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Cytokine expression profiles in the brain of non-demented control patients with increasing Alzheimer's disease pathology, in comparison with normal control and Alzheimer's disease patients

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Abstract

Evidence of the critical importance of inflammatory mediators in Alzheimer's disease (AD) pathology has been provided by basic, clinical and epidemiological studies. These studies have indicated that nonsteroidal anti-inflammatory drugs (NSAIDs) delay the onset and slow the progression of AD. Herein we examined what inflammatory mediators are involved in AD pathology and how early they begin to be linked to the development of AD pathology before clinical manifestation of overt dementia. To address this issue, we analyzed cytokine expression profiles of the brain, with focus on nondemented control patients with increasing AD pathology, referred to as high pathology control (HPC) cases. If some cytokines are more primarily involved in HPC cases, which provide an intermediate subset between AD and normal control cases, the target of inflammatory mediators implicated more in the progress of AD could be pinpointed for the development of AD therapies. With semi-quantitative analysis of cytokine mRNA, we found that interleukin-1 (IL-1) β and IL-1 receptor antagonist showed somewhat of an increment in the HPC; IL-6, IL-10 and tumor necrosis factor (TNF) α, expressed under normal conditions, had a tendency to decrease in the HPC, leading to the assumption that temporal expression of the IL-1 family and the decline in IL-6, IL-10 and TNFα expression play key roles in the manifestation of overt dementia with AD progression. *Tottori J. Clin. Res.* 1(1), 152-168, 2008

Key words : cytokines, Alzheimer's disease, neuroinflammation, dementia

Introduction

The pathogenesis of AD has typically not been explained by alterations in immune function. Several studies have shown the importance of T cell and macrophage secretory products, and provided epidemiological evidence detailing the critical importance of inflammatory mediators in AD[1)]. Direct and tangential evidence of a neurodegenerative role for the inflammatory process in the AD brain has been provided from basic research. Clinical research has widely suggested that conventional nonsteroidal anti-inflammatory drugs (NSAIDs) may delay the onset and slow the progression of AD. The hallmarks of AD, amyloid β peptide (Aβ), neurofibrillary tangles (NFT), and neuronal degeneration, are the most likely sources for inflammation in the AD brain[2)]. Actually, β-plated, fibrillar Aβ and tau-containing NFT have been shown to directly activate the classical complement pathway fully in vitro, and to do so even in the absence of antibodies[3,4)]. A variety of molecules,

defined as key mediators in peripheral immune reactions, have been found to occur in high concentration in the AD brain[2].

Thus, neuroinflammation clearly occurs in pathologically vulnerable regions of the AD brain, and it generally seems to be accepted that AD neuroinflammation significantly contributes to AD pathogenesis[2, 5]. However, many issues regarding this contribution require clarification. Do inflammatory mechanisms actually cause damage in the AD brain? Are they more primary AD pathologic processes that occur before neuronal damage becomes significant? Is neuroinflammation always a secondary response to the hallmarks of AD, occurring in the later symptomatic stages of AD[6]? In fact, HPC patients without a history of dementia but who nonetheless exhibit sufficient neocortex Aβ deposits and entorhinal cortex NFT at autopsy to otherwise qualify for the diagnosis of AD show elevations of inflammatory markers, although only modest and partial, that are significantly greater than in nondemented elderly (ND) patients but dramatically less than in AD patients[7-9]. This matter is particularly important because if inflammatory mediators begin to change in the latent phase of AD, anti-inflammatory therapy may interrupt or slow the pathological progression of latent AD and thereby delay or even prevent the onset of dementia[6]. In addition, investigations of what inflammatory or immune mechanisms, or mediators are earlier or more significantly involved in the developmental process of AD pathology could be expected to lead to the development of therapeutic strategies to pinpoint the inflammatory mechanisms involved in AD pathological progression.

In the present study, we examined what inflammatory mediators are involved in AD pathology and how early they begin to be linked to the development of AD pathology before clinical manifestation of overt dementia. To address this issue, we analyzed cytokine expression profiles of the brain, with focus on HPC cases. HPC cases provide an intermediate subset between AD and a low pathology control[7-9]. Low pathology control (LPC) cases, also referred to as ND patients, have limited AD pathology and no prior medical history of dementia.

Materials and methods

Patient samples

From among the 100 routine brain autopsies per year of patients who had prospectively enrolled in the Sun Health Research Institute Tissue Donation Program and given premortem consent, four to six typical cases were selected from each of the early and late stages of AD, HPC and LPC patients, based on prior medical records with antemortem neuropsychological test scores, including data of the Folstein Mini Mental Status Examination (MMSE) and the Clinical Dementia Rating Scale (CDR), and postmortem neuropathological records with the Consortium to establish a registry for AD (CERAD) pathological criteria[10] and the Braak staging[11]. In addition, our study was approved by the ethical committees of both the Shiga University of Medical Science and the National Hospital Organization Tottori Medical Center.

Tissue preparation

Brain tissue sections, which had originally been prepared for immunohistochemical procedures, were used for the present study. Briefly, brain tissue was removed within four hours of death, sectioned coronally at 1 cm intervals, fixed for 48 hours in ice-cold 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4), cut at 40 μm on a freezing microtome, cyroprotected for 36-48 hours in a 0.1 M phosphate buffer (pH 7.4) containing 2% dimethylsulfoxide (DMSO) and 10% glycerol, and stored in a 20 mM phosphate buffer (pH 7.4) containing 30% ethylene glycol and 30% glycerol at -20°C until use, as described elsewhere in detail[8].

Extraction of total RNA

Three sections from the temporal cortex of each case were incubated for one hour at 45°C with 200 μl of a homogenate buffer consisting of 3.5 mg/ml of proteinase K (Wako Pure Chemicals), 2% sodium