

A Pathophysiological Role for Selective Alteration of the Cytokine–Chemokine Network – Inflammatory Theory in Alzheimer's Disease

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Abstract

There is growing evidence that an altered metabolism of the amyloid β ($A\beta$) precursor protein (APP) with progressive deposition of its $A\beta$ fragment is a crucial event in the pathogenesis of AD. Increasing evidence suggests that inflammation and alteration of the cytokine–chemokine network contributes to the pathophysiology of AD. Activated microglia produce multiple pro-inflammatory cytokines, chemokines and reactive oxygen species (ROS); additionally, $A\beta$ itself can stimulate microglia, astrocytes and oligodendrocytes to secrete pro-inflammatory cytokines, chemokines and ROS, which can lead to neuronal damage. The concomitant release of pro-inflammatory cytokines, which influence neurodegenerative pathways, and anti-inflammatory cytokines may contribute to the chronicity of the disease. It is the balance of pro-inflammatory products and anti-inflammatory products that may be essential in the degenerative process. Influencing this balance may help in slowing the disease. Promising results for neurological disease treatment may be achieved by targeting cytokines and chemokines in the development of antagonists and synthesis inhibitors.

Keywords

Alzheimer's disease, amyloid β , cytokines, chemokines, neuroinflammation, Parkinson's disease

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Alzheimer's disease (AD) is the most common form of dementia, and worldwide affects 20–30 million individuals over 60 years of age. In 1907 Alois Alzheimer first described AD following an autopsy on the brain of a 55-year-old woman who had died following progressive mental deterioration, increasing confusion and memory loss.

Researchers from the Epidemiology and Prevention of Dementia group (EURODEM) have estimated that the prevalence increases from ~2% in the 65–69-year-old population to ~22% of those 85–89 years of age.¹ Independent of the aetiological agents, on a histopathological level AD is characterised by extracellular deposition of amyloid β ($A\beta$) protein in senile plaques and intraneuronal accumulation of paired helical filaments (PHFs) in neurofibrillary tangles, dystrophic neurites and neuropil threads. There is growing evidence that altered metabolism of the $A\beta$ precursor protein (APP) with progressive deposition of its $A\beta$ fragment is a crucial event in the pathogenesis of AD.² The fibrillar $A\beta$ can bind the complement factor C1 and activate the classic complement pathway. The activated complement products play a key role in the recruitment and activation of microglia at the sites of fibrillar $A\beta$ deposits.³ In turn, this activated microglia produces multiple pro-inflammatory cytokines, chemokines and reactive oxygen species that can ultimately influence plaque and tangle formation and can lead to neuronal damage.⁴ Additionally, $A\beta$ itself can stimulate microglia, astrocytes and oligodendrocytes to secrete pro-inflammatory cytokines, chemokines and reactive oxygen species (ROS), which can lead to neuronal damage.⁵

Qin et al. showed that the entry of pro-inflammatory factors such as tumour necrosis factor- α (TNF- α) to the brain induced the activation of microglia and subsequent production of more inflammatory factors, which may then cause neuronal death.⁶ This has clinical implications and, additionally, provides a link between peripheral inflammation and neuroinflammation. Support of the hypothesis that peripheral inflammation may amplify the neuroinflammation, contributing to AD pathogenesis, and that its inhibition may slow the disease progression can be argued from epidemiological findings indicating that prior long-term non-steroidal anti-inflammatory drugs (NSAIDs) are associated with a low risk of developing AD.⁷ Increased levels of interleukin (IL)-1, IL-6 and TNF- α have been found both in autopsy specimens and in the peripheral blood of patients with AD.^{8,9} IL-6 has been implicated in the transformation of diffuse neuritic plaques in the AD brain. IL-1 has also been linked to amyloid plaque transition from the diffuse to the dense core stage and the propagation of the inflammatory signal.

Transforming growth factor- β 1 (TGF- β 1) has been shown to promote $A\beta$ deposition in transgenic mouse models and therefore may exacerbate the amyloidogenic pathology. However, TGF- β 1 may also have non-inflammatory functions and may play an important role in the growth and survival of neurons in the AD brain. The expression of the cytokine TNF- α is decreased in the frontal cortex, superior temporal gyrus and entorhinal cortex of AD patients compared with non-AD controls, and has both protective and destructive functions.

Cytokines and Chemokines in the Alzheimer's Disease Brain

It is known that the central nervous system (CNS) has an endogenous immune system in which the classic signs of inflammation such as redness, swelling, heat and pain are absent. The microglial cells, the morphological 'delegates' of the immune system in the brain, play a significant role in the endogenous immune response. *Post mortem* investigations cannot easily address the inflammatory hypothesis of AD, in particular the question of whether inflammation is an early component in the pathogenesis of the disease or a common final step of the neurodegenerative process in AD.^{10,11}

Recent evidence suggests that bidirectional communication occurs between cells of the nervous and immune systems. The aberrant regulation of one system by the cells and products of the other may be responsible for the development of pathological conditions. After activation, microglia secrete pro-inflammatory cytokines and chemokines, followed by reactive oxygen species and complement protein.¹² The function of all of these molecules is diverse, and likely regulates the intensity and duration of an inflammatory response and plays an important role in orchestrating the behaviour of immune cells that can cross the blood–brain barrier (BBB) via their secretion of both cytokines and chemokines.¹³ In fact, the interactions between activated T cells and microglia induce the production of inflammatory cytokines. In the brain, IL-1 induces IL-6 and macrophage colony-stimulating factor (M-CSF) production in astrocytes and enhances neuronal acetylcholinesterase activity, microglial activation and additional IL-1 production. IL-6 activates microglia and promotes astrogliosis. In addition to the upregulation of interleukins, an association of AD with several polymorphisms of pro-inflammatory genes, such as IL-1, TNF- α and IL-6, has been described.^{14–16} Intracerebral production of anti-inflammatory cytokines such as IL-1 receptor antagonist, IL-4 and IL-10 are consistent with induction of homeostatic mechanisms in neuroinflammation.

Peripheral Cytokines and Chemokines in Alzheimer's Disease

The evidence that a systemic inflammatory reaction may contribute to the pathogenesis of AD is supported by many epidemiological data, by *in vitro* studies and by the hypothesis that anti-inflammatory agents may delay the onset and slow the progression of the disease.

One mechanism by which blood-borne cytokines might affect the functions of brain regions is by crossing the blood–brain barrier for direct interaction with the central nervous system tissue.

The role of peripheral inflammation in AD is not yet fully elucidated and remains controversial. Thus, one hypothesis is that inflammation starts in the CNS; a second hypothesis is that inflammation first develops in the periphery, as a result of different causes, and then contributes to brain damage and, finally, neurodegeneration.¹⁷

In the serum and cerebrospinal fluid (CSF) of AD patients, the upregulation of IL-1, IL-6 and TNF- α has been described. Chemokines

and nitric oxide are also increased in the same clinical patterns, while IL-4, a modulatory cytokine, has shown a parallel downregulation.

The identification of reciprocal interactions between the brain and the peripheral immune system permits comparison of the functions of the two different districts.¹⁸ An important finding was that cytokines and chemokines, as well as their receptors, are endogenous to both

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the brain and the immune system. These findings lead to the view that the immune system and brain speak a common biochemical language. There are both morphological and humoral pathways for bi-directional communication between the two systems.¹⁹

One mechanism by which blood-borne cytokines might affect the functions of brain regions is by crossing the BBB for direct interaction with the central nervous system tissue. A saturable transport system from the blood to the central nervous system has been described for IL-1 α , IL-1 β , interleukin-1 receptor antagonist, IL-6 and TNF- α .²⁰ In the past decade it has been demonstrated that abnormalities in both humoral and cellular immune response are supported in AD, suggesting a faulty immune regulation in this pathological pattern. Th1 responses are increased while Th2 activity is attenuated in AD patients, and MCP-1 is involved in the induction of polarised type Th2 responses and in the enhancement of IL-4 production. A possible feedback loop for Th2 activation would be the production of IL-4 and IL-13 by Th2, which stimulates MCP-1 production and leads to further recruitment of Th2 cells. The impaired Th1/Th2 peripheral release of cytokines could characterise the pathological state of lymphocytes in AD patients.²¹

Recent evidence suggest that systemic inflammation can induce behavioural changes, and may induce local inflammation in the diseased brain, leading to exaggerated synthesis of inflammatory mediators in the brain. Inflammatory mediators pass from the blood to the brain via macrophage populations associated with the brain, the perivascular macrophages and microglia. Such interactions suggest that a systemic challenge that promotes a systemic inflammatory response may contribute to the outcome or progression of a chronic neurodegenerative disease.²²

The activation of the peripheral immune system may be both a cause and a consequence of the pathogenetic process of dementia, with a self-enhancing cascade. This cascade includes A β deposit formation that leads to local inflammation within the brain, resulting in the activation of the peripheral immune system that leads to increased A β deposit formation. Recently, we have used an experimental model based on *in vitro* analysis of total peripheral mononuclear cells that may represent the whole response among different immune cells. The results have shown that in AD there is an imbalance of cytokine and chemokine expression and

production that is not restricted to the brain, but involves also the peripheral system, and that the administration of acetylcholinesterase inhibitor (AChEI) downregulates IL-1, IL-6 and TNF- α and upregulates the expression and production of IL-4.^{23,24} This result may suggest that inhibitors of AChE lead to the remodelling of the cytokine network, probably acting on the lymphocyte cholinergic system.

Concluding Remarks

Increasing evidence suggests that inflammation and alteration of the cytokine–chemokine network contributes to the pathophysiology of AD. The concomitant release of pro-inflammatory cytokines that influence neurodegenerative pathways and anti-inflammatory cytokines may contribute to the chronicity of the disease. It is the balance of pro-inflammatory and anti-inflammatory products that may be essential in the degenerative process; influencing this balance may help in slowing the disease.

Although the interpretation of the results of many AD studies has been somewhat controversial and confounded by limitations in sampling, in general all demonstrated an activation of the peripheral immune status that was probably linked to an inflammatory condition present in the brain. Assuming that the brain's pathological events are replicated in some manner in the periphery, it would seem rational to propose a link between the cytokine profiles of the brain and the systemic circulation.

Certainly the most important limitation of the majority of the research reviewed is the acknowledged cross-sectional design of the studies, from which it is not possible to define any 'cause–effect relationship'. In particular, such studies cannot distinguish whether or not high cytokine levels might contribute to the pathogenesis of AD or represent a protective response. The two are not mutually exclusive, and could be time-, location- and concentration-dependent. Moreover, dementia may lead to high cytokine levels, or other as yet unknown factors may contribute to both cognitive impairment and systemic inflammation. Although the precise molecular and cellular relationship between neurodegeneration and inflammation remains ambiguous, there is strong evidence that cytokines may induce

activation of signalling pathways that lead to further inflammation and neuronal injury, and both pharmacological and immunological tools are now available to confirm or refute this.

An interesting further question is whether or not systemic inflammation influences the progression of a chronic neurodegenerative disease, or is simply a consequence of tissue degeneration. In order to account for these different scenarios, two not mutually exclusive possibilities have been proposed. On the one hand, the overproduction of brain cytokines may contribute to the pool of peripheral cytokines by a spill-over from the CNS, whereas on the other hand peripheral cytokines may affect brain functions by crossing the BBB and directly interacting with CNS targets.

In the end, however, the greatest potential to aid the development of mechanistically driven treatments will likely derive from identifying the crucial factors responsible for the detrimental neuroinflammatory responses that occur in neurodegenerative diseases. In this regard, promising results for neurological disease treatment may be derived by targeting cytokines and chemokines in the development of antagonists and synthesis inhibitors. ■

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- Jorm AF, Jolley D, The incidence of dementia :a metanalysis, *Neurology*, 1998;51:728–33.
- Selkoe D, Deciphering the genesis and fate of amyloid beta protein yields novel therapies for Alzheimer's disease, *J Clin Invest*, 2002;110:1375–81.
- Eikelenboom P, Veerhuis R, The role of complement and activated microglia in pathogenesis of Alzheimer's disease, *Neurobiol Aging*, 1996;17:673–80.
- Streit WJ, Mrak RE, Griffin WS., Microglia and neuroinflammation: a pathological perspective, *J Neuroinflammation*, 2004;1:14.
- Sambamurti K, Greig NH, Lahiri DK, Advances in the cellular and molecular biology of the beta-amyloid protein in Alzheimer's disease, *Neuromolecular Med*, 2002;1(1):1–31.
- Qin L, Wu X, Block ML, Liu Y, et al., Systemic LPS Causes Chronic Neuroinflammation and Progressive Neurodegeneration, *Glia*, 2007;55:453–62.
- Selkoe D, Alzheimer's Disease :genes ,proteins and therapy, *Physiol Rev*, 2001;81:741–66.
- Luterman J, Haroutunian V, Yemul S, et al., Cytokine gene expression as a function of the clinical progression of Alzheimer disease dementia, *Arch Neurol*, 2000;57:1153–60.
- Singh VK, Guthikonda P, Circulating cytokines in Alzheimer's Disease, *J Psychiat Res*, 1997;31:657–60.
- Rojo LE, Fernández JA, Maccioni AA, et al., Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease, *Arch Med Res*, 2008;39(1):1–16.
- Tuppo EE, Arias HR, The role of inflammation in Alzheimer's disease, *Intern J Biochem Cell Biol*, 2005;37:289–305.
- Cacquevel M, Lebeurrier N, Chéenne S, Vivien D, Cytokines in neuroinflammation and Alzheimer's disease, *Curr Drug Targets*, 2004;5(6):529–34.
- Cardona AE, Li M, Liu L, et al., Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation, *J Leukoc Biol*, 2008;84(3):587–94.
- Tarkowski E, Liljeroth AM, Nilsson A, et al., TNF gene polymorphism and its relation to intracerebral production of TNF α and TNF β in AD, *Neurology*, 2000;54:2077–81.
- Papassotiropoulos A, Bagli M, Jessen F, et al, A genetic variation of the inflammatory cytokine interleukin-6 delays the initial onset and reduces the risk for sporadic Alzheimer's disease, *Ann Neurol*, 1999;45(5):666–8.
- Nicoll JA, Mrak RE, Graham DI, et al, Association of interleukin-1 gene polymorphism with Alzheimer's disease, *Ann Neurol*, 2000;47(3):365–8.
- McGeer PL, Rogers J, McGeer EG, Inflammation, anti-inflammatory agents and Alzheimer's disease :the last 12 years, *J Alzheimers Dis*, 2006;9:271–6.
- Perry VH, The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease, *Brain Behav Immun*, 2004;18(5):407–13.
- Goncharova LB, Tarakano AO, Molecular networks of brain and immunity, *Brain Res Rev*, 2007;55:155–66.
- Banks WA, Kastin AJ, Broadwell RD, Passage of cytokines across the blood-brain barrier, *Neuroimmunomodulation*, 1995;4:241–8.
- Reale M, Iarlori C, Gambi F, et al., The acetylcholinesterase inhibitor, Donepezil, regulates a Th2 bias in Alzheimer's disease patients, *Neuropharmacology*, 2006;50(5):606–13.
- Guerreiro R, Santana I, Bras JM, et al., Peripheral Inflammatory Cytokines as Biomarkers in Alzheimer's Disease and Mild Cognitive Impairment, *Neurodegenerative Dis*, 2007;4:406–12.
- Reale M, Iarlori C, Feliciani C, et al., Chemokine involvement in Alzheimer's disease pathogenesis: implication for peripheral inflammatory process. In: Linkers WP (ed.), *Progress in Chemokine Research*, Nova Science Publishers, Inc., 2007;12:169–77.
- Reale M, Iarlori C, Gambi F, Feliciani C, et al., Treatment with acetylcholinesterase inhibitor in Alzheimer patients modulates the expression and production of the pro-inflammatory and anti-inflammatory cytokines, *J Neuroimmunol*, 2004;148:162–71.