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Protein and gene expression of tumour necrosis factor receptors I and II and their promoter gene polymorphisms in Alzheimer's disease

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Abstract

The levels of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), are increased in the brain in Alzheimer's disease (AD). Most of the biological properties of TNF-α are mediated through its two receptors, tumour necrosis factor receptors I and II (TNF-RI and TNF-RII). We have used immunohistochemistry, Western blotting and real time-PCR (RT-PCR) on frontal (BA 6/24) and temporal (BA 20–22) neocortex and hippocampus from AD and control brains to determine if both receptor proteins were present and expressed in AD and if sequence variations (SNPs) in the promoter regions of the two genes are associated with AD. Expression of TNF-RI exceeded that of TNF-RII in AD and control brains at protein and mRNA levels. The level of TNF-RI protein varied considerably in individual brains but not between AD and control brains. None of the identified TNF-RI and -RII SNPs in the promoter regions of the genes was linked with AD. Our findings suggest that TNF-RI and -RII promoter gene polymorphisms and variations in protein and gene expression of these receptors are unlikely to play a major role in the development of AD.

Keywords: Genetics; SNP's; Immunohistochemistry; TRAF-2

1. Introduction

Alzheimer's disease (AD) is the most common human neurodegenerative disorder. It is characterized clinically by progressive loss of memory and cognitive function, and pathologically by plaques of amyloid β-protein (Aβ), neurofibrillary tangles (consisting of abnormally phosphorylated tau protein), degeneration of synapses and loss of neurons.

Although the cause of the loss of neurons is not completely understood, inflammatory mediators, such as cytokines, are thought to play a role. Multiple cytokines are produced in response to Aβ-dependent activation of microglia and are up-regulated in the brain in AD (McGeer and McGeer, 2002). TNF-α is one of the main pro-inflammatory cytokines and its role in the pathogenesis of AD has been the subject of a number of studies (Perry et al., 2001). TNF-α has been reported to influence the expression or metabolism of several molecules that are implicated in the development of AD, including Aβ, apolipoprotein E and α2-macroglobulin (Perry et al., 2001). Most of the biological properties of TNF-α are mediated through its two receptors, TNF-RI (CD120a) and TNF-RII (CD120b). Many cell types co-express TNF-RI and TNF-RII and require cooperation between the 2 receptors to generate a response to TNF-α (Chan Francis Ka-Ming, 2000).

The TNF-R signalling pathways involve complex interactions between several proteins and can lead to either cell survival or cell death. For neurons, this may depend on the particular subtype of TNF-R that is predominantly expressed (Yang et al., 2002). The main difference between the 2 receptors is the presence of an intracellular 'death

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domain' (DD) in TNF-RI but not TNF-RII. *In vitro*, the DD was shown to contribute to neuronal cell death when the TNF-RI signalling pathway was activated whereas activation of the TNF-RII signalling pathway was neuroprotective (Shen et al., 1997). However, binding to TNF-RI can cause recruitment and activation of two alternate intracellular signalling complexes, with opposite outcomes. The first (complex 1) is bound to the plasma membrane and consists of TNF-RI, TRADD (TNF-R-associated DD), RIP1 (receptor interacting protein 1) and TRAF-2 (TNFR-associated factor 2); this complex mediates rapid activation of NF-κB and JNK, leading to cell survival. If complex 1 is not activated, a second complex is formed – complex 2, comprising TRADD, RIP1, FADD (Fas-associated DD) and caspase 8 – which activates downstream caspases, leading to apoptosis (Micheau and Tschopp, 2003).

The reasons why the complex 1 sometimes fails to be activated are not known. Chan Francis Ka-Ming (2000) reported that TNF-RII plays a role in determining TNF-RI-mediated cellular responses and potentiates TNF-RI-induced cell death (Chan Francis Ka-Ming, 2000).

We recently reported that TRAF-2 is associated with neuritic plaques and neurofibrillary tangles in AD (Culpan et al., 2004) whereas another study reported an increased expression of TNF-RI and TRADD in AD cases compared to age matched controls (Zhao et al., 2003). These data support the role of the TNF-α pathway in AD pathogenesis.

Genetic factors may influence the expression of TNF-RI and TNF-RII by neurons. SNPs in the regulatory regions of several inflammatory cytokines such as IL-1α, IL-1β, IL-6 and TNF-α have been reported to influence the risk of AD, possibly by influencing the level of expression of the protein (McGeer and McGeer, 2001). The genes encoding TNF-RI and TNF-RII are located on chromosomes 12p13.2 and 1p36.3-2, respectively (Beltinger et al., 1996; Fuchs et al., 1992). Both genes are in areas of suggested linkage for late-onset AD (Myers et al., 2002).

Our aim in this study was to investigate if TNF-RI and TNF-RII are present at protein and mRNA level in AD and age-matched control brains and whether sequence variations in the promoter regions of the 2 genes are associated with the presence of neuropathologically confirmed AD.

2. Materials and methods

2.1. Patients

This retrospective case-control study used brain tissue that had been donated to the South West Dementia Brain Bank, Bristol. The tissue was from 55 neuropathologically confirmed cases of AD (mean age 81.4y, SD = 7.2, (66–96y) male:female 22:33) and 45 non-demented elderly controls (mean age 81.1y, SD = 7.1, (65–95y), male:female 27:18) (screening cohort). All brains were assessed by an experienced neuropathologist. Those with AD conformed to CERAD criteria for a diagnosis of definite AD (Mirra et al., 1991). The study was approved by Frenchay Local Research Ethics Committee.

2.2. Immunostaining

Paraffin sections of formalin-fixed frontal and temporal lobe from 20 cases (8 controls and 12 with AD) of the 100 used for genotyping were immunostained for TNF-RI and TNF-RII. Sections 7 μm in thickness were collected on glass slides, dewaxed, dehydrated through graded alcohols, immersed for 30 min in methanol containing 3% H_2O_2 to quench non-specific peroxidase activity, and then incubated overnight at room temperature in 1:1000 sc7895 rabbit polyclonal TNF-RI (Santa Cruz Biotechnology, CA) or 1:200 sc-8041 mouse monoclonal antibody to TNF-RII (Santa Cruz Biotechnology). Sections were rinsed, incubated with secondary antibody (Vectastain Biotinylated Universal antibody), then with VectaElite ABC complex and DAB, counterstained with haematoxylin and mounted.

2.3. Western blotting

Protein was extracted from homogenates of frozen tissue from 3 different regions of brain, frontal cortex (BA 6/24), temporal cortex (BA 20–22) and hippocampus, from 10 AD cases (mean age 81.9y, SD = 10.1) and 5 controls (mean age 79y, SD = 6.8). Approximately 0.5 g of tissue was homogenized in PBS and spun for 15 min at 15,000*g*. In addition, 0.5 g of homogenized tissue from 4 AD cases and 2 controls was further separated using ProteoExtract® Subcellular Proteome Extraction Kit (Merck) into 4 fractions: (1) cytosolic proteins, (2) membranes and membrane organelles, (3) nucleic proteins and (4) components of the cytoskeleton. Protein concentration in supernatants and individual fractions was measured with Bio-Rad protein assay kit.

Thirty microgram of protein was loaded onto a 4–12% pre-cast SDS–PAGE gel (Bio-Rad) and electrophoresed for 60 min at 150 V in a Bio-Rad Mini-Protean II apparatus. The proteins were electrophoretically transferred (overnight) onto nitrocellulose paper in a Bio-Rad Mini-Trans-Blot chamber (30 V) at 4 °C. To block non-specific binding, the membranes were incubated in TBS/Tween (0.05%) (TTBS) containing 10% non-fat milk for 60 min at room temperature.

Primary antibody was diluted in 5% non-fat milk/TTBS (TNF-RI: 1:1000 and TNF-RII 1:200) and applied to the membranes for 60 min at room temperature. Blots were washed in TTBS and incubated with the 2nd antibody (1:5000) diluted in 5% MP/TTBS for 60 min at room temperature. Finally the membranes were washed three times in TTBS and specific labelling was detected by enhanced chemiluminescence (ECL, Amersham Biosciences). The membranes were exposed to film (Kodak) and developed. The films were scanned and analyzed using the 'UN-