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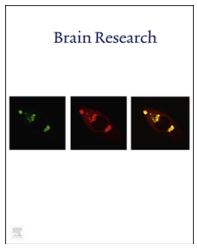

Research Report

Involvement of nitric oxide in the induction of interleukin-1 beta in microglia

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ABSTRACT

In response to in vitro stimulation with lipopolysaccharide (LPS), microglia induce the production of the inflammatory cytokine interleukin-1 beta (IL-1β) together with nitric oxide (NO) and superoxide anion (O_2^-). Here we investigated the role of NO and O_2^- in the signaling mechanism by which IL-1β is induced in microglia. The LPS-inducible IL-1β was significantly suppressed by pretreatment with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide, but not by pretreatment with the O_2^- scavenger *N*-acetyl cysteine, suggesting the close association of NO with IL-1β induction. The pretreatment of microglia with the inducible NO synthase inhibitor 1400W prior to LPS stimulation significantly reduced the production of IL-1β, and the addition of the NO donor S-nitroso-*N*-acetyl-DL-penicillamine (SNAP) into microglia led to the induction of IL-1β. These results suggested that NO induces IL-1β through a specific signaling cascade. LPS-dependent IL-1β induction was significantly suppressed by inhibitors of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and nuclear factor kappaB (NFκB), indicating that ERK/JNK and NFκB serve in the cascade of IL-1β induction. As expected, ERK/JNK and NFκB were all activated in the SNAP-stimulated microglia.

Abbreviations: ACM, astrocytic conditioned medium; AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; ALS, amyotrophic lateral sclerosis; Carboxy-PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; CBB, Coomassie brilliant blue; CREB, cAMP response element binding protein; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immuno-sorbent assay; ERK, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; Iba1, ionized Ca^{2+}-binding adapter molecule 1; IκB, inhibitor of NFκB; IL-1β, interleukin-1 beta; iNOS, inducible NO synthase; JNK, c-Jun *N*-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCM, microglial conditioned medium; NAC, *N*-acetyl cysteine; NFκB, nuclear factor kappa B; NMDA, *N*-methyl D-aspartate; NO, nitric oxide; O_2^-, superoxide anion; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PD, Parkinson's disease; PI, propidium iodide; PI3K, phosphatidyl inositol 3-kinase; proNGF, pro-nerve growth factor; RLU, relative light unit; SDS, sodium dodecyl sulfate; SNAP, S-nitroso-*N*-acetyl-DL-penicillamine; SOD, superoxide dismutase; TNFα, tumor necrosis factor alpha

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Taken together, these results indicate that NO is an important signaling molecule for the ERK/JNK and NFκB activations, which are requisite to the induction of IL-1β in microglia.

1. Introduction

Interleukin-1beta (IL-1β) is generally grouped into the proinflammatory cytokines, and is induced in the nervous system of individuals undergoing acute stress (Nguyen et al., 1998; O'Connor et al., 2003) or cerebral ischemia (Touzani et al., 1999; Adibhatla and Hatcher, 2007) or suffering from Alzheimer's disease (AD) (Griffin et al., 2006; Liu and Chan, 2014), acquired immunodeficiency syndrome (AIDS) dementia (Brabers and Nottet, 2006; Xing et al., 2009), amyotrophic lateral sclerosis (ALS) symptoms (Meissner et al., 2010), or Parkinson's disease (PD) (Leal et al., 2013). IL-1β is known as a pleiotrophic cytokine, and it contributes to various biological activities including the development of febrile seizures (Heida et al., 2009; Saghazadeh et al., 2014), the regulation of glucose concentration (Besedovsky and del Rey, 2010), the pain states (Ren and Torres, 2009; Sim et al., 2013), the regulation of blood–brain barrier permeability (Argaw et al., 2006; Wang et al., 2014), the promotion of remyelination (Mason et al., 2001) and regeneration (Temporin et al., 2008), and the formation of aggressive behavior (Pesce et al., 2011).

IL-1β also has various deleterious effects, such as the promotion of *N*-methyl D-aspartate (NMDA)-evoked cell death (Ma et al., 2002–2003; Ye et al., 2013), injury-dependent hippocampal neuronal cell death (Lu et al., 2005), glia-triggered dopaminergic cell death (Long-Smith et al., 2010), and pro-nerve growth factor (proNGF)-mediated neuronal cell death in the CA1 hippocampus (Choi and Friedman, 2014). These actions of IL-1β have been implicated in the progression of brain diseases such as AD, AIDS dementia, ALS and PD, as noted above.

Among the various cell types in the nervous system parenchyma, microglia have been recognized as a key cell type with significant effects on pathological states by inducing IL-1β (Yates et al., 2000; Lull and Block, 2010; Lambertsen et al., 2012). The production of IL-1β in microglia in vitro is known to be upregulated by certain molecules, including endotoxin and amyloid β (Meda et al., 1999; Wu et al., 2013; Peng et al., 2014). Microglia-derived IL-1β has thus been proposed as a therapeutic target in many brain disorders (Basu et al., 2004; Gabay et al., 2010; Lambertsen et al., 2012; Liu and Chan, 2014). However, the molecular mechanism by which IL-1β is induced in the microglia has not been sufficiently analyzed. In particular, the signaling molecules and related transduction cascade associated with IL-1β induction have not been accurately defined.

Previously, we observed that superoxide anion (O_2^-) and nitric oxide (NO) were produced along with the induction of tumor necrosis factor alpha (TNFα) in lipopolysaccharide (LPS)-stimulated microglia (Yoshino et al., 2011), and our findings clarified that the O_2^- produced in microglia is closely associated with the induction of TNFα. In the present study, we report that NO produced in the microglia by LPS stimulation or by an NO donor contributes to the induction of IL-1β through the activation of specific signaling cascades.

Fig. 1 – Induction of IL-1β concomitant with NO and O_2^- in LPS-stimulated microglia. (A) Microglia and astrocytes in vitro. Microglia (Micro) at 1 day after the recovery from the primary culture and astrocytes (Ast) 3 days after the subculture were immunoblotted for GFAP, Iba1 and actin, as described in Section 4 (B) Production of NGF and/or IL-1β in astrocytes and microglia. Two astrocytic cultures (1.5×10^6 cells/dish; Ast) and two microglial cultures (1.5×10^6 cells/dish; Micro) were prepared, and each dish was stimulated with LPS (0.5 μg/mL). The two LPS-stimulated dishes (+LPS) and two non-stimulated dishes (−) were maintained for 24 h. The medium from each dish was then recovered and subjected to immunoblotting for NGF and IL-1β as described in Section 4. (C) Protein profile of ACM and MCM. Each CM sample shown in panel B was transblotted, and the Immobilon membrane was stained with CBB. (D) Time course of IL-1β production in microglia. Microglia were stimulated with LPS (0.5 μg/mL), and at 0, 3, 6, 12 and 24 h the microglial conditioned medium (MCM) was recovered and analyzed for IL-1β by immunoblotting. A typical result is shown. (E) Protein profile of MCM. Each MCM sample in panel D was transblotted, and the Immobilon membrane was stained with CBB. (F) Quantification of IL-1β in MCM. The amounts of the IL-1β in MCMs (D) were measured by ELISA as described in Section 4. The results are the mean ± SD of three independent experiments. Differences between the MCM (0 h) and the MCMs (3, 6, 12 and 24 h) were assessed by Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$. (G) NO production in LPS-stimulated microglia. Microglia on a 12-well plate were stimulated with LPS (0.5 μg/mL), and at 0, 6, 12 and 24 h the MCM was recovered and analyzed for NO using an NO_2/NO_3 assay kit as described in Section 4. The results are the mean ± SD of three independent experiments. Differences between the control (0 h) and the stimulated groups (6, 12 and 24 h) were assessed by Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$. (H) Production of O_2^- in LPS-stimulated microglia. Microglia on a 12-well plate were stimulated with LPS (0.5 μg/mL), and at 0, 6, 12 and 24 h the O_2^- in each well was measured by an O_2^- assay kit as described in Section 4. Ten units of SOD was added to one of the microglial wells after stimulation with LPS (0.5 μg/mL) for 24 h, and then the O_2^- level was determined (+SOD). The results are the mean ± SD from three independent experiments. Differences between the control (0 h) and the stimulated groups (6, 12 and 24 h) were assessed by Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$.