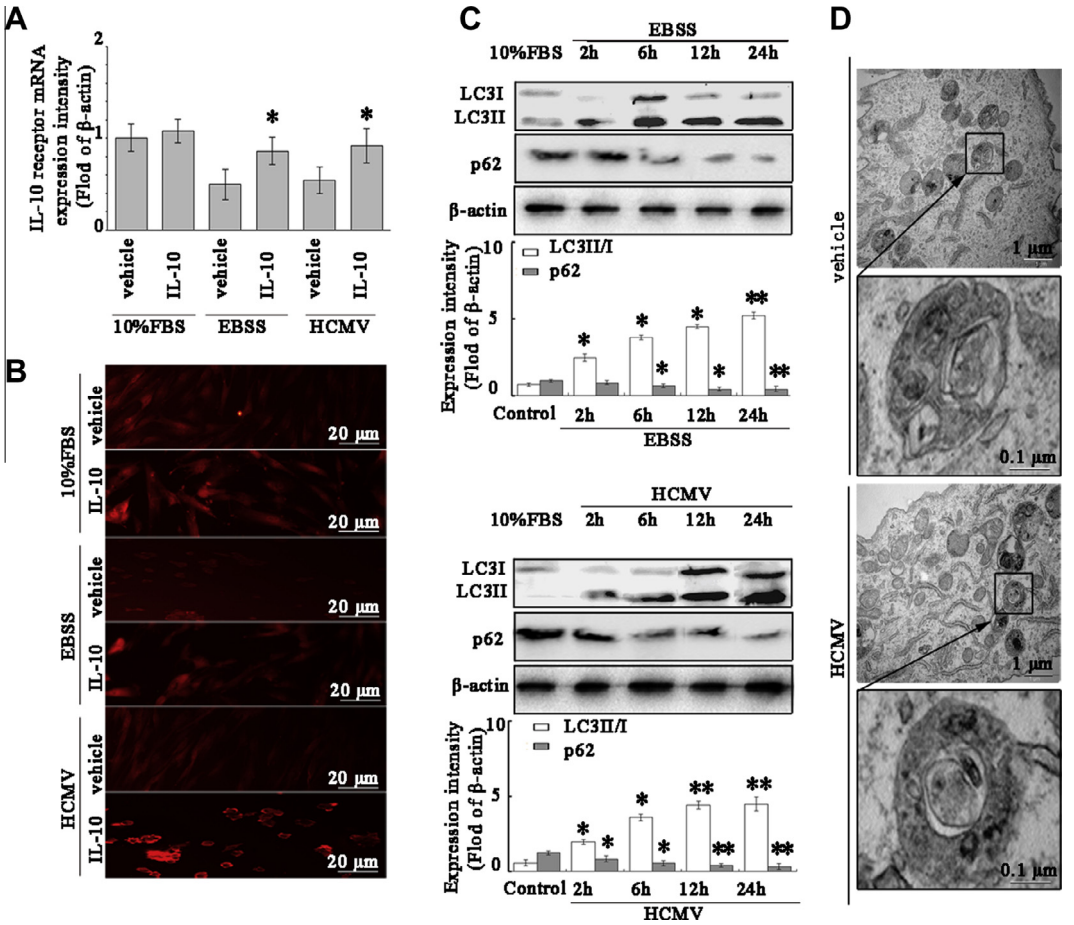

Fig. 1. IL-10 receptor expression in the MRC5 cells and autophagy induced by starvation and HCMV infection. (A) IL-10R mRNA expression determined by qRT-PCR. MRC5 cells were pretreated with IL-10 (100 ng/mL) or vehicle (0.5% DMSO) for 10 h, and then exposed to EBSS or HCMV for 2 h. Thereafter, the IL-10R mRNA expression was determined. The relative expressions were normalized against β-actin, and compared with the vehicle group. $^{*}P < 0.05$. (B) IL-10R expression in MRC5 cells determined by IF. MRC5 cells were pretreated with IL-10 for 10 h, then exposed to EBSS or HCMV; MRC5 cells treated with 10% FBS for 2 h were served as control. (C) Autophagy related proteins determined by Western blot. MRC5 cells were treated with EBSS for 2 h, 6 h, 12 h and 24 h or inoculated with HCMV for 2 hpi, 6 hpi, 12 hpi and 24 hpi. Cells treated with 10% FBS were served as control. The protein expression levels were normalized against β-actin and LC3II/I conversion is the ratio of LC3II to LC3I. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control. (D) TEM images of cells inoculated with HCMV at a MOI of 3, for 6 hpi and control. Much more autophagolysosomes were observed in cells infected with HCMV. Black arrows pointed to autophagolysosomes.

Autophagic flux is enhanced in MRC5 cells in the early stages of infection with HCMV, but nothing is currently known about the exact role of IL-10 in regulating HCMV-induced autophagy. A part of our study was to ascertain whether IL-10 can modulate HCMV-induced autophagy. Firstly, we found that IL-10 could significantly reduce the autophagy level induced by 24 h starvation in MRC5 cells. This was substantiated by a decrease in LC3II/I conversion and an increase of p62 at 24 h post starvation. The inhibitory effect of IL-10 on starvation-induced autophagy increased in a dose dependent manner, reaching maximal effect at the concentration of 100 ng/mL (Fig. 2A). In addition, CFP-LC3 plasmids were transfected into MRC5 cells to assess autophagic flux level by counting LC3 puncta under fluorescence microscope. IL-10 significantly decreased the puncta of CFP-LC3 under starvation (Fig. 2B), although our preliminary data showed that IL-10 did not alter the autophagy under serum condition (data was not shown). These data reveal that IL-10 inhibits autophagy induced by starvation in MRC5 cells.

Secondly, to confirm the role of IL-10 in autophagy triggered by HCMV, the expression of autophagic markers was analyzed by western blots. Following treatment with IL-10, the expression of LC3-I and LC3-II decreased while p62 level increased (Fig. 2C). As illustrated in Fig. 2D, the LC3 puncta accumulated in the cytoplasm after HCMV infection while the number of LC3 puncta was reduced after IL-10 treatment. These data suggest that IL-10 inhibits autophagy triggered by HCMV in the early stages of infection.

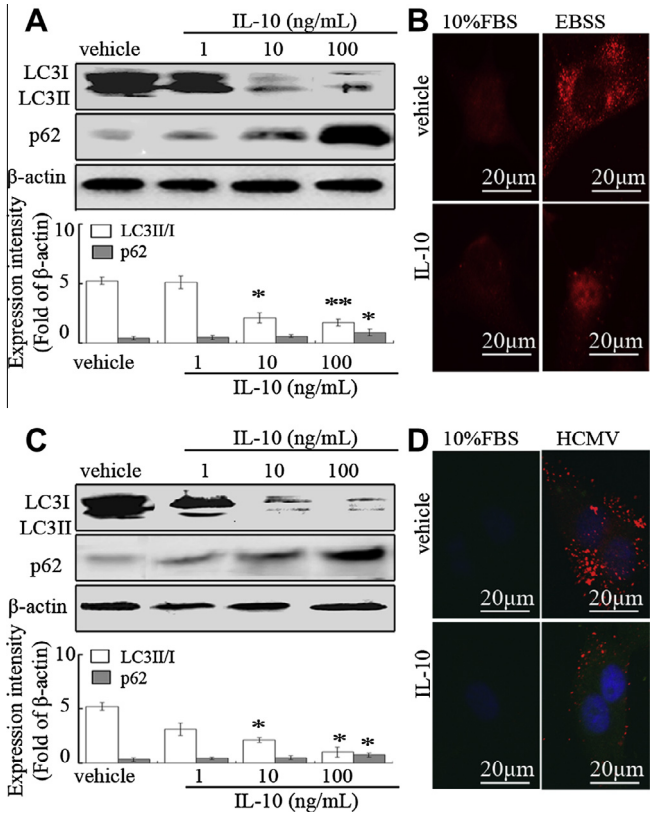

Fig. 2. IL-10 inhibits HCMV induced autophagy. (A) Autophagy related proteins during starvation determined by western blot. MRC5 cells were treated with IL-10 at 1 ng/mL, 10 ng/mL, 100 ng/mL respectively for 10 h. Autophagy was induced by EBSS medium for 4 h. LC3II/I conversion and p62 were counted, $^{**}P < 0.01$, $^{*}P < 0.05$. (B) Autophagosome formation after treated with EBSS for 4 h. MRC5 cells were transfected with CFP-LC3 for 48 h, and then pretreated with IL-10 or vehicle (0.5% DMSO) for 10 h followed by EBSS for 4 h. (C) Western blot analysis for autophagy triggered by HCMV infection. MRC5 cells were treated with IL-10 at 1 ng/mL, 10 ng/mL, 100 ng/mL for 10 h. Autophagy was induced by HCMV infection for 4 h. LC3II/I conversion and p62 were analyzed, $^{**}P < 0.01$, $^{*}P < 0.05$. (D) Autophgosomes formation observed using confocal microscope. MRC5 cells were transfected with CFP-LC3 for 48 h, and then pretreated with IL-10 (100 ng/mL) for 10 h followed by HCMV infection at a MOI of 3 for 4 h. Cells were fixed and immunostained for pp65 viral protein (green). LC3II/I conversion were compared to cells treated with 10% FBS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. IL-10 suppresses HCMV replication by downregulating autophagy

First, we investigated whether cell viability was affected by IL-10 and our data showed that stimulation by varying concentration of IL-10 did not alter the cell viability (Fig. 3A). Next, the role of IL-10 on HCMV replication was investigated. As shown in Fig. 3B, IL-10 repressed HCMV replication in MRC5 cells as demonstrated by viral titer analysis, while the viral protein IE1 also decreased consistently (Fig. 3C). To explore whether IL-10 inhibits virus replication by impairing autophagy, rapamycin (Rap) was used. Rap partly reversed the inhibitory effect of IL-10 on HCMV-induced autophagy (Fig. 3C). Rap also reversed the inhibitory effects of IL-10 on HCMV replication, evidenced by viral titer analysis and IE1 expression.

To further confirm our hypothesis, LV-BECN1 or vector was transfected into MRC5 cells (Fig. 3D). As expected, BECN1 and LC3II/I proteins were significantly increased in LV-BECN1 cells (BECN1) as compared with blank vector (vector) (Fig. 3D). Then, BECN1-overexpressed MRC5 cells and vector cells were treated with IL-10 or vehicle. LC3II/I expression was partly inhibited by IL-10 while BECN1 expression was not altered by IL-10 in vector cells. However, both BECN1 and LC3II/I protein expressions were not affected by IL-10 treatment in BECN1-overexpressed MRC5 cells (Fig. 3D). These data suggested that IL-10 cannot inhibit the autophagy in BECN1-overexpressed cells. More important, there was no significant difference in IE1 protein expression (Fig. 3D) and HCMV replication (Fig. 3E) between the IL-10 and vehicle groups in BECN1-overexpressed cells, suggesting that IL-10 suppressed HCMV replication possibly through inhibition of autophagy in MRC-5 cells.

3.4. IL-10 represses autophagy in MRC5 cells in the early stages of HCMV infection through activation of the PI3K/Akt pathway

The PI3K/Akt pathway is not only involved in regulating autophagy, but also participates in cellular response to microbes [6,9]. Therefore, we observed the phosphorylation of PI3Kp85α and Akt during HCMV infection. As expected, western blot analysis showed that phosphorylation of Akt and PI3Kp85α expression was inhibited by HCMV while the effect could be partly reversed by IL-10. The results (Fig. 3F) suggested that IL-10 could repress HCMV-induced autophagy in MRC5 cells possibly through PI3K/Akt signaling.

4. Discussion

IL-10 acts through multiple immunosuppressive modes, mainly affecting the expression of proinflammatory cytokines and chemokines, modulating the function of antigen-presenting cells, and suppressing effector T cell and NK cell responses [10]. IL-10 receptor complex includes two parts, IL-10 receptor 1 (IL-10R1) and IL-10 receptor 2 (IL-10R2), both of which are necessary for signal transduction of IL-10. Whether IL-10R existed in the MRC5 cells and affected by virus infection or IL-10 treatment is uncertain. Our results showed that its expression was not only induced by the presentation of exogenous IL-10, but also enhanced by HCMV infection. The results are consistent with a previous report stating that non-hematopoietic cells, such as fibroblasts and epithelial cells can respond to stimuli by upregulating IL-10R1 [11].

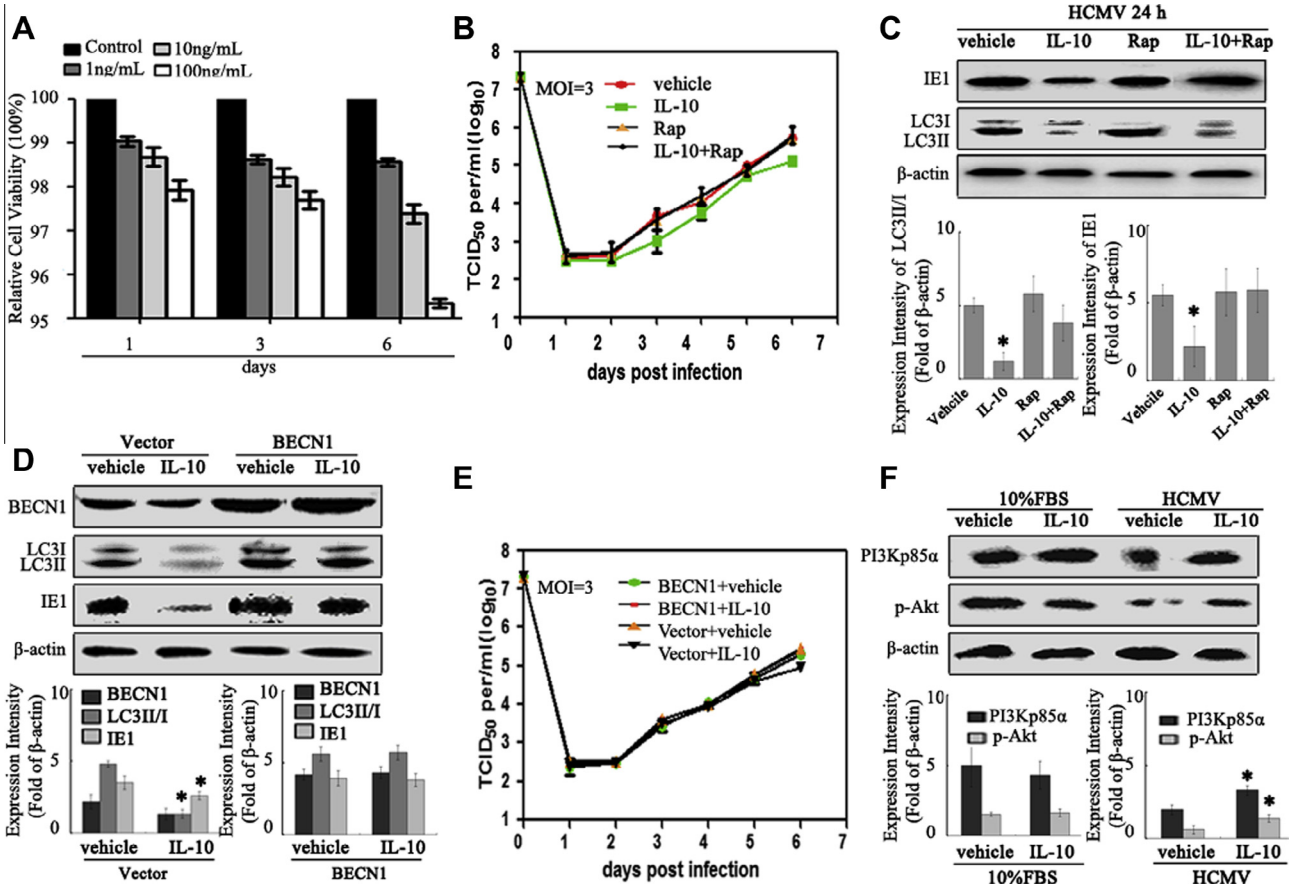

Fig. 3. IL-10 suppresses HCMV replication through down-regulation of autophagy. (A) WST-1 assay for cell viability. MRC5 cells were treated with IL-10 at 1 ng/mL, 10 ng/mL, 100 ng/mL for 1 d, 3 d and 6 d. (B) Single-step growth analysis of HCMV in MRC5 cells. MRC5 cells were infected with HCMV in different culture conditions, vehicle (1% BSA), IL-10 (100 ng/mL), Rap (100nM) and IL-10 + Rap. (C) Western blot analysis for IE1 and LC3I/II expression. MRC5 cells were pretreated with different culture conditions (as mentioned above) before infected with HCMV at a MOI of 3 for 24 h. Expressions of IE1 viral protein and LC3II/I conversion were determined by western blot. $^{*}P < 0.05$ (*t* test). (D) Western blot analysis for BECN1, IE1 and LC3I/II expression in BECN1 and vector cells. BECN1 over-expression cells (BECN1) and blank control cells (vector) were pretreated with IL-10 (100 ng/mL) or vehicle (1% BSA), and then inoculated with HCMV at a MOI of 3 for 4 h. Western blots were performed to determine the expression of BECN1, LC3II/I and IE1. $^{*}P < 0.05$ (*t* test). (E) Single-step growth analysis of HCMV in BECN1 overexpression cells. BECN1 over-expression cells (BECN1) and blank control cells (vector) were treated with IL-10 (100 ng/mL) or vehicle (1% BSA), and then infected with HCMV at a MOI of 3. (F) Western blot analysis for phosphorylation of Akt and PI3K in MRC5 cells infected with HCMV. MRC5 cells were pretreated with IL-10 (100 ng/mL) for 10 h, and then inoculated with HCMV. Phosphorylation of Akt and PI3Kp85α were determined by western blot. Cells treated with 10% FBS were served as positive control, $^{*}P < 0.05$.

Research on IL-10 function is often focused on its production or its effects on immune cells. We chose fibroblasts, one of the typical host cells infected by HCMV, as IL-10 targets. Previous studies have shown that IL-10R blockade impaired cytotoxic NK cell responses and elevated NK cell apoptosis [9]. In addition, IL-10 activates mTORC1 through the PI3K/Akt pathway in macrophages under conditions of starvation-induced autophagy [12]. IL-10 can modulate immune cell viability and responses to autophagic stimuli. In order to determine its effects on host cells, we treated cells with different concentrations of IL-10 prior to infection. Our data showed that 100 ng/mL of IL-10 most effectively inhibited HCMV-induced autophagy. Western blot analysis for autophagy-related proteins showed that the ratio of LC3II to LC3I (LC3I/II conversion) reduced about 3-fold in HCMV + IL-10 group, compared with the HCMV group. The result was consistent with autophagosomes formation studied by CFP-LC3 puncta counting, indicating that IL-10 partly blocked HCMV-induced autophagy. Once the role of IL-10 in modulating HCMV-induced autophagy was ascertained, we began investigating the effects of IL-10 on HCMV replication.

Some studies have reported that HCMV can trigger autophagy in host cells in the early stages of infection [2]. First, we repeat the tests to ascertain autophagic responses of MRC5 cells to starvation and early infection by HCMV. Consistent with previous reports, infection with HCMV at MOI 3 caused a gradual increase in LC3II/I conversion from 6 to 12 hpi, reaching the maximum at 12 hpi and maintaining the level until 24 hpi. Simultaneously, the expression of p62 decreased as autophagy flux increased. Viruses have evolved mechanisms to utilize aspects of host autophagy for their own replication, morphogenesis, cellular egress, or pathogenicity [12]. For instance, pharmacological stimulation of autophagy in HIV-infected macrophages increases extracellular viral yields [13]. whereas pharmacological or genetic inhibition of autophagy decreases extracellular viral yields. We tried to use IL-10 to interfere with the autophagy induced by HCMV for inhibiting the virus replication.

Our data indicated that IL-10 reduced HCMV replication in the early stages of infection. The data suggested that the virus titer differences between the HCMV group and HCMV + IL-10 group were more significant within 3 days than that within last 3 days. This is the first study about the effects of IL-10 on infected cells. Previous studies mostly focused on its role in modulating immune cells during acute CMV infection, especially the activity of NK cells [4]. In acute mouse cytomegalovirus (MCMV) infection, IL-10 not only restricts the activation-induced death of NK cells, but also protects the liver from inflammatory injury by NK production. The protective role of IL-10 in acute MCMV infection was verified in most studies, but the data on whether it affects virus replication was not comparable in different laboratories. The possible reason is

that the role of IL-10 on virus replication varied with the different stage of infection. Consistently, the autophagic protein expressions induced by HCMV were down-regulated by IL-10, which suggested that IL-10 suppressed viral replication in the early stages of infection through inhibition of HCMV-induced autophagy in MRC5 cells. The possible mechanism for virus replication inhibited by IL-10 is that autophagy may supply the building for viral replication. To further confirm our hypothesis, promotion of autophagy with either pharmacologic inducer (rapamycin) or Beclin-1 over-expressing technique was used. The results showed that they significantly reversed IL-10 effects on autophagy and virus replication.

The class I PI3K signaling inhibits autophagy through its downstream molecule, which can activate mTOR1 [14]. Previous study has shown that IL-10 inhibits the starvation induced autophagy through not only promotion of p70SK6 phosphorylation, which is activator of mTOR1, but also activation of Akt phosphorylation [6]. In addition to IL-10, other Th2 type cytokines including IL-4 and IL-13 also inhibits the autophagy induced by starvation through activation of class I PI3K signaling [15]. After infected with HCMV, the phosphorylation of PI3Kp85α and Akt increased with IL-10 treatment indicated that class I PI3K/Akt signaling activation. The results suggested that the PI3K signaling was vital for IL-10 inhibiting autophagy.

Conclusively, our results explain the inhibitive effects of IL-10 on host cell autophagy during the early stages of HCMV infection. It sheds light on possible treatment approaches for acute HCMV infection.

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