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Research Report

Nonparticipation of nuclear factor kappa B (NFκB) in the signaling cascade of c-Jun N-terminal kinase (JNK)- and p38 mitogen-activated protein kinase (p38MAPK)-dependent tumor necrosis factor alpha (TNFα) induction in lipopolysaccharide (LPS)-stimulated microglia

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ABSTRACT

The molecular mechanism of cytotoxic cytokine tumor necrosis factor α (TNFα) induction in microglia remains to be clarified. We have previously reported that p38 mitogen-activated protein kinase (p38MAPK) is an important signaling molecule for the induction of TNFα in lipopolysaccharide (LPS)-stimulated microglia. Recently, we have shown that c-Jun N-terminal kinase (JNK) is associated with the induction of TNFα. Furthermore, using an NFκB inhibitor (SN50), we discovered that activation of nuclear factor κB (NFκB) may also be linked to TNFα induction. We therefore examined the relationship between NFκB and the two MAPKs (p38MAPK and JNK) in the signaling cascade of TNFα induction in LPS-stimulated microglia. NFκB inhibitor SN50 decreased the induction of TNFα under the suppressed NFκB activation. However, SN50 was found to prevent the activation of MKK3/6-p38MAPK and MKK4-JNK pathways. On the other hand, the other NFκB inhibitor ammonium pyrrolidine dithiocarbamate (APDC) neither prevented the activation of p38MAPK and JNK nor inhibited TNFα induction in LPS-stimulated microglia, although it was confirmed to serve as an NFκB inhibitor. These results suggest that both MKK3/6-p38MAPK and MKK4-JNK pathways are important signaling cascades leading to the induction of TNFα in LPS-stimulated microglia, but that NFκB itself is not required for this induction.

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Abbreviations:
TNFα, tumor necrosis factor alpha
CNS, central nervous system
PNS, peripheral nervous system
MAPK, mitogen-activated protein kinase
JNK, c-Jun N-terminal kinase
LPS, lipopolysaccharide
NFκB, nuclear factor kappa B
ERK, extracellular signal-regulated kinase
APDC, ammonium pyrrolidine dithiocarbamate
IκB, inhibitor of NFκB
MKK, MAPK kinase
HRP, horseradish peroxidase
FITC, fluorescent isothiocyanate
DMEM, Dulbecco's modified Eagle medium
Iba1, ionized calcium binding adapter molecule 1
PAGE, polyacrylamide gel electrophoresis
PBS, phosphate-buffered saline
IKK, IκB kinase
IL-1β, interleukin 1beta
VIP, vasoactive intestinal peptide
HIV-1, human immunodeficiency virus-1
MLK, mixed lineage kinase
ASK1, apoptosis signal-regulated kinase 1

1. Introduction

Microglia are believed to produce a variety of deleterious molecules, including hazardous cytokines, reactive oxygen radicals, and neurotoxins, all of which greatly affect the pathological and/or regenerative state of the brain (Banati et al., 1993; Kreutzberg, 1996; Nakajima et al., 2003). Tumor necrosis factor alpha (TNFα) is a representative inflammatory and harmful cytokine that induces the cell death of oligodendrocytes (Selmaj and Raine, 1988) and some neurons (Venters et al., 2000; Zassler et al., 2003) in the central nervous system (CNS) as well as motoneurons (Sedel et al., 2004) in the peripheral nervous system (PNS). On the other hand, proliferative effects on oligodendrocyte progenitors (Arnett et al., 2001) have been reported, as have neurosupportive effects (Cheng et al., 1994; Liu et al., 1999). Furthermore, this cytokine is known to exhibit a variety of effects on CNS cells as a pleiotrophic factor in the brain, modulating neural progenitor cells (Wu et al., 2000) and neuronal function (Cunningham et al., 1996; Pan et al., 1997), stimulating angiogenesis (Leibovich et al., 1987), glial proliferation (Barna et al., 1990), and glial activation (Aloisi et al., 1992; Merrill, 1992; Munoz-Fernandez and Fresno, 1998; Panek et al., 1994; Romero et al., 1996), and regulating microglial phagocytosis (von Zahn et al., 1997). Activated microglia are plausible candidates for the specific cell types that express or produce TNFα in the pathological brain (Banati et al., 1993; Benveniste, 1997; Gregersen et al., 2000; Hofman et al., 1989; Medana et al., 1997; Perry et al., 1993). However, the precise details of the signaling cascade involved in TNFα induction/suppression in microglia have not yet been established. It is therefore necessary to study the molecular mechanism by which TNFα is induced or suppressed in microglia. Of the three mitogen-activated protein kinases (MAPKs) (Kyosseva, 2004), p38MAPK has been recognized as a major MAPK in the signaling cascade of TNFα induction in lipopolysaccharide (LPS)-stimulated microglia (Nakajima et al., 2004). Recently, c-Jun N-terminal kinase (JNK) has been added to the MAPKs responsible for the induction of TNFα, as reported by Waetzig et al. (2005). In such LPS-stimulated microglia, nuclear factor kappa B (NFκB) has also been shown to be activated (Nakajima et al., 2002), and LPS-dependent TNFα induction appears to be prevented by treatment with an NFκB inhibitor (SN50), suggesting the role of NFκB in the induction of TNFα.

Thus, two MAPKs (JNK and p38MAPK) and NFκB have been highlighted as the important signaling molecules for the induction of TNFα in LPS-stimulated microglia. In the present study, therefore, we examine whether or not NFκB activation is associated with the JNK and p38MAPK activation cascade leading to TNFα induction in LPS-stimulated microglia, and whether or not NFκB activation is required for the induction of TNFα in LPS-stimulated microglia.