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IL-8 enhancement of amyloid-beta ($A\beta_{1\text{-}42}$)-induced expression and production of pro-inflammatory cytokines and COX-2 in cultured human microglia

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Abstract

The effects of the chemokine IL-8 on amyloid beta peptide ($A\beta_{1\text{-}42}$)-induced responses in cultured human microglia were investigated using RT–PCR, ELISA and immunocytochemistry. $A\beta_{1\text{-}42}$ (5 μM) applied for 8 h induced the expression and increased the production of the pro-inflammatory cytokines IL-6, IL-1β, TNF-α, the inducible enzyme COX-2 and chemokine IL-8. Microglial treatment with IL-8 added (at 100 ng/mL) with $A\beta_{1\text{-}42}$ led to enhancement in both expression and production of all of these pro-inflammatory factors compared with peptide alone. Stimulation with IL-8 itself was effective in increasing microglial expression of pro-inflammatory cytokines, IL-8 and COX-2, however, had no effect on protein levels of all these factors. The expression of the anti-inflammatory cytokines IL-10 and $TGF\beta_1$ remained unchanged from basal levels with stimulation using either $A\beta_{1\text{-}42}$, IL-8 or the peptide together with IL-8. The actions of IL-8 to potentiate $A\beta_{1\text{-}42}$-induced inflammatory mediators may have particular relevance to Alzheimer disease brain which exhibits elevated levels of the chemokine.

Keywords: Human microglia; Amyloid beta; Interleukin-8; Brain inflammation; Cytokine; Cyclooxygenase-2

1. Introduction

Microglia are resident immune cells of the brain which become activated in neurological disorders, such as Alzheimer's disease (AD). The progressive pathology associated with AD may be a consequence of local inflammatory reactions, and results from clinical studies have indicated that prolonged treatment with anti-inflammatory drugs can reduce the incidence of dementia (Rogers et al., 1993; In t' Veld et al., 2001). Amyloid beta (Aβ) peptide is the main component of senile plaques found in AD brain, and high numbers of reactive microglia are associated with plaques (Frautschy et al., 1998). It has been proposed that inefficient phagocytosis of peptide by microglia could lead to hyper-activation of cells and release of inflammatory mediators and neurotoxic factors, thereby contributing to neurodegenerative processes (Akiyama et al., 2000). Stimulatory actions of Aβ on microglia include cellular secretion of the pro-inflammatory cytokines, respiratory burst activity, increased phagocytosis and chemotaxis (Davis et al., 1992; McDonald et al., 1997; Murphy et al., 1998; Meda et al., 1999), as well as release of an unidentified neurotoxin (Giulian et al., 1995).

The pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and the inducible enzyme cyclooxygenase-2 (COX-2) play important roles in modulating inflammatory responses (Benveniste, 1992; O'Neill and Ford-Hutchinson, 1993), and increased levels of these factors have been observed in AD brain (Griffin et al., 1998; Pasinetti and Aisen, 1998; Ho et al., 1999). Microglia are a major source of pro-inflammatory cytokines (Griffin et al., 1998) with TNF-α

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(Giulian et al., 1993) and IL-1β (Griffin and Mrak, 2002) directly implicated in neurodegenerative processes. Selective inhibition of COX-2 was found to prevent glial activation and neuronal cell death in an animal model of AD (Giovannini et al., 2002).

Another factor which plays an important role in inflammation is the chemokine IL-8 (Harada et al., 1994). IL-8 is an autocrine agent for microglia inducing chemotaxis of cells to sites of injury (Cross and Woodroofe, 1999) and is also released by microglia (Ehrlich et al., 1998). Microglial production of IL-8 with Aβ as a stimulus has been reported (Nagai et al., 2001). Elevated levels of IL-8 (Galimberti et al., 2003) and IL-8 receptors (Xia et al., 1997) have been detected in AD brain. In a detailed analysis of gene expression profiling of $A\beta_{1\text{-}42}$-stimulated postmortem adult human microglia isolated from nondemented individuals, IL-8 was observed to be strongly up-regulated (Walker et al., 2001). These results suggest that IL-8 may play a role in the network of inflammatory responses which contribute to the pathogenesis of AD.

In the present study, we have investigated the effects of IL-8 on $A\beta_{1\text{-}42}$-induced expression of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, the inflammatory enzyme COX-2 and the anti-inflammatory cytokines IL-10, $TGF\beta_1$. In addition, in cases where expression was altered, we then examined IL-8 as a modulator of $A\beta_{1\text{-}42}$ actions to alter production of the agents.

2. Materials and methods

2.1. Isolation of human fetal microglia

The procedures for the isolation of human fetal microglia have been reported previously (Satoh et al., 1995). Use of embryonic human tissue was approved by the Clinical Screening Committee for Human Subjects of the University of British Columbia. Briefly, embryonic brain tissues 12–18 weeks gestation were incubated in phosphate-buffered saline (PBS) containing 0.25% trypsin and DNase (40 μg/mL) for 30 min at 37 °C and subsequently dissociated into single cells by gentle pipetting. After 7–10 days of growth in culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 5% horse serum, 5 mg/mL glucose, 20 μg/mL gentamicin and 2.5 μg/mL amphotericin B, freely floating microglia were collected from a medium of mixed cell cultures and plated into 12-well culture dishes for use in RT–PCR and ELISA assays and on Aclar coverslips for COX-2 immunocytochemistry. CD11b was used to confirm the purity of microglial cultures which was in excess of 98%.

2.2. Semiquantitative reverse transcriptase–polymerase chain reaction (RT–PCR)

Human fetal microglia were seeded into poly-L-lysine-coated 12-well plates at a density of 1×10^4 cells/well. Following preincubation in serum-free conditions for 48 h, human microglia were treated for 8 h with stimuli. Total RNA was isolated using TRIzol (GIBCO-BRL, Gathersburg, MD) subjected to DNase treatment and then processed for the first strand complementary DNA (cDNA) synthesis using Moloney murine leukemia virus (M-MLV) reverse transcriptase (GIBCO-BRL). cDNA products were then amplified by PCR using a GeneAmp thermal cycler (Applied Biosystems, Foster City, CA). Specific sense and antisense primers with the expected product size were: COX-2 sense 5′-TTCAAATGAGATTGTGGGAAAATTGCT-3′, COX-2 antisense 5′-AGATCATCTCTGCCTGAGTATCTT-3′ (305 bp); IL-1β sense 5′-AAAAGCTTGGTGATGTCTGG-3′, IL-1β antisense 5′-TTTCAACACGCAGGACAGG-3′ (179 bp); IL-6 sense 5′-GTGTGAAAGCAGCAAAGAGGC-3′, IL-6 antisense 5′-CTGGAGGTACTCTAGGTATAC-3′ (159 bp); IL-8 sense 5′-ATGACTTCCAAGCT-GGCCGTG-3′, IL-8 antisense 5′-TATGAATTCTCAGCCCTCTTCAAAA-3′ (301 bp); IL-10 sense 5′-AGATCTCCGAGATGCCTTCAGCAGA-3′ IL-10 antisense 5′-CCTTGATGTCTGGGTCTTGGTTCTC-3′ (194 bp); TNF-α sense 5′-CAAAGTAGACCTGCCCAGAC-3′ TNF-α antisense 5′-GACCTCTCTCTAATCAGCCC-3′ (490 bp); $TGF\beta_1$ sense 5′-TTGCAGTGTGTTATCCGTGCTGTC-3′, $TGF\beta_1$ antisense 5′-CAGAAATACAGCAACAATTCCTGG-3′ (185 bp); G3PDH sense 5′-CCATGTTCGTCATGGGTGTGAACCA-3′, G3PDH antisense 5′-GCCAGTAGAGGCAGGGATGATGTTC-3′ (251 bp). PCR consisted of an initial denaturation step of 95 °C for 6 min followed by a 30- to 40-cycle amplification program consisting of denaturation at 95 °C for 35 s, annealing at 55–59 °C for 1 min and elongation at 72 °C for 1 min. A final extension was carried out at 72 °C for 10 min. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a reaction standard. The amplified PCR products were identified by electrophoresis using 1.5% agarose gels containing ethidium bromide and then visualized under UV light. The intensities of each band were measured by densitometry using the NIH Image J 1.24 software (National Institutes of Health, Bethesda, MD, USA) as has been outlined previously (Heneka et al., 2003). The band intensities of PCR products in control and with stimuli were measured from n=5 independent experiments and expressed as relative mRNA levels (mRNA values normalized to G3PDH).

2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISAs were carried out according to manufacturer's instructions (R&D Systems, Minneapolis, MN, USA) and were able to detect low levels of cytokines (as low as 4.4 pg/mL of TNF-α, 1 pg/mL IL-1β, 0.7 pg/mL IL-6 and 10 pg/mL IL-8). Cells (approximately 1×10^5 cells/well) were placed in serum-free conditions for 48 h followed by incubation with stimuli for 24 h. Under serum-free conditions, levels of IL-6 were undetectable. Therefore, to generate a measurable level of basal IL-6 production, cells