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The endoplasmic reticulum: a sensor of cellular stress that modulates immune responses

Fabio Martinon*

Dept. of Biochemistry, University of Lausanne, 155 Ch. Des Boveresses, Epalinges 1066, Switzerland

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Abstract

Many inflammatory and infectious diseases are characterized by the activation of signaling pathways steaming from the endoplasmic reticulum (ER). These pathways, primarily associated with loss of ER homeostasis, are emerging as key regulators of inflammation and infection. Recent advances shed light on the mechanisms linking ER-stress and immune responses.
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1. Introduction

Loss of cellular integrity is a hallmark of severe pathogenic insults and leads to the activation of immune defenses aimed at repairing tissue damage and fighting infections. It has been postulated that the release of danger signals by damaged cells could lead to the mounting of immune defenses [1−3]. These danger signals also known as damage associated molecular pattern (DAMPs) are molecules that trigger specific receptors including receptors that initiate inflammatory and immune responses. Many DAMPs are nuclear proteins such as HMGB1 or cytosolic molecules including, for example, uric acid and ATP. When released outside of the cell following tissue injury, DAMPs are sensed by immune cells and trigger innate immune receptors such as the Toll-like receptors (TLRs) or the NLRP3 inflammasome [4,5]. The release of DAMPs by cells is often described as a passive process, occurring as a consequence of sustained cell damage. This process is irreversible and correlates with the death and elimination of the damaged cell. However, before losing membrane integrity, cells can experience various states of malfunction that are reversible. We can define these cellular states as stress. Typically, stressed cells are malfunctioning, but are also characterized by the activation of adaptation responses to the stress aimed at repairing cell damage. Stress can be a consequence of perturbations in basic cellular functions including the availability of nutrients and oxygen as well as the capacity to communicate with neighboring cells and to respond to changes in the microenvironment. If the cell is able to sense these perturbations it mounts a response (hereby referred to as a stress response) that, by definition, is an active cellular adaptation to the abnormal conditions. If this repair/adaptation fail or if the stress is too severe, the cell will eventually die possibly releasing DAMPs. Tissue stress and malfunction promotes low-grade inflammation which helps the tissue to adapt to the damage and restore tissue function [6,7]. This low-grade inflammation also termed para-inflammation illustrates how stress and possibly stress response pathways can contribute to immune responses (Fig. 1). However the mechanisms and the role of stress responses in controlling innate immunity and inflammatory pathways are poorly understood.

The endoplasmic reticulum (ER) is emerging as a key organelle that maintains cellular homeostasis and contributes to the regulation of innate immune response [1,8,9]. Perturbations that affect the ER trigger a specific response known as the ER-stress response. In this review we discuss how

* Tel.: +41 21 692 5695; fax: +41 21 692 5705.
E-mail address: fabio.martinon@unil.ch.

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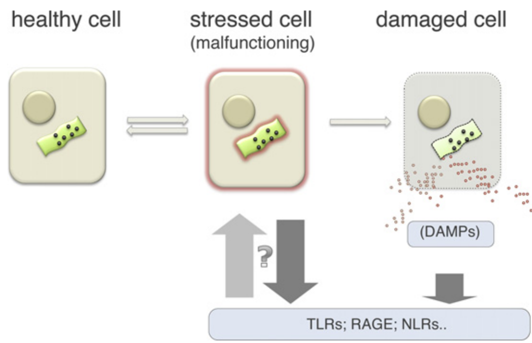

Fig. 1. Regulation of innate immune signaling by stressed and damaged cells. The state of a tissue can range from healthy, to stressed or malfunctioning, to irreversibly damaged. Damaged cells release DAMP, that directly engage innate immune receptors such as TLRs, NLRs or RAGE receptors. In contrast, tissue stress or malfunction is a reversible state that triggers an adaptation response aimed at restoring tissue homeostasis. Stressed tissue may also trigger danger signals or stress signals that could contribute to the upreglation of inflammatory and innate immune responses.

infectious and inflammatory signals affect the ER and focus on how perturbations and signaling pathways emerging from this organelle regulate innate and inflammatory pathways.

2. The endoplasmic reticulum (ER)

The ER is an essential organelle that controls the biogenesis of cellular components including proteins, lipids and carbohydrates. It is connected with the nuclear membrane and via mitochondria associated membranes (MAMs) to the power source of the cell: the mitochondria. In eukaryotes, resident proteins of the endocytic and exocytic organelles as well as cell surface and secreted proteins are synthetized on polysomes anchored to ER membrane and then, translocated across the membrane into the ER lumen. The ER lumen is rich in calcium and its oxidizing environment catalyzes post-translational modifications such as the addition of N-linked glycans and the formation of disulfide bonds [10,11]. The nascent protein must fold via a complex process that is monitored by resident ER–protein chaperones and enzymes that prevent aggregation and regulate proper folding of newly synthetized proteins. This process being essential for the production of virtually every secreted protein and membrane receptor, the ER is therefore an essential hub controlling various aspect of cellular biology including virtually all aspects of cell–cell communication and signal transduction pathways steaming from membranes. In Immunology the ER is best known for its role in antigen presentation, it is the main site for the assembly of MHC class I and MHC class II molecules and is the location of peptide loading onto MHC class I molecules.

3. The ER-stress response

Conditions of ER stress occur when the amount of proteins entering the ER exceeds its folding capacity. This imbalance induces a protective signaling cascade collectively termed the unfolded protein response (UPR) or the ER-stress response [12,13]. Experimentally, many conditions can trigger the ER-stress response. For example, conditions that inhibit glycosylation including low glucose as well as pharmacological compounds such as tunicamycin, affect the maturation and transport of most secreted proteins causing ER-stress. Other drugs and conditions that perturb ER Ca^{2+} levels have profound effects on ER homeostasis and protein folding. Drugs such as thapsigargin can trigger depletion of ER calcium stores leading to acute ER-stress responses. The ER presents an oxidizing environment that can favor protein folding and the formation of disulfide bonds. Reducing agents such as dithiothreitol (DTT) affect the oxidizing potential of the ER leading to the accumulation of misfolded proteins and the subsequent engagement of the ER-stress response. In addition, various physiological and pathological insults that perturb the cell protein homeostasis network, a group of interconnected pathways collectively termed the proteostasis network may trigger the ER-stress response [14,15]. These insults include alterations in cellular pH, inhibition of the proteasome pathways, infections with various pathogens as well as metabolic changes associated with cancer. ER-signaling pathways and the ER-stress response are among the major and best-characterized components of the proteastasis network pathways.

The ER-stress response is a highly controlled process that adapt to specific insults to optimize and orchestrate an appropriate response. While this response may differ from one condition to another, a general picture of signaling events emerge. The attenuation of translation is among the first things that are observed upon induction of ER-stress. This occurs within minutes to hours of ER-stress activation and prevent further translational overload of the ER. Perturbation of ER-homeostasis also results in the up-regulation of genes involved in ER biogenesis, in chaperoning and folding of proteins as well as in the quality control mechanisms that target and ensure elimination of malfunctioning proteins including the endoplasmic-reticulum-associated protein degradation (ERAD) pathway. The aim of these responses is to help the cell cope with the stress, remove the accumulated protein load, increase ER capacity and restore normal function of the ER. In conditions of acute and prolonged stress, the response changes from promoting survival to inducing cell death. The point at which the 'apoptotic switch' is activated has not yet been determined, and may vary form cell to cell. The mechanisms of ER-stress mediated apoptosis are yet poorly understood but may involve the intrinsic apoptotic pathway that lead to release of cytochrome *c* from the mitochondria and activation of the caspase-9 apoptosome [16–18].

4. ER-signaling pathways

In mammalian cells, the ER-stress response is mediated by at least three signaling pathways that operate in parallel (Fig. 2). Each pathway steams from a class of transmembrane proteins anchored at the ER: IRE1 (Inositol-requiring enzyme 1) PERK (PKR-like ER kinase), and ATF6 (activating

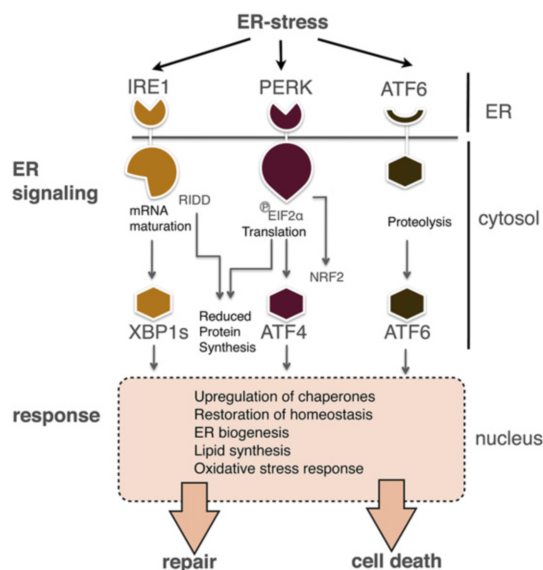

Fig. 2. ER-stress signaling pathways. Accumulation of unfolded proteins or loss of homeostasis in the ER triggers a stress response that activates ER-signaling pathways including IRE1, ATF6, and PERK. Active IRE1 lead to an unconventional splicing of the transcription factor XBP1 and the translation of an active transcription factor. IRE1 can also activate additional pathways such as the degradation of ER-related mRNAs through a process termed RIDD. PERK activation induces the translation of the transcription factor ATF4 and dampen the translation initiator factor ElF2α activity, leading to decreased translation and protein synthesis. PERK also promotes the activation of the transcriptional regulator NRF2. The transcriptionally active form of ATF6 is produced by proteolysis. When these three main pathways are activated simultaneously they orchestrate the ER-stress response characterized by the transcriptional induction of genes aimed at restoring ER homeostasis. The outcome of the response is repair or, if repair fails, cell death and apoptosis.

transcription factor 6). Activation of these three signaling branches control the expression of a few proximal ER-stress related transcription factors and signaling events that orchestrate the transcriptional upregulation of the universe of genes involved in restoring ER functions [16,19,20].

IRE1 is the most conserved branch, the only one present in lower eukaryotes and probably the best-studied branch of the ER-stress response. In yeast, IRE1 triggers the full ER-stress response program, whereas in higher eukaryotes the program requires cooperation between the three branches of the ER-stress response. IRE1 transmembrane region is flanked by a sensor unit at its N-terminus that localize in the ER lumen and a C-terminal portion that reside in the cytosol. The cytosolic portion of IRE1 contains two functional units: a Ser/Thr protein kinase and an endoribonuclease. Oligomerization of IRE1 at the ER, triggers its ribonuclease activity and cleaves, at two closely located and distinct sites, the mRNA of the X-box binding protein-1 (XBP1) gene. This cleavage results in the excision of a mini intron of 26 nucleotides in mammals. Upon ligation, mature XBP1 mRNA encodes a functional transcription factor. IRE1 mediated removal of the 26 nucleotides mini intron open a new translation reading frame at the C-terminus of the protein coding for a potent transactivation domain. Therefore, by a unique mechanism in mammals, unrelated to regular splicing, IRE1 converts an inactive XBP1 protein sequence into an active transcription factor. Active XBP1 referred hereby as XBP1s, contribute to the regulation of genes involved in different processes including protein folding, mechanisms controlling protein quality as well as a broad array of genes involved in almost every aspect of ER function, physiology and biogenesis [21,22]. Early studies on XBP1 highlighted the role of the ER-stress response pathways in the development and regulation of secretory cells including plasma B cells. XBP1-deficient B cells are unable to differentiate into antibody-secreting plasma cells [23,24] and antibody production *in vivo* in response to antigenic challenge is impaired by XBP1 deficiency. Importantly it was shown that activated B cells undergo XBP1 mRNA maturation to produce XBP1s in order to promote ER expansion and develop the secretory capacity of plasma cells [25,26]. However, what drives IRE1 activation in B cells is still unclear. In B cells, XBP1 activation is not impaired by IgM deficiency [27,28] suggesting that massive immunoglobulin production *per se* is not the cause of XBP1s activation and that IRE1/XBP1 activation may by part of a preemptive differentiation program rather than as a consequence of ER overload. In addition to XBP1 activation, IRE1 ribonuclease activity has other functions. Upon sustained and acute ER-stress, IRE1 may contribute to the degradation of membrane-associated mRNAs through a process known as regulated IRE1 dependent decay or RIDD [27,29,30]. The exact role of RIDD is unknown. It is unclear if it solely contribute to decreasing protein load in the ER or is part of the induction of the apoptosis phase as suggested by experiments showing increased ER-stress induced apoptosis in cells in which IRE1 activity was manipulated to promote RIDD activation [30,31].

PERK is an ER anchored kinase that upon activation oligomerizes and autophosphorylate. It kinase activity phosphorylates the translation initiation factor EIF2α, transiently decreasing overall protein translation. This helps reducing folding requirements in the ER. By phosphorylating EIF2α at serine 51 PERK inhibits the guanine nucleotide exchange factor EIF2B, a factor involved in the recycling of EIF2α to its active GTP-bound form [32–34]. EIF2α phosphorylation can be targeted by phosphatases, such as Growth arrest and DNA-damage inducible protein-34 (GADD34) [35,36] that contribute to the resolution of the stress responses. Some mRNA containing short open reading frames in their 5′ untranslated region are induced when EIF2α is inhibited. EIF2α phosphorylation can therefore promote the synthesis of a specific subset of genes by increasing the translation of selective mRNAs whose translation is inhibited in unstressed cells. For example this mechanisms promote the translation of activating transcription factor 4 (ATF4) a major regulator of ER-stress. ATF4 is involved in various cellular responses including, glutathione biosynthesis, amino acid import, and resistance to oxidative stress [37–39]. PERK also phosphorylates Nuclear Factor (erythroid-derived 2)-like 2 (NRF2), a transcription factor that regulates oxidative stress responses and a critical regulator of innate immune responses [32,40,41].

The third branch of the ER-stress pathway is mediated by ATF6, the best-known member of a transmembrane protein

family that encodes membrane anchored bZIP transcription factors. Upon ER-stress, ATF6 translocates to the Golgi where it is processed by site 1 and site 2 proteases that sequentially remove the luminal domain and the transmembrane anchor. These cleavages release the transcriptionally active N-terminal fragment of ATF6 (ATF6N) which relocates to nucleus where it upregulates ER-stress induced genes related to protein folding and lipid synthesis [34,42–44].

5. ER-stress and ER-signaling regulation of inflammation and immune responses

Beyond the adaptation phase that restore ER homeostasis, ER-stress as well as the activation of ER-signaling pathways can impact on host immune functions and promote inflammation [9,45]. ER-stress observed in inflammatory pathologies determine the size, nature and duration of immune response [1,3,46].

Several studies have showed that ER-stress might augment inflammation. Mutations in mice that produce defects in protein folding or in the ER-stress response pathways lead to spontaneous inflammation. Mice with a mutation impairing the folding of MUC2, a mucin expressed in the Paneth and goblet cells, show signs of ER-stress in the intestine and develop an inflammatory phenotype similar to the inflammation observed in inflammatory bowel disease (IBD) patients [4,47]. Patients that carry misfolding mutations in the surfactant protein-C (SFTPC) gene are characterized by ER-stress activation in epithelial cells and develop hyper-inflammatory responses upon viral infections [6,48]. Viruses are believed to contribute to the onset of idiopathic pulmonary fibrosis (IPF) in these patients. However the exact contribution of the infection in the induction of ER-stress or the contribution of ER-stress to the upregulation of inflammation-mediated lung fibrosis in these patients are still open questions. Similarly mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), an ion transporter, may cause ER-stress in bronchial epithelia cells that become hyper-responsive and more susceptible to respiratory infection. Hyper-responses to inflammatory stimuli have also been shown in macrophages. Studies showed that HLA-B27 misfolding can induce ER-stress responses in macrophages resulting in enhanced cytokine production and increased release of type I interferon, upon stimulation with the TLR4 agonist LPS. HLA-B27 misfolding is associated with susceptibility to spondyloarthritides, a group of heterogeneous autoinflammatory diseases [8,49]. In an other autoinflammatory disease, ER-retention of the TNF receptor (TNFR1) was shown to contribute to increased cytokines production observed in TNFR1-associated periodic syndrome (TRAPS) patients [10], suggesting that perturbations in the ER could be directly linked to the development of autoinflammatory syndromes. Most autoinflammatory diseases have been associated with aberrant inflammasome activation [12]. The inflammasome is a cytosolic multiprotein complex that senses pathogens and cellular damage and leads to the activation of a central proteolytic enzyme, caspase-1. The main function of the inflammasome is the cleavage and maturation of a few key cytokines, including IL-1β. These cytokines drive the initiation of inflammatory cascades in patients. Because the inflammasome has been shown to become activated upon ER-stress [14], it is possible that loss of ER-homeostasis could directly drive IL-1β dependent inflammation and contribute to the pathogenesis of diseases including autoinflammatory syndromes such as TRAPS, by directly driving inflammasome activation.

ER-stress is also involved in regulating another important branch of the innate immune system: The Toll-like receptors (TLRs) response. TLRs are type I transmembrane proteins characterized by an extracellular leucine-rich domain and a cytoplasmic domain harboring a TIR domain similar to the cytoplasmic domain of the mammalian interleukin 1 receptor (IL-1R). TLRs activation upon recognition of "Pathogen-Associated Molecular Patterns" or PAMPs, results in initiation of cellular signaling events including activation of transcription factors, cytokine modulation, and upregulation of interferon-stimulated genes, leading to inflammatory responses and the release of antimicrobial molecules. Regulatory loops connecting TLRs and ER-signaling have been described. For example, TLR4 and TLR2 promote the mRNA maturation of XBP1s by selectively triggering the IRE1 branch of the ER-stress response while inhibiting the other branches of the ER-stress pathway [16,18]. Therefore the activation of IRE1 and XBP1s by TLRs does not trigger a full ER-stress response but an XBP1s dependent program that synergizes with TLR signaling pathways. The induction of XBP1s amplifies TLR responses leading to increased cytokine production [16,18]. This demonstrates that specific engagement of ER-signaling pathways in absence of ER-stress can contribute to immunity. XBP1s activation in the context of ER-stress can also enhance TLRs responses. Macrophages treated with pharmacological agents disrupting ER homeostasis display a dramatically enhanced response to TLR4 and TLR2 activation [16,20]. The mechanisms by which XBP1s enhances cytokine production is still unclear but may likely involve XBP1 binding at cytokine promoters [21].

In addition to XBP1, the ER stress-induced transcription factor C/EBP homologous protein (CHOP), is involved in the upregulation of cytokines including IL-23 in the context of dendritic cells undergoing both ER stress and TLR stimulation [23]. Knockdown of CHOP in a monocytic cell line significantly reduced the synergistic effects of ER-stress and TLRs on IL-23 expression. Therefore diverse ER stress-related transcriptional pathways can affect the nature of immune responses (Fig. 3).

The ER-stress response and inflammation are interconnected through various additional mechanisms. The production of reactive oxygen species (ROS), the activation of the transcription factor nuclear factor-κB (NF-κB) and the induction of acute-phase proteins have being linked to both ER-stress and inflammatory responses [25]. Immune cells can also respond to stress signals released by other tissues. A recent report suggests that stressed cells can transmit (via a yet to be defined stress signal) ER-stress conditions to macrophages, increasing inflammatory responses [27]. Incubation of macrophages with cultured conditioned medium from ER-stressed cells promotes IRE1 activation and protein

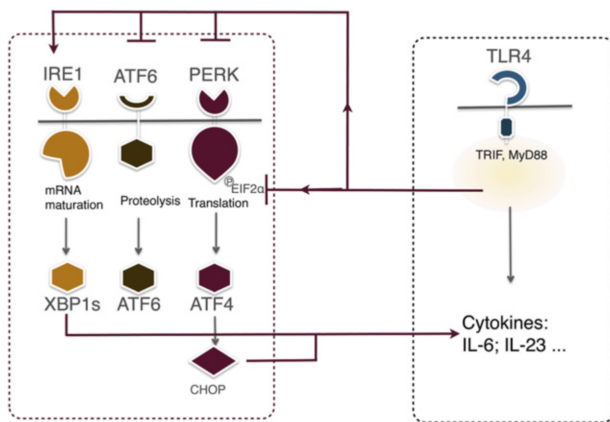

Fig. 3. TLR4 responses and ER-signaling are connected and synergize to promote cytokine production. TLR4 activate the transcription factor XBP1 by selectively activating the IRE1 kinase while decreasing the activation of ATF6 and PERK pathways as well as ElF2α activation. The activation of IRE1 and XBP1 does not contribute to an ER-stress response. However, activated XBP1 amplifies TLR signaling by enhancing cytokine production. Similarly, if CHOP and XBP1 are induced upon treatment with pharmacological agents, they both may contribute to cytokine production.

chaperones upregulation in the macrophages that deploy a more sustained proinflammatory response. Similarly injection into mice of media supernatant harvested from ER-stressed cells elicit the upregulation of genes typically associated with ER stress responses in the liver [27]. These observations may indicate that loss of ER homeostasis in tissues can be transmitted to the microenvironment and immune cells to augment immune responses [31]. Whether this phenomenon is relevant to the pathology of inflammatory diseases characterized by the loss of ER-homeostasis in tissues is an exciting question that remains to be addressed.

ER-stress pathways are also implicated in regulating immunogenicity. It has been shown that in tumor cells dying upon treatment with anticancer therapy, ER-stress promotes cell-surface localization of factors involved in immunogenic cell death [32,34]. Calreticulin is the best-characterized immunogenic signal emerging from the ER. This ER-resident chaperone delocalize on the cell surface where it can act as an “eat me” or “danger” signal that primes immune responses directed against the stressed cell [35]. The exposure of calreticulin on the cell surface is associated with the induction of ER-stress [37,39] and may directly involve the activation of ER-stress signaling pathways such as the PERK pathway [32]. Therefore ER-stress specific signal may increase immunogenicity by tagging the cell as stressed [34,34,42].

6. Induction of ER-stress and ER-signaling pathways by pathogens

Pathogens activate ER-signaling pathways such as IRE1 by directly engaging innate immune receptors as it was shown for TLRs, however live pathogens affect also directly ER functions as part of their infectious and replicative cycle [9].

In plants, the ntbZIP60 gene (a signaling branch of the plant ER-stress response related to ATF6) is activated upon infection of tobacco leaves with *Pseudomonas cichorri*. Moreover, silencing of ntbZIP60 allows higher multiplication of *P. cichorri* compared to control plants [46], suggesting that ER-stress pathways may represent an ancestral mechanism of immune regulation. In *Caenorhabditis elegans* infected with bacteria expressing pore-forming toxins, XBP1s and ATF6 are induced [47]. Genetic studies have shown that these ER-stress signaling pathways augment survival of infected worms probably by promoting a protective restoration of ER homeostasis or by increasing immune responses. Similarly in *C. elegans* infected with *Pseudomonas aeruginosa*, activation of IRE1 and XBP1 was noted [48]. In this model, XBP1 deficiency decreases viability, possibly due to a reduced ability to cope with loss of ER-homeostasis in these worms [49].

Several types of virus, parasite and bacterium that infect humans have been shown to perturb the ER and to manipulate ER-signaling pathways as a mechanism aimed at interfering with host immune responses and supporting replication. Viruses are obligate intracellular pathogens that depend on the host machinery to produce large amounts of infectious viral particles. Many viruses depend on ER-membranes for translation and budding of viral particles, a process that can result in the alteration of ER-homeostasis and therefore affect immune responses [50]. In humans, ER-stress was detected in tissues infected with different viruses. For example, XBP1 splicing and CHOP upregulation was detected in duodenal biopsies from individuals infected with HIV compared to uninfected persons [51]. ER-stress was also observed in the liver of patients with chronic hepatitis C (HCV) [52]. Parasites such as *Toxoplasma gondii* activate the ER-stress response [53]. Loss of ER-homeostasis was also observed upon infection with different bacteria. CHOP induction, EIF2α phosphorylated and IRE1 activation was observed in macrophage-rich areas of granulomas in lungs of mice infected with virulent *Mycobacterium tuberculosis* (Mtb) [54]. ER-stress activation by live bacteria can also occur prior to cell host invasion. The facultative intracellular pathogen *Listeria monocytogenes* (Lm) was found to induce ER expansion and ER-stress signaling pathways prior to host cell entry [55].

The monitoring of genes typically associated with ER-stress responses is the main feature used to identify the activation of ER-signaling pathways. However recent studies demonstrate that pathogens can trigger a specific branch of the ER-stress program independently of the other branches without engaging a full ER-stress response and upregulating genes characteristic of ER-stress responses. Influenza A virus activates the IRE1 pathway with little or no parallel activation of the PERK and the ATF6 pathways. IRE1 activation is apparently important for viral replication. Inhibition of IRE1 blocked Influenza A virus replication [56], demonstrating that the virus may manipulate the IRE1 and benefit from it. Acute infection with lymphocytic choriomeningitis virus (LCMV) was shown to trigger a selective induction of the ATF6-regulated branch of the ER-stress response, whereas PERK and IRE1 pathways are neither activated nor blocked [57].

How viruses trigger specific ER-signaling pathways is unclear. In the case of LCMV, activation of ATF6 may involve the viral glycoprotein precursor (GPC) [57], further suggesting that some viruses may directly regulate these pathways independently of the induction of ER-stress.

While many pathogens trigger ER-stress and/or features associated with the ER-stress response, the exact function of these pathways are still poorly understood. Activation of ER-stress and ER-signaling pathways may increase immunity and reduce the cellular stress associated with the infection. This is a recent hypothesis and few experimental evidences demonstrate the physiological importance of these pathways in regulating pathogen replication and immunity *in vivo*. Importantly, the key role of these pathways is also highlighted by the fact that pathogen have evolved mechanisms to manipulate ER-signaling pathways optimizing survival and replication.

7. Regulation of ER-signaling pathways by pathogens

The role of the ER-stress pathways in shaping immunity is underscored by the fact that pathogens as well as innate immunity pathways regulate ER-stress responses. It has been shown for example that the ATF4-CHOP branch of the PERK pathway is specifically inhibited by TLR signaling [58]. Activation of TRIF by TLRs activates a phosphatase, PP2A, that dephosphorylate a subunit of EIF2B (guanine nucleotide exchange factor for EIF2α) leading to the inhibition of phosphorylated EIF2α activity and downregulation of PERK mediated ATF4 and CHOP upregulation [59]. In macrophages TLR signaling has been found to inhibit ATF6 and PERK activation by a mechanism yet to be identified [16]. This regulation of ER-signaling responses by innate immunity may explain why TLRs in macrophages only activate IRE1 and in absence of concomitant activation of PERK and ATF6, therefore, favoring an inflammatory response rather than a full ER-stress response.

Viruses are also able to manipulate the ER-stress response by altering specific pathways or changing cellular homeostatic setpoints. Dengue fever virus (DENV) for example, manipulates the ER-signaling pathways to specifically activate and suppress the three different branches of the ER-stress response. Importantly each branch is regulated differently depending on timing and the infectious stage [60], suggesting that the virus can specifically manipulate the system to increase survival and prolong the viral life cycle. HCV provides us with another example of time-dependent regulation of the ER-stress response. This virus triggers the ER-stress response in a wave-like fashion, which peaks a few days after infection. Then the hepatocytes become tolerant to the stress induced by the virus or by chemical agents. Suppression of viral replication restore the ability to engage an ER-stress response suggesting that, the virus itself is can regulate the response [61]. In line with these observations, livers from patients with untreated chronic hepatitis C exhibit activation of the three ER-stress branches without apparent induction of downstream ER-related responsive genes [52]. These findings could be explained by specific dampening of the downstream response by viral effectors. It has been shown that HCV reduces XBP1 transcriptional activity [62] and that the HCV envelope protein E2 may regulate PERK activity [63]. Similar observations were reported for coronavirus mouse hepatitis virus (MHV). Infection by MHV induces IRE1-mediated splicing of *XBP1* mRNA and the maturation of ATF6, without upregulating significant downstream target genes [64]. Likewise, the influenza virus and the Enterovirus 71 (EV71) modulate the stress response in the setting of a preexisting stress by dampening the activation and processing of the ATF6 as indicated by its inhibitory effect on ATF6-dependent genes [56,65]. Parasites can also modulate ER-signaling responses. The host ATF6 pathway is targeted by the *T. gondii* virulence factor ROP18 [53]. ROP18 is a kinase that triggers proteasome-dependent degradation of ATF6 a process that apparently is required for parasite virulence.

These examples demonstrate that pathogens have evolved distinct mechanisms to regulate and benefit from ER-signaling pathways. Cells that undergo acute pressure on the ER may trigger an apoptosis program as part of a cellular response to acute dysfunction. It is therefore likely that the dampening of this response by pathogens may increase it life cycle by promoting survival of host cells. Alternatively, the pathogen may also manipulate the system to specifically control transcriptional programs triggered by the ER-stress signaling branches independently of the ER-stress response. The exact function of these programs in the context of an infection *in vivo* is unclear. The regulation of inflammatory and immune functions as well as the induction of pathogen specific transcriptional programs may represent key mechanisms orchestrated by pathogens takeover of ER-signaling pathways.

8. Conclusion

The overall message of this review is that ER-signaling pathways steaming from the ER are often tightly linked to inflammation and immunity. The ER is therefore a central stress sensor that detects cellular insults and trigger specific responses involved in restoring homeostasis as well as promoting immunity. However, there are many knowledge gaps that need to be investigated in order to have a comprehensive picture of how innate immune signaling pathways intersect with signaling branches emerging from the ER. The challenge for the future is to characterize at the molecular level the proximal pathways that emerge from the ER-stress signaling pathways and to understand how these branches are activated and regulated by pathogens, pathogen virulence factors as well as innate immune signaling component such as TLRs. ER-stress is often defined by the induction of the ER-stress response, however it is now clear that pathogen as well as inflammatory condition can operate ER-signaling pathways in absence of ER-stress. The development of new techniques and reagents to monitor the stress in the ER as well as the activation of ER-signaling branches will foster a better characterization of these pathways in immunity. Deficiencies in the main components of the ER-signaling pathways are generally lethal in mice. The development of new mouse

models with conditional deletion of specific ER-signaling pathways may therefore help assess the physiological importance of these responses during the course of infections as well as in autoimmune and autoinflammatory diseases. It will be also important to understand how ER-signaling pathways affect immune responses in cooperation with other stress responses related to the proteostasis network such as autophagy and the oxidative stress response. Finally, it is tempting to speculate that the development of drugs to alleviate ER-stress or regulate specific branches of the ER-stress response may provide new therapeutic approaches in the regulation of infectious and immune-related diseases.

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