ELSEVIER

Journal of Hepatology 46 (2007) 1075–1088

Journal of Hepatology

www.elsevier.com/locate/jhep

In vivo altered unfolded protein response and apoptosis in livers from lipopolysaccharide-challenged cirrhotic rats

Khalid A. Tazi[1,2], Ivan Bièche[3], Valérie Paradis[4,5], Cécile Guichard[6], Ingrid Laurendeau[3], Delphine Dargère[5], Agnès Legrand[7], Michèle Fay[1,2], Eric Pedruzzi[1,2], Marie-Anne Robin[1,2], Dominique Cazals-Hatem[4], Zéra Tellier[8], Dominique Bernuau[9], Gérard Feldmann[1,2], Michel Vidaud[3], Didier Lebrec[1,2], Eric Ogier-Denis[1,2,†], Richard Moreau[1,2,*,†]

[1]*INSERM, U773, Centre de Recherche Bichat-Beaujon CRB3, Paris 75018, France*
[2]*Université Denis Diderot-Paris 7, site Bichat, 75018, France*
[3]*INSERM, U745, Paris 75006, France*
[4]*Service d'Anatomie Pathologique, Hôpital Beaujon, Clichy 92118, France*
[5]*Faculté de Pharmacie, CNRS UMR 8149, Paris 75006, France*
[6]*Department of Pathology, Immunology, and Clinical Pathology, Centre Medical Universitaire, Geneva, Switzerland*
[7]*INSERM, U591, Paris 75015, France*
[8]*Laboratoire français du Fractionnement et des Biotechnologies, Courtaboeuf, France*
[9]*INSERM, U697, Paris 75010, France*

***Background/Aims*: Endoplasmic reticulum (ER)-related unfolded protein response (UPR) is mediated by PKR-like ER kinase (PERK), ATF6 and IRE1. PERK phosphorylates eukaryotic translation initiation factor-2α (eIF2α) to attenuate protein synthesis, including in NF-κB-dependent antiapoptotic proteins. We hypothesized that an altered UPR in the liver may sensitize cirrhotic livers to LPS-induced, TNFα-mediated apoptosis. Thus, we examined in vivo UPR and NF-κB activity in livers from cirrhotic and normal LPS-challenged rats.**

***Methods*: Livers were harvested in rats that did or did not receive LPS.**

***Results*: Under baseline conditions, no UPR was found in normal livers while PERK/eIF2α and ATF6 pathways were activated in cirrhotic livers. After LPS, in normal livers, the PERK/eIF2α pathway was transiently activated. ATF6 and IRE1 were activated. In cirrhotic livers, the PERK/eIF2α pathway remained elevated. ATF6 and IRE1 pathways were altered. LPS-induced, NF-κB-dependent antiapoptotic proteins increased in normal livers whereas their expression was blunted at the posttranscriptional level in cirrhotic livers.**

***Conclusions*: Cirrhotic livers exhibit partial UPR activation in the basal state and full UPR, although altered, after LPS challenge. Sustained eIF2α phosphorylation, a hallmark of cirrhotic liver UPR, is associated with a lack of LPS-induced accumulation of NF-κB-dependent antiapoptotic proteins which may sensitize cirrhotic livers to LPS/TNFα-mediated apoptosis.**

***Keywords*: Endoplasmic reticulum; PERK; ATF6; IRE1; LPS; Cirrhosis; Programmed cell death**

1. Introduction

In cells, including hepatocytes, newly synthesized secretory and membrane-associated proteins are correctly folded and assembled in the endoplasmic

Received 28 August 2006; received in revised form 10 January 2007; accepted 16 January 2007; available online 28 February 2007

* Corresponding author. Tel.: +33 (1) 40 87 55 13; fax: +33 (1) 47 30 94 40.
E-mail address: rmoreau@bichat.inserm.fr (R. Moreau).
† These authors contributed equally to this work.

0168-8278/$32.00
doi:10.1016/j.jhep.2007.01.034

reticulum (ER) [1]. Once ER homeostasis is perturbed by various pathological conditions, newly synthesized unfolded proteins accumulate in the ER, resulting in ER stress [1]. To cope with accumulated unfolded ER proteins, mammalian cells trigger a specific adaptive response called the unfolded protein response (UPR) [1]. There are three distinct signaling pathways that are induced by ER stress, mediated by three ER-resident stress sensors, i.e., the double-stranded RNA-activated protein kinase-like ER kinase (PERK) [2], the activating transcription factor 6 (ATF6) [3], and the inositol requiring enzyme (IRE) 1 [3]. PERK is a serine/threonine protein kinase that phosphorylates Ser^{21} of the α subunit of eukaryotic translation initiation factor 2 (eIF2α) [1]. Phosphorylation of eIF2α subsequently inhibits protein synthesis to prevent further influx of nascent proteins into an already saturated ER lumen [1,2,4]. Paradoxically, eIF2α phosphorylation induces translation of a basic-region leucine zipper (bZIP) transcription factor ATF4 [1] and subsequent expression of the ATF4 target genes, *growth arrest and DNA damage-inducible* (*GADD*) *34* (*GADD34*) [1,5–8] and *C/EBP homologous protein-10* (*CHOP*, another bZIP transcription factor also known as *GADD153*) [1,6–9]. The second signaling pathway is mediated by the bZIP transcription factor ATF6 [1,3,10], which is activated by a process called regulated intramembrane proteolysis [11]. ATF6 is synthesized as a precursor protein with a molecular mass of 90 kDa (p90ATF6), and anchored to the ER membrane where it is retained by BiP (also known as glucose-related protein (GRP) 78) [1,3]. In response to ER stress, ATF6 is released from BiP and transported to the Golgi complex, where ATF6 undergoes sequential cleavages by two proteases, S1P and S2P [1,10,11]. The processed form of ATF6 (p50ATF6) translocates to the nucleus and activates target genes [1]. The third signaling pathway is composed of IRE1 and XBP1 [1,3,12]. IRE1 contains both serine/threonine kinase and ribonuclease domains. Under normal conditions, only the unspliced form of *XBP1* [*XBP1*(*U*)] mRNA is translated, but its product is a weak transcriptional activator with a short protein half-life [1]. Upon ER stress, activated IRE1 cuts 26 nucleotides out from *XBP1*(*U*) mRNA to generate spliced *XBP1* [*XBP1*(*S*)] mRNA, which encodes the more stable and transcriptionally active XBP1(S) protein [1,3,12], which belongs to the family of bZIP transcription factors [1]. XBP1(S) activates target genes, including BiP [1]. XBP1 is a transcription factor essential for hepatocyte growth and homozygote XBP1-deficient embryos have massive liver apoptosis [13].

The UPR also promotes an adaptive response which involves activation of the transcription factor nuclear factor (NF)-κB [14]. However, when the adaptive responses are not sufficient to relieve the ER stress, apoptotic pathway(s) are activated [1]. Several mechanisms may contribute to ER stress-induced apoptosis, including the activation of c-Jun NH_2-terminal kinase (JNK) [15,16], the induction of CHOP [9,16,17], and/or the activation of the ER-associated caspase-12 [18].

Recently, significant increases in the expression of *XBP1(S)*, *ER degradation-enhancing α-mannosidase-like protein* (*EDEM*), *BiP* and *CHOP* mRNAs have been shown in normal mice livers harvested 24 h after the administration of lipopolysaccharide (LPS, a Gram-negative bacteria byproduct) [19]. Unlike *CHOP* mRNA, whose expression can be induced by other stress signals [20], *XBP1* splicing (i.e., IRE1 activation) is specific to the UPR [3,21]. Moreover, *EDEM* and *BiP* mRNAs are XBP1(S) target mRNAs [1,22]. Together, these findings show that the UPR is induced in vivo in the liver 24 h after LPS challenge. The administration of proinflammatory cytokines interleukin (IL) 6 (IL6) or IL1β to normal mice also triggers the hepatic UPR in vivo [19]. The proinflammatory cytokine tumor necrosis factor α (TNFα) elicits full UPR activation in murine fibrosarcoma L929 cells [23] by activating PERK, ATF6 and IRE1/XBP1. Since LPS administration stimulates the in vivo production of IL6, IL1β, and TNFα [24], these cytokines may mediate, at least in part, the induction of the UPR in the liver from LPS-challenged mice. The finding that TNFα induces the UPR in a reactive oxygen species (ROS)-dependent fashion [23] suggests that TNFα-elicited ROS may play a role in the accumulation of unfolded proteins in the ER [23].

In patients with cirrhosis, the mechanisms that cause Gram-negative bacterial infections to worsen hepatocyte function are unclear [25]. The LPS-induced increase of TNFα levels in plasma is more marked in cirrhosis than under normal conditions [26,27]. There is preliminary evidence that cirrhotic rats are abnormally sensitive to in vivo LPS-induced, TNFα-mediated hepatocyte apoptosis within the first hours after LPS challenge [28]. However, the mechanisms for this are unknown. Isolated cells in which protein synthesis is inhibited by cycloheximide or by eIF2α phosphorylation in vitro are abnormally sensitive to TNFα-mediated apoptosis [29]. Inhibition of protein synthesis is thought to favor TNFα toxicity, at least in part, by inhibiting de novo synthesis of NF-κB-dependent antiapoptotic proteins [29]. In fact, NF-κB is a major negative regulator of apoptosis [31–37], especially in response to TNFα and in vitro ER stress sensitizes cells to TNFα-induced apoptosis through downregulation of the NF-κB-dependent protein TNF receptor-associated factor 2 (TRAF2) [30]. The present in vivo study aimed to investigate the induction of UPR and NF-κB-dependent expression of antiapoptotic genes in livers from normal and cirrhotic rats with or without LPS.