Endothelial cells are damaged by autophagic induction before hepatocytes in Con A-induced acute hepatitis

Ming-Chen Yang¹, Chih-Peng Chang² and Huan-Yao Lei²,*

¹Institute of Basic Medical Sciences and ²Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan, Republic of China

*Correspondence to: H.-Y. Lei; E-mail: hylei@mail.ncku.edu.tw

Received 16 December 2009, accepted 27 April 2010

Abstract

We have reported both T-cell-dependent and -independent hepatitis in immunocompetent and immunodeficiency mice, respectively, after intravenous injection of Con A in mice. The mode of hepatocyte cell death is different: autophagy for T-cell-independent hepatitis in contrast to apoptosis for T-cell-dependent one. In this study, we further demonstrate that liver blood vessels are the first target in both modes. The infused Con A bond to the hepatic vascular endothelial cells and cause its damage with autophagy. Before the elevation of the serum alanine aminotransferase at 6 h post-injection, the plasma leakage and hemorrhage occur at 1–3 h without inflammation. Con A induces autophagy of endothelial cells and hemorrhage that is enhanced by IFN-γ. Using the endothelial cell line HMEC-1, a dose- and time-dependent cell death with autophagic LC3-II (microtubule-associated protein light chain 3) conversion was induced by Con A and was enhanced by IFN-γ. In conclusion, Con A induced autophagy on hepatic endothelial cells; the damage of liver blood vessel occurs before the induction of T-cell-dependent hepatitis via apoptosis or T-cell-independent hepatitis via autophagy.

Keywords: acute hepatitis, autophagy, Con A, endothelial cells

Introduction

Con A, a plant lectin isolated from Canavalia ensiformis (Jack bean) seeds, can agglutinate cells with its mannose-binding specificity. Con A is one of the most widely used lectins. It is a T-cell mitogen and has been used to induce hepatitis in mice through the triggering of NK T cells and subsequent activation of CD4⁺ T cells (1–2). Con A-induced T-cell-dependent liver injury has been considered as an experimental mouse model of human autoimmune hepatitis. However, we have reported that Con A can also induce acute hepatitis in SCID/non-obese diabetic (NOD) mice (3). The mechanism of acute hepatitis after Con A injection in immunodeficient SCID/NOD mice was different from that observed in immunocompetent BALB/c mice. The dose required to induce acute hepatitis in SCID/NOD mice is higher than that in BALB/c mice. No lymphocyte infiltrations were found in SCID/NOD mice, and the cytokine production profile was also different. In the NKT/CD4⁺ T-cell-mediated acute hepatitis, the hepatocyte injury is caused by apoptosis, whereas in the Con A-induced T-cell-independent acute hepatitis in SCID/NOD mice, the hepatocytes death is mediated by autophagy (3). In that study, Con A was found to deposit on the hepatic sinusoidal endothelial cells and hepatocytes at 0.5 h post-injection. The liver tissue was stained with anti-LC3-II (microtubule-associated protein light chain 3) antibody, and the punctate staining (an indicator of autophagy characteristics) was found to be present on the hepatocytes beginning at 6 h, reaching a peak at 12 h post-injection of Con A in SCID/NOD mice. However, according to western blot analysis, the LC3-II conversion was detected as early as 3 h and peaked at 12 h. Although we concluded that Con A can induce autophagy in the liver of SCID/NOD mice (3), we suspected that hepatic sinusoidal endothelial cells would also be affected. Therefore, in this study, the effects of Con A on endothelial cells were further evaluated. We report that endothelial cells are damaged with autophagic induction before hepatocytes in the Con A-induced acute hepatitis and IFN-γ participates in the enhancement of this process.

Materials and methods

Mice

Eight- to 10-week-old male BALB/c, NOD/SCID and C57BL/6 mice were from National Cheng Kung University (NCKU)
Con A-induced autophagy on endothelial cells

662

laboratory animal center. B6.129S7-Ifngtm1Ts/J (IFNGR1−/−) and B6.129S7-Ifngr1tm1Agt/J (IFNGR1−/−) mice were from Jackson Laboratories (Bar Harbor, ME, USA). The mice were maintained in NCKU laboratory animal center. The animals were raised and cared for according to the guidelines set up by the National Science Council, Republic of China. The mouse experiments were approved by the Institutional Animal Care and Use Committee. To induce hepatitis, mice were injected intravenously with various doses of Con A, the serum being collected at various time points post-injection. The activities of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were determined by Hitachi type 717 automatic analyzer (Hitachi) as previously described (3).

Con A binding to endothelial cells

Human endothelial cell line HMEC-1 and mouse hepatoma cell line ML1 were incubated with different doses of Con A-FITC (Sigma, St Louis, MO, USA) (1–10 μg ml−1) at 37°C for 30 min, after being washed by PBS, and the Con A binding activity was then determined by FACS Calibur. The binding can be blocked by adding 125 mM methyl-α-D-mannopyranoside (MMA) (Sigma) mixed with Con A-FITC to the cells. For the in vivo experiment, Con A-FITC (10 mg kg−1) was intravenously injected to BALB/c mice, and at 1–6 h post-injection, the mice liver harvested to investigate the Con A binding feature. The liver was perfused by 10 ml of 4% paraformaldehyde to fix the binding. The frozen liver tissue section was then further fixed by 0.15 M NaCl solution 24 h prior to Con A injection. The membranes were blocked with 5% skim milk and incubated with primary antibodies, including LC-3, BNIP3, BECLN and β-actin, at 4°C overnight. After incubation with peroxidase-conjugated secondary antibodies at room temperature for 2 h, the blots were visualized by enhanced chemiluminescence reagents (PerkinElmer, Waltham, MA, USA).

Results

Con A preferentially binds to hepatic sinusoidal endothelial cells before the induction of hepatic inflammation

Con A is a carbohydrate-binding protein that can bind to cell membrane glycoprotein with mannose/glucose moiety. We tested whether endothelial cells can be bound by Con A. Using the human endothelial cell line, HMEC-1, Con A can specifically bind to endothelial cells dose dependently (Fig. 1A). The ML1 hepatoma cell line was used as a positive control as reported previously (4). The binding can be blocked by MMA, indicating its mannose specificity. Then the in vivo binding was investigated. After intravenous injection of Con A-FITC into BALB/c mice, Con A-FITC was found to deposit on the hepatic sinusoidal endothelial cells as identified by anti-CD31 antibody staining at 1 h post-injection. The endothelial cell surface molecule CD31 is co-localized with the Con A-FITC (Fig. 1B, 1 h). With passage of time, the Con A-positive staining on endothelial cells gradually decreased, probably due to the internalization of Con A and degradation after binding to the cell membrane. Most of the Con A-FITC were first preferentially bound to endothelial cells, but after some time (3 or 6 h post-injection), Con A bound to hepatocytes was observed. Con A was also internalized into hepatocytes (Fig. 1B, 3 and 6 h).

Con A-induced endothelial cell damage before hepatitis

The consequences of Con A binding to hepatic sinusoidal blood vessels was then evaluated. Since the lethal dose for immunocompetent BALB/c mice is at the dose >20 mg kg−1 (3), we used both a non-lethal dose (20 mg kg−1) and induction in endothelial cells. For the staining of Transferase dUTP nick end labeling (TUNEL-positive) apoptotic cells, the formalin-fixed and paraffin-embedded liver tissue sections were stained with the ApoAlert DNA Fragmentation Assay Kit (Clontech Laboratories, Mountain View, CA, USA). The experimental procedure proceeded according to the manufacturer’s instructions.

Cell viability determination

The HMEC-1 cell viability was determined by propidium iodide (PI) staining. The cells were washed by PBS once and re-suspended in 5 μg ml−1 PI, incubated at room temperature for 10 min and then analyzed by FACScan. The PI-positive cells were considered to be dead.

Western blotting

HMEC-1 cells were treated with different doses of Con A with or without IFN-γ (PeproTech, Rocky Hill, NJ, USA); total cell protein was obtained by incubation in lysis buffer (Cell Signaling Technology, Danvers, MA, USA). Proteins were separated by 12% SDS–PAGE and transferred to PVDF membranes. The membranes were blocked with 5% skim milk and incubated with primary antibodies, including LC-3, BNIP3, BECLN and β-actin, at 4°C overnight. After incubation with peroxidase-conjugated secondary antibodies at room temperature for 2 h, the blots were visualized by enhanced chemiluminescence reagents (PerkinElmer, Waltham, MA, USA).

Evans blue blood vessel leakage determination

BALB/c, SCID/NOD, C57BL/6, B6.129S7-Ifngtm1Ts/J (IFNGR1−/−) and B6.129S7-Ifngr1tm1Agt/J (IFNGR1−/−) mice treated with or without Con A were intravenously injected 50 mg kg−1, Evans blue dye in Dulbecco’s Phosphate Buffered Saline (Invitrogen) at 30 min prior to killing. The liver tissue section was perfused with 10 ml PBS, then formamide 4 ml g−1 tissue added and further incubated at 37°C for 48 h. Centrifuge at 3000 r.p.m. for 10 min; harvest the supernatant to determine the blood vessel leakage by detection OD 630 of spectrophotometer.

Deplete liver macrophage by GdCl3

Eight- to 10-week-old male BALB/c mice were intravenously treated with 30 mg kg−1 GdCl3 (Sigma–Aldrich) dissolved in 0.15 M NaCl solution 24 h prior to Con A injection. The macrophage depletion efficiency was confirmed by immunohistochemistry staining of F4/80 expression.

Immunohistochemical staining for autophagy and apoptosis in endothelial cells

The frozen liver tissue sections were stained with anti-LC3 antibody (Abgent, San Diego, CA, USA), anti-rabbit conjugate Alexa 594 (Invitrogen), anti-CD31 and anti-rat conjugate Alexa 594 (Invitrogen) to determine the autophagy...
Fig. 1. Con A-FITC binding to endothelial cells. (A) Human endothelial cell line HMEC-1 and mouse hepatoma cell line ML1 cells were treated with 1, 5 and 10 μg ml⁻¹ Con A-FITC at 37°C for 30 min and its binding activity was determined by flow cytometry. The binding is mannose specific because it can be blocked by 125 mM MMA. The numbers on each peak represent the mean fluorescence intensity. (B) In vivo binding of Con A to sinusoid endothelial cells. Con A-FITC (10 mg kg⁻¹) was intravenously injected into BALB/c mice; the liver tissue was perfused with 10 ml 4% paraformaldehyde at 1–6 h post-injection. Liver endothelial cells were stained with anti-CD31 antibody (red). The co-localization of Con A-FITC and CD31 showed Con A preferentially bound to endothelial cells, as indicated by white arrow. The blue arrow indicates the Con A internalized in hepatocytes.
a lethal dose (30 mg kg\(^{-1}\)) of Con A to induce acute hepatitis. Acute hepatitis was observed as early as 6 h, increasing gradually and peaking at 24 h post-intravenous injection as expected (Fig. 2A). Using Evans blue as a plasma leakage marker from liver blood vessels, we determined whether there was any endothelial cell damage in the liver. A significant leakage of Evans blue into the liver was observed at 3 h after Con A injection in BALB/c mice (Fig. 2B). The hematoxylin and eosin (H&E) stain of liver tissue also showed hemorrhage and necrosis in the liver (Fig. 2C). The RBCs leaked into the tissue beginning at 3 h, becoming severe at 12–24 h that was associated with intense inflammation and necrosis, which is compatible with the data of Evans blue leakage. We further used a lethal dose of 30 mg kg\(^{-1}\) of Con A to evaluate its effect on the hepatic blood vessels. The mice will die within 5–6 h, and no significant hepatitis was observed at 3 h post-injection (Fig. 2D). However, hepatic blood vessel leakage and hemorrhage were observed at as early as 2 h post-injection (Fig. 2E). Although no increase of Evans blue was detected at 1 h, mild hemorrhage with RBC leakage was found at 1 h on H&E staining (Fig. 2F). Con A at 30 mg kg\(^{-1}\) causes more damage to hepatic blood vessels than at 20 mg kg\(^{-1}\). It is generally known that Con A induces acute hepatitis through the CD4+ T-cell-mediated inflammation that occurs at 6–24 h post-injection. Hemorrhage with blood vessel damage is associated with the inflammation and necrosis at this time point. However, this study is the first to report that hepatic blood vessel damage and hemorrhage is induced at 2–3 h after Con A injection without lymphocyte infiltration and hepatitis.

We have reported a T-cell-independent acute hepatitis in SCID/NOD mice (3). Con A at 40 mg kg\(^{-1}\) was injected intravenously into SCID/NOD mice, and the hepatitis and hemorrhage were analyzed kinetically. No alanine transaminase elevation was observed until 12 h post-injection (Fig. 2G), but Evans blue leakage was detected as early as 3 h, with a higher level at 6 h post-injection, and maintained a significant level at 12–24 h (Fig. 2H). The H&E stain of liver tissue confirmed the hemorrhage beginning at 3 and 6 h without the inflammatory cell infiltration. Necrosis was observed at a later time of 12–24 h (Fig. 2I). Thus, we conclude that Con A can cause hemorrhage before the induction of hepatitis. The hepatic blood vessel can therefore be directly damaged by Con A in either immunocompetent or immunodeficiency mice.

**Con A-induced autophagy of endothelial cells**

To further understand how the blood vessel is damaged by Con A to cause hemorrhage, we stained liver tissue with both autophagy and apoptosis markers. To induce acute hepatitis in SCID/NOD mice, the dose of Con A needs to be >20 mg kg\(^{-1}\) (3). As shown in Fig. 3(A), LC3-II punctate formation was observed on hepatic blood vessels of SCID/NOD mice at dose of 30 mg kg\(^{-1}\), but not 20 mg kg\(^{-1}\). Dose of 40 mg kg\(^{-1}\) showed more LC3-II punctates. When tested on BALB/c mice with the dose of 30 mg kg\(^{-1}\) of Con A, the autophagy LC3 markers were co-localized with the CD31+ endothelial cells at 4 h post-injection (Fig. 3B). On the other hand, no TUNEL-positive cells were detected in the liver at 3 h post-injection, whereas TUNEL-positive apoptosis was observed in hepatocytes at 12–24 h following a 20 mg kg\(^{-1}\) injection in BALB/c mice (Fig. 3C). Apparently, hepatic endothelial cells were stimulated by Con A to undergo autophagy but not apoptosis. The signal pathway was further studied on the human endothelial cell HMEC-1. Con A can induce dose- and time-dependent death of HMEC-1 cells (Fig. 4A and B). The autophagic marker of LC3 II conversion was demonstrated by western blot analysis (Fig. 4C). It is concluded that Con A induces autophagy on hepatic endothelial cells.

**IFN-γ enhanced the Con A-mediated autophagy of endothelial cells**

We have reported an early transient elevation of IFN-γ in the liver and serum at 1–3 h after Con A injection (3); therefore, the role of IFN-γ on Con A-induced autophagy of endothelial cells was further studied. Using the IFN-γ and IFNγ receptor knockout mice, the Con A-induced acute hepatitis was ameliorated in these knockout mice (IFNG−/− or IFNGR−/−) comparing with the wild type (Fig. 5A). Moreover, there was low level of Evans blue extravasation on IFNGR−/− mice. When comparing with the wild-type and IFN-γ and IFNγ receptor knockout mice, the IFN-γ significantly enhanced the Evans blue extravasation and hemorrhage with RBC leakage in hepatic blood vessels at 1–6 h after Con A injection (Fig. 5B and C). On HMEC-1 cell, Con A-induced cell death can be enhanced by IFN-γ dose dependently (Fig. 4D). Autophagic LC3-II conversion from LC3-I was enhanced by IFN-γ (Fig. 4E). IFN-γ treatment seems to sustain the autophagy process by maintaining the level of LC3-I. Based on the data above, we conclude that Con A induces autophagy on hepatic endothelial cells, which can be enhanced by IFN-γ, and this liver blood vessels damage occurs before the induction of hepatitis.

**Discussion**

Severe hepatic injury can be induced by Con A administration in mice. This experimental acute hepatitis model, since being established by Tiegs et al. in 1992, has been studied extensively. The scenario of Con A-induced acute hepatitis can be depicted as follows: after intravenous injection of Con A, Kupffer cells were stimulated to secrete TNFα, IL-12 and IL-18 and activate T cells including NKT and CD4+ T cells. T cells produce IFN-γ and further activate Kupffer cells in a positive feedback loop. The superoxide/ROS, TNFα and IFN-γ are thought to be responsible for the hepatocyte apoptosis. Several accessory molecules or cells such as IL-4, IL-6, IL-10, MIF, adhesion molecules of selectin and ICAM-1, neutrophils or Treg cells also participate to modulate the hepatocyte damage (5–13). Con A-induced hepatitis is thought to be NKT/CD4+ T-cell dependent, but a T-cell-independent hepatitis was also reported in SCID/NOD mice with a higher dose stimulation (3). The mechanism of T-cell-independent hepatitis is distinct from that of T-cell-independent hepatitis with regard to dose used, cytokine produced and mode of cell death. We previously proved that Con A can induce autophagic cell death of hepatocytes in its direct effect (3, 4, 14). In this study, we further demonstrate that liver blood vessels are the first target of Con A.
Fig. 2. Con A induces hemorrhage before hepatitis in murine liver. Groups of four mice were intravenously injected with Con A at 20 mg kg\(^{-1}\) (A–C), 30 mg kg\(^{-1}\) (D–F) in BALB/c mice or 40 mg kg\(^{-1}\) (G–I) in SCID/NOD mice. At various hours post-injection, the liver injury was evaluated by serum ALT level (A, D and G). The hemorrhage status was determined by Evans blue dye leakage from blood vessels (B, E and H). At 30 min prior to sacrifice time point 50 mg kg\(^{-1}\) Evans blue was intravenously injected. Liver was perfused with 15 ml PBS, the tissue was incubated with formamide to extract the Evans blue and its amount was determined by spectrophotometry at 630 nm. The H&E staining of liver tissue (in \(\times 100\) or \(\times 200\) magnification) is shown (C, F and I). Arrow indicates the hemorrhage site (*\(P < 0.05\)).
The role of macrophage on the endothelial cell damage is excluded because the GdCl3 depletion of liver macrophage does not inhibit the Con A-induced plasma leakage (Supplementary Figure 1 is available at International Immunology Online). The damage of hepatic vascular endothelial cells will precede the subsequent lymphocyte infiltration and cytokine-mediated hepatocyte injury. This new finding adds an in-depth understanding on the pathogenesis of Con A-induced hepatitis, especially the fulminant hepatitis.

Using Con A-FITC to trace its deposition, it was found to primarily deposit on the blood vessels that bear the endothelial cell marker CD31+. Con A-induced endothelial cell damage is particularly severe in liver, comparing with spleen, kidney and lung (data not shown). The liver can trap blood-born foreign substances, and its anatomic location makes it a good site to concentrate Con A. Con A trapped in the liver binds to the endothelial cells leads to the induction of autophagic cell death. The further activation of immune cells will subsequently induce the adaptive immune response against the hepatocytes and cause acute hepatitis. The autophagy in endothelial cells at 3–4 h is different from the TUNEL-positive apoptosis of hepatocytes at 12–24 h post-injection. The earliest time to observe the hepatocyte death with the elevation of the serum ALT is at 6 h post-injection and then increased at 12–24 h. This is caused by cytokine-caused apoptosis. On the other hand, the autophagy

![Fig. 3. Con A induces autophagy of liver endothelial cells. Groups of four mice were injected intravenously with Con A. Livers were collected at 3 or 4 h post-injection and stained with anti-LC3 or anti-CD31 antibodies. (A) LC3 punctate staining on liver endothelial cells of SCID/NOD mice at 3 h after 20–40 mg kg⁻¹ Con A treatment. (B) Double staining of anti-CD31 (green) and anti-LC3 antibody (red) on liver of BALB/c mice treated with 30 mg kg⁻¹ Con A. (C) TUNEL staining (green) and propidium iodine counter-staining (red) of BALB/c liver in 20 mg kg⁻¹ Con A treatment. Arrow indicates TUNEL-positive apoptotic hepatocytes.](https://academic.oup.com/intimm/article-abstract/22/8/661/774675)
of endothelial cells occurs at 1–3 h and causes plasma leakage and hemorrhage without cell infiltration (Fig. 2C and F). Therefore, Con A-induced disruption of blood vessels by autophagy is a prerequisite for further recruitment of inflammatory cells and subsequent hepatocyte death.

Autophagy is an evolutionarily conserved lysosomal pathway involved in cytoplasmic homeostasis to control the turnover of long-lived proteins and can be stimulated in response to various stimuli (15–20). The sensitivity to Con A in mice (BALB/c > SCID > SCID/NOD mice with the lethal dose at 20, 40 and 50 mg kg⁻¹, respectively) reflects the degree and combination of direct autophagic cytotoxicity and indirect immunomodulation. B6 mice is more sensitive to Con A than BALB/c mice. The direct autophagic induction needs a dose >30 mg kg⁻¹, and IFN-γ can enhance the susceptibility to Con A-induced autophagy. Both endothelial cells and hepatocytes can be induced by Con A to undergo autophagy, but endothelial cells are more susceptible than hepatocytes. Plasma leakage and hemorrhage is observed at 1–3 h post-injection because of endothelial cell disruption, whereas hepatitis occurs at 6–12 h post-injection. The hepatocyte damage can be either autophagy (high dose, 40 mg kg⁻¹ in SCID/NOD) or apoptosis (low dose, 20 mg kg⁻¹ in BALB/c). Con A, due to its specific mannose/glucose binding, can bear the dual properties of autophagic cytotoxicity and immunomodulation on T cells to cause acute hepatitis. The hepatocyte damage mechanism is therefore dependent on the immune status of the host. A synergistic effect of autophagy induction and immunomodulation in immunocompetent mice will make a dose >30 mg kg⁻¹ lethal for this host, which can be considered as an induction of fulminant hepatitis.

The role of autophagy in hepatitis is worthy of further investigation—whether autophagy is beneficial for adaptation and the survival of hepatocytes or whether it is detrimental in that it leads to death. The autophagy on endothelial cells can be induced by advanced glycation end products of glycated collagen I, apolipoprotein L1, kringle 5 of plasminogen...
This study of Con A-induced autophagy of endothelial cell that can be enhanced by IFN-\(\gamma\) is a new example. IFN-\(\gamma\) is an important immunomodulator that enhance the macrophage of T cells (25–27). The effect of IFN-\(\gamma\) on Con A-induced acute hepatitis is ascribed to enhance the T cell and therefore increase hepatocyte damage (28–29). In this study, we further extend that IFN-\(\gamma\) can enhance the Con A-induced endothelial cell autophagy.

Several lectins of LEL, RCA-1, UEA-1 and Con A have been shown to bind to sinusoidal endothelium of the liver and bone marrow in a patchy granular pattern after intravenous injection (30). The bound lectins were endocytosed and accumulated within the endothelial cells. We confirmed the same binding and further showed the consequence of Con A binding, i.e. the endothelial cells will undergo autophagic cell death. Kind and Petersen (31) have reported in 1968 that Con A could induce hemorrhagic skin lesions in mice, as they observed congestion of capillaries and free RBCs at 4 and 24 h after skin injection. But they described it as an Arthus-like reaction because of the accumulation of leukocytes at 24 h. The hemorrhage observed at the early 4-h point is indeed similar to our finding that it is directly caused by Con A and does not involve the cell infiltration. Knolle et al. (32) reported that sinusoidal endothelial cells after Con A binding will be killed by activated T cells. This can explain the initiating events in T-cell-mediated liver injury after recruiting T cells from circulation. Miyazawa et al. also reported an altered intrasinusoidal hemostasis that consisted of erythrocyte agglutination, lymphocyte/neutrophil sticking to endothelial cells and platelet agglutination and degranulation, resulting in a marked decrease in the intrahepatic blood flow and elevation of portal perfusion at 6 h after Con A injection (33). Our finding provides a new mechanism that endothelial cells are damaged via Con A-induced autophagy and causes altered hemostasis before T-cell infiltration.

**Fig. 5.** IFN-\(\gamma\) enhanced the Con A-mediated hemorrhage in liver. Groups of four B6, IFNG\(^{-/-}\) or IFNGR\(^{-/-}\) mice were intravenously injected with Con A at 30 mg kg\(^{-1}\). At various hours post-injection, the liver injury was evaluated by serum ALT level (A). The hemorrhage status was determined by Evans blue dye leakage from blood vessels (B). At 30 min prior to sacrifice time point 50 mg kg\(^{-1}\) Evans blue was intravenously injected. Liver was perfused with 15 ml PBS, the tissue was incubated with formamide to extract the Evans blue and its amount was determined by spectrophotometry at 630 nm. The H&E staining of liver tissue (in ×100 magnification) is shown (C). Arrow indicates the hemorrhage site (**\(P < 0.05\)).
Con A-induced autophagy on endothelial cells

Supplementary data

Supplementary data are available at International Immunology Online.

Funding

National Science Council, Taiwan (NSC91-2320-B006-036, NSC94-3112-B006-005).

Disclosure

The authors have no financial conflicts of interest.

References