2012

Chikungunya-induced cell death is limited by ER and oxidative stress-induced autophagy

Pierre-Emmanuel Joubert
Institut Pasteur

Scott Werneke
Washington University School of Medicine in St. Louis

Claire de la Calle
Institut Pasteur

Florence Guivel-Benhassine
Institut Pasteur

Alessandra Giodini
Institut Pasteur

See next page for additional authors

Autophagy 8:8, 1261–1263; August 2012; © 2012 Landes Bioscience

Chikungunya-induced cell death is limited by ER and oxidative stress-induced autophagy

Pierre-Emmanuel Joubert,[1,5] Scott Werneke,[6] Claire de la Calle,[2] Florence Guivel-Benhassine,[4] Alessandra Giodini,[1,5] Lucie Peduto,[3] Beth Levine,[7] Olivier Schwartz,[4] Deborah Lenschow[6] and Matthew L. Albert[1,2,5,*]

[1]Unité Immunobiologie des Cellules Dendritiques; Institut Pasteur; Paris, France; [2]Centre d'Immunologie Humaine; Institut Pasteur; Paris, France; [3]Laboratory of Lymphoid Tissue Development; Department of Immunology; Institut Pasteur; Paris, France; [4]Unité Virus et Immunité; Institut Pasteur; Paris, France; [5]INSERM U818; Paris, France; [6]Department of Pathology and Immunology; Department of Medicine; Washington University School of Medicine; St. Louis, MO USA; [7]Howard Hughes Medical Institute; University of Texas Southwestern Medical Center; Dallas, TX USA

It has been recognized that macroautophagy constitutes an important survival mechanism that allows both the maintenance of cellular homeostasis and the regulation of programmed cell death pathways (e.g., apoptosis). Although several pathogens have been described to induce autophagy, the prosurvival function of this process in infectious models remains poorly characterized. Our recent studies on chikungunya virus (CHIKV), the causative agent of major epidemics in India, Southeast Asia and southern Europe, reveal a novel mechanism by which autophagy limits the cytopathic effects of CHIKV by impinging upon virus-induced cell death pathways.

Keywords: chikungunya, autophagy, apoptosis, endoplasmic reticulum stress and oxidative stress

Submitted: 04/25/12

Revised: 05/09/12

Accepted: 05/14/12

http://dx.doi.org/10.4161/auto.20751

*Correspondence to: Matthew L. Albert; Email: albertm@pasteur.fr

Punctum to: Joubert PE, Werneke SW, de la Calle C, Guivel-Benhassine F, Giodini A, Peduto L, et al. Chikungunya virus-induced autophagy delays caspase-dependent cell death. J Exp Med 2012; 209:1029–47; PMID:22508836; http://dx.doi.org/10.1084/jem.20110996

Chikungunya virus is the causative agent of the mosquito-transmitted disease, referred to as chikungunya fever. Infection of humans with CHIKV results in an illness characterized by high fever, rash, arthritis and an erratic relapsing and arthralgia (joint pain). The disease was first described after the 1952 outbreak in Tanzania, and the clinical features led to it being named by the Makonde term for "that which bends up," a reference to the contorted position of the patient secondary to their joint and bone pain. CHIKV is a member of the *Alphaviridae* genus of the family *Togaviridae*, which are characterized by being enveloped, single-stranded positive polarity RNA viruses. As observed for other alphaviruses (e.g., Sindbis virus and Ross River virus), CHIKV infection has the capacity to induce apoptotic cell death, which has been suggested to be associated with its disease pathogenesis.

Our recent work demonstrated that CHIKV infection leads to enhanced autophagic flux. In contrast to other viruses, which escape and/or modulate autophagic processes by inhibiting the maturation of the autophagosome, CHIKV-induced autophagy is a complete process, culminating with lysosomal degradation of the contents of autophagic vesicles. Using single-cell multispectral cytometric analysis, we demonstrated that autophagy is triggered in a cell-intrinsic manner (i.e., direct activation of autophagy secondary to CHIKV infection and replication). This results in the induction of both endoplasmic reticulum (ER) and oxidative stress. The screening of the three major pathways known to regulate ER stress induction implicated the ERN1/IRE1α-XBP1 pathway, which is activated during the first 48 h of infection. CHIKV infection also results in an increased intracellular presence of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Whereas the ERN1-XBP1 pathway is known to directly induce autophagy, we provided evidence for ROS-induced autophagy as a mechanism for achieving MTORC1 inhibition, a key regulator of autophagy. Notably, the timing of MTORC1 inhibition and ERN1-XBP1 activation correlates with the time period of peak autophagy induction. Using complimentary strategies of ROS inhibition and

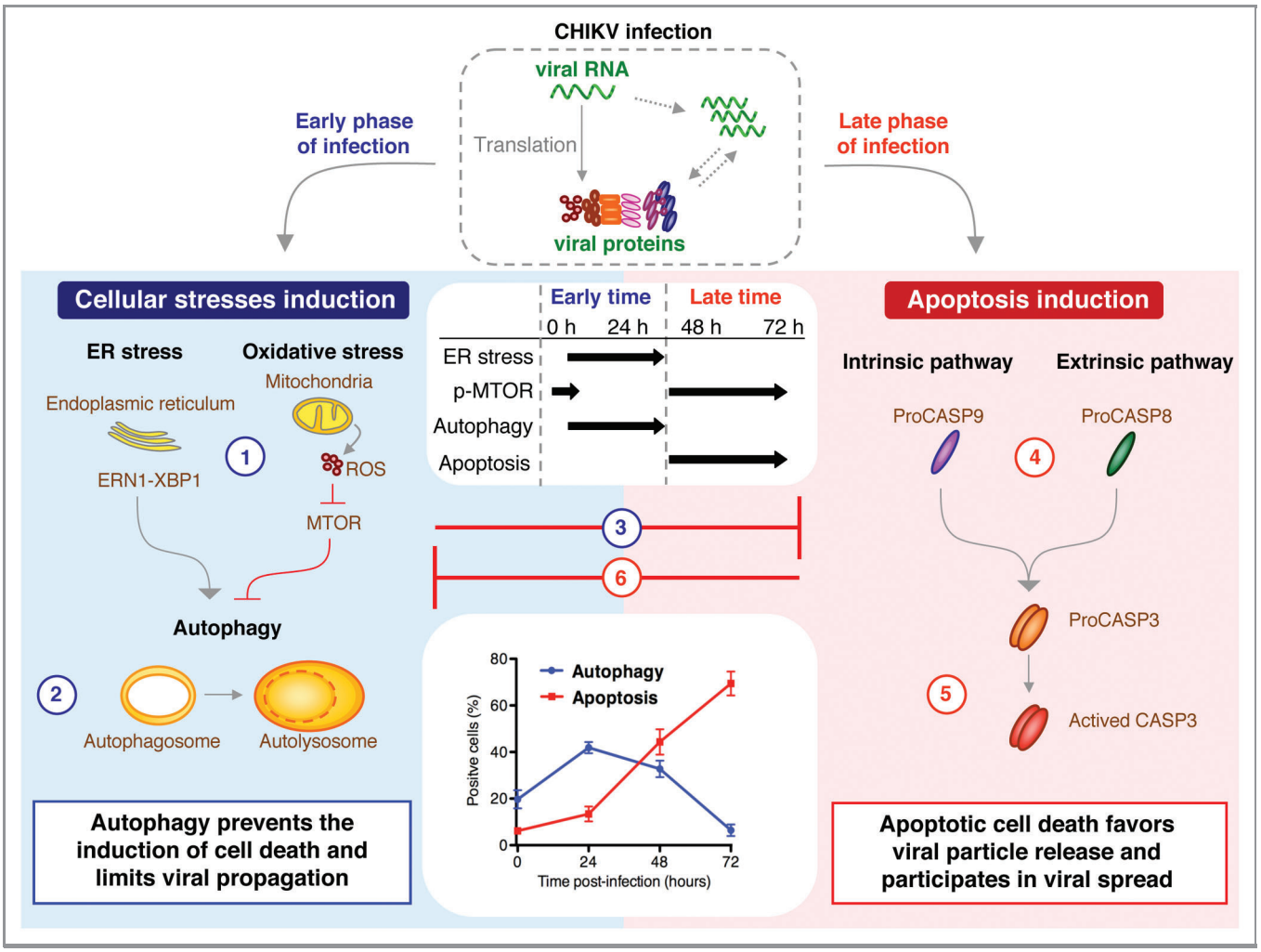

Figure 1. Stress and death during chikungunya viral infection. (1) During the early phase of chikungunya virus (CHIKV) infection, viral replication results in the induction of both endoplasmic reticulum (ER) and oxidative stress. ER stress results in activation of the ERN1-XBP1 pathway. (2) Both stress pathways act in an interdependent manner to enhance autophagic flux in CHIKV-infected cells. Whereas the ERN1-XBP1 pathway directly impinges upon the autophagy pathway, ROS induces autophagy indirectly via its inhibition of MTORC1, a key negative regulator of autophagy. (3) CHIKV-induced autophagy impedes the initiation of apoptotic cell death, and in turn limits viral propagation. (4) During late phase of infection (i.e., 48 h post-infection), decreased autophagic flux was observed, correlating with evidence of enhanced apoptotic cell death. At least two apoptotic pathways are induced during CHIKV infection: the intrinsic pathway, which is dependent on virus replication, and an extrinsic pathway that may involve the induction of cell surface or soluble death effector ligands (e.g., TNFSF10/TRAIL). (5) Both death effector pathways result in activation of CASP3 and apoptotic cell death, which favors the release of viral particles. (6) Apoptosis induction is correlated with decreased activity of cell stress pathways, marked by the degradation of ERN1 and the hyperphosphorylation of MTORC1. Thus, in the context of CHIKV infection, there is an intricate crosstalk between cell stress and apoptosis with important consequences for viral propagation.

small interfering RNA, we formally demonstrated that both stress pathways act in an interdependent manner to enhance autophagic flux in CHIKV-infected cells (**Fig. 1**).

In contrast to autophagy, apoptosis is observed in both infected and uninfected bystander cells. This result suggested that apoptotic cell death is triggered in both cell-intrinsic and cell-extrinsic manners. To characterize these pathways, we demonstrated a role for CASP9 activation in CHIKV infected cells and CASP8 activation in uninfected bystander cells. These results confirmed that CHIKV infection can induce apoptotic cell death via an intracellular pathway, which is dependent on virus replication, and/or an extracellular mechanism that remains to be defined, but may involve the induction of cell surface or soluble death effector ligands (e.g., TNFSF10/TRAIL). According to the kinetics of apoptosis induction, we argue that the intrinsic pathway is induced during a first wave of cell death (activated-CASP9-positive cells are detectable after 16 h of infection), and that secondary to the host cell response (e.g., interferon-induced TRAIL), there is the subsequent induction of the extrinsic pathway (**Fig. 1**). Mechanisms implicated in apoptosis induction were found to be independent of ER and oxidative stress, and as such the precise mechanism of CHIKV-induced cell death remains to be determined.

In order to provide in vivo relevance for our findings, we studied mice hypomorphic in their expression of ATG16L1, with reduced induction of autophagy (referred to as ATG16L1^{HM}). Strikingly, ATG16L1^{HM} mice show a higher sensitivity to CHIKV infection and exhibit a 2-fold increase in lethality as compared with wild-type mice. These observations supported our conclusion that autophagy, as a host response to infection, acts to limit the cytopathic effects of CHIKV and regulates the pathogenesis of acute chikungunya disease.

How autophagy regulates apoptosis and how apoptosis ultimately succeeds in overwhelming autophagy's inhibitory effects remain open questions. What our study does clarify, however, is the role for autophagy in serving as a cell intrinsic, prosurvival mechanism that is capable of preventing the early induction of apoptosis. Regarding the anti-apoptotic function of CHIKV-induced autophagy, several possible mechanisms may be considered: (i) autophagy sequesters and degrades damaged mitochondria, thus preventing release of cytochrome c and the activation of the intrinsic apoptosis pathway; (ii) autophagy could result in the degradation of pro-apoptotic protein complexes, including the apoptosome; or (iii) autophagy-related gene products may directly regulate viral replication, limiting the cytopathic effects of virus. Whereas our in vitro data suggest an inhibitory effect of autophagy on virus propagation, the specific factors implicated in this process remain to be defined. Our studies also support the second hypothesis, as higher levels of proCASP3 are found in cells deficient for autophagy as compared with wild-type cells.

During the late phase of infection (i.e., 48 h post-infection), we observed decreased autophagic flux, which correlates with increased apoptotic cell death (**Fig. 1**). The switch between autophagy and apoptosis was not clearly defined, but our in vitro data may suggest new directions for future investigation. Specifically, MTOR and ERN1-XBP1 complexes appear to be important modulators, marking the switch between autophagy and apoptosis. Prior to infection (i.e., resting state for the host cell), MTOR is activated and serves as a negative regulator of autophagy induction. At early time points post-infection with CHIKV, MTOR is dephosphorylated, and, together with inducers of ER stress, MTOR inhibition enhances survival through the derepression of autophagic flux. By 48 h post-infection, we observed degradation of ERN1 and hyperphosphorylation of MTOR (leading to increased activity), which correlate with decreased autophagic flux and increased levels of apoptotic cell death. Our model for the cascade of events triggered by CHIKV infection implicates viral protein regulation of MTOR and ERN1, facilitating during late phase infection the inhibition of autophagy, induction of apoptosis and the consequent release of newly formed virions.

In sum, our work established a functional connection between cell stresses and cell death in the context of CHIKV infection and may allow the establishment of new therapeutic strategies for limiting the pathogenesis of chikungunya disease.