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Research Report

Inhibition of inducible nitric oxide synthase and interleukin-1β expression by tunicamycin in cultured glial cells exposed to lipopolysaccharide

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ABSTRACT

Endoplasmic reticulum (ER) stress has recently been implicated in human diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). However, the link between the immune system, ER stress, and the development of neurodegenerative diseases has not yet been clarified in detail. Mouse primary cultured astrocytes were treated with lipopolysaccharide (LPS) and/or tunicamycin (Tm), and inducible nitric oxide synthase (iNOS) and interleukin (IL)-1β levels were then measured using RT-PCR, ELISA, and Western blotting. Activation of the immune system by LPS triggered inflammatory responses in astrocytes, as measured by the induction of iNOS and IL-1β. Tm-induced ER stress inhibited the LPS-induced expression of IL-1β and iNOS at the protein level. On the other hand, ER stress alone did not induce the expression of IL-1β or iNOS. The inhibitory effect of ER stress on iNOS and IL-1β may not be mediated transcriptionally as we did not observe inhibition at the mRNA level. LPS-induced iNOS protein levels were attenuated by the Tm post-treatment in the absence of LPS. Overall, these results suggest that ER stress negatively regulates the expression of IL-1β and iNOS in LPS-activated astrocytes.

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1. Introduction

Brain inflammation is known to be involved in neurodegenerative diseases. Glial cells such as microglia and astrocytes play critical roles in regulating immune reactions in the central nervous system (CNS). The accumulation of Aβ has previously been reported to stimulate immune responses by releasing cytokines such as tumor necrosis factor-α (TNF-α) and IL1-β, or enzymes such as iNOS from microglia and astrocytes in the AD brain (Weiner and Selkoe, 2002; Dorheim et al., 1994; Akama and Van Eldik, 2000). This immune activation may contribute to neurotoxicity. For example, iNOS has been shown to stimulate dopaminergic neurodegeneration in the PD brain (Liberatore et al., 1999).

ER is an organelle that is involved in accelerating protein folding. However, when cells are exposed to stress conditions, which perturb ER function, unfolded proteins accumulate, resulting in ER stress. Cells activate unfolded protein responses (UPR) to cope with ER stress conditions. These UPR include (1) the IRE1-mediated induction of X-box binding protein 1 (XBP-1) splicing and subsequent induction of glucose-regulated protein 78 (GRP78), and (2) the protein kinase RNA-like endoplasmic reticulum kinase (PERK)-mediated phosphorylation of eIF2α and subsequent induction of the C/EBP homologous protein (CHOP) (Walter and Ron, 2011; Hosoi and Ozawa 2010). Evidence to suggest that ER stress is involved in the pathogenesis of neurodegenerative diseases is increasing (Lindholm et al., 2006; Hosoi and Ozawa, 2010, 2012). UPR were shown to be activated in the AD brain (Hoozemans et al., 2005), and the presenilin-1 mutation in the AD brain has been associated with ER stress (Katayama et al., 1999). UPR were also activated in a cellular model of PD (Ryu et al., 2002) as well as in the dopaminergic neurons of the substantia nigra in the PD brain (Hoozemans et al., 2007). These findings suggest a key role for ER stress in the development of neurodegenerative diseases. Although the involvement of immune function as well as ER stress in neurodegenerative diseases has been reported, the link between these two phenomena has yet to be clarified in detail. In the present study, we demonstrated that ER stress may attenuate the activation of immune reactions, which suggests that ER stress may be a negative regulator of inflammation in the CNS.

2. Results

2.1. ER stress attenuated LPS-induced inflammatory responses in astrocytes

As glial cells play a central role in regulating brain immune reactions, we examined mouse primary cultured astrocytes in the present study. We investigated the possible link between immune reactions and ER stress by measuring iNOS and IL-1β levels under ER stress conditions. RT-PCR analysis revealed that astrocytes expressed IRE1α, a stress sensor protein that detects the accumulation of unfolded proteins in the ER lumen (Fig. 1A). Activated IRE1 has been shown to induce XBP1 splicing, thereby inducing molecular chaperone such as GRP78

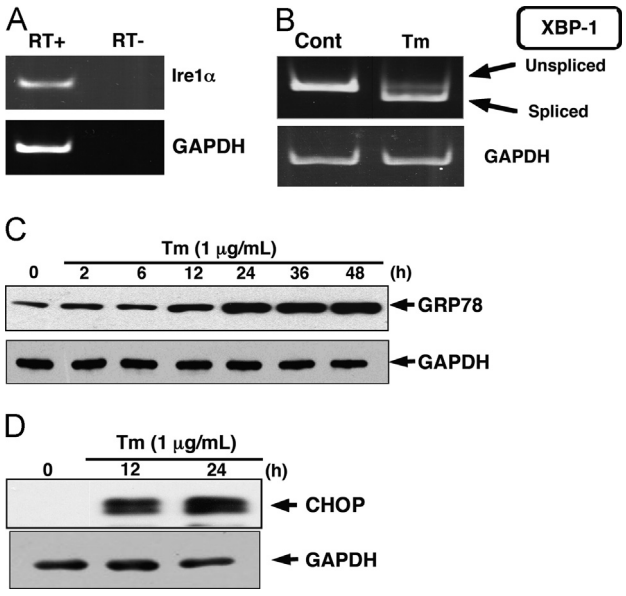

Fig. 1 – Activation of ER stress in mouse primary cultured glial cells. (A) The expression of IRE1 α in primary cultured astrocytes as assessed by RT-PCR. Note the presence of a PCR product in the presence of RT (reverse transcriptase), but not in the absence of RT, which indicated that there was no contamination by genomic DNA. (B) The induction of XBP1 splicing by Tm. Tm (6 h) increased XBP1 splicing in primary cultured astrocytes, as assessed by RT-PCR. (C) Tm induced GRP78 expression. Cells were treated with Tm (1 μg/mL) for the indicated times and the expression levels of GRP78 were analyzed by Western blotting. (D) Tm induced CHOP expression. Cells were treated with Tm (1 μg/mL) for the indicated times and the expression levels of CHOP were analyzed by Western blotting.

(Yoshida et al., 2001). Tm blocked protein N-glycosylation, which increased the accumulation of unfolded proteins and caused ER stress. The Tm treatment with led to a marked increase in XBP1 splicing and GRP78 expression in primary cultured astrocytes (Fig. 1B and C). CHOP is an apoptotic transcription factor that is induced with the accumulation of unfolded proteins in the ER (Zinszner et al., 1998). We found a marked increase in the expression of CHOP in Tm-treated astrocytes (Fig. 1D). These results indicated that ER stress was activated under Tm-treated conditions in primary cultured astrocytes.

To determine the possible role of ER stress in immune function, astrocytes were treated with Tm, followed by the assessment of iNOS and pro-IL-1β levels. Although LPS increased iNOS and pro-IL-1β levels, we did not detect the induction of iNOS or pro-IL-1β levels in Tm-treated cells at either time point examined (Fig. 2A). Therefore, we investigated the possibility that ER stress may not activate immune function, but rather inhibit it. Immune-activated astrocytes were treated with Tm and iNOS and IL-1β levels were then analyzed. LPS-induced iNOS levels were markedly attenuated by the treatment with Tm. There were no obvious cytotoxic effects of Tm under the present condition. The effects observed were dose-dependent, and were inhibited at 1 and 10 μg/mL of Tm. LPS-induced IL-1β levels were also