The Role of Neuroimmunomodulation in Alzheimer's Disease

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The idea that alterations in the brain immunomodulation are critical for Alzheimer's disease (AD) pathogenesis provides the most integrative view on this cognitive disorder, considering that converging research lines have revealed the involvement of inflammatory processes in AD. We have proposed the damage signal hypothesis as a unifying scheme in that release of endogenous damage/alarm signals, in response to accumulated cell distress (dyslipidemia, vascular insults, head injury, oxidative stress, iron overload, folate deficiency), is the earliest triggering event in AD, leading to activation of innate immunity and the inflammatory cascade. Inflammatory cytokines play a dual role, either promoting neurodegeneration or neuroprotection. This equilibrium is shifted toward the neurodegenerative phenotype upon the action of several risk factors that trigger innate damage signals that activate microglia and the release of tumor necrosis factor-α, interleukin-6, and some trophic factors. In this neuroimmunomodulatory hypothesis we integrate different risk factors with microaglial activation and the resulting neuronal alterations and hyperphosphorylations of tau protein. The progression of AD, with slowly increasing damage in brain parenchyma preceding the onset of symptoms, suggests that tissue distress triggering damage signals drives neuroinflammation. These signals via toll-like receptors, receptors for highly glycosylated end products, or other glial receptors activate sensors of the native immune system, inducing the anomalous release of cytokines and promoting the neurodegenerative cascade, a hallmark of brain damage that correlates with cognitive decline.

Key words: **Alzheimer's disease; immunomodulation hypothesis; neuroinflammation; innate immunity; cytokines; glial cells; neurons; tau protein; hyperphosphorylations; neuronal degeneration**

Introduction

Alzheimer's disease (AD) is the principal cause of dementia throughout the world and the fourth cause of death in developed economies after cancer, cardiovascular diseases, and vascular stroke. However, the set of disorders that cause cognitive impairment and that include vascular brain disorders, stroke, and brain trauma, accounts for one of the largest factors of morbidity and mortality.[1,2] In the United States approximately 5 million people are affected by AD, and mortality from this disease is near 100,000 per year, with a cost to the economy of over $100 bn.

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Considering that AD accounts for the largest number of dementia cases, including dementia from Lewy bodies, frontotemporal dementia (FTD-17), and vascular dementia, and that age is the main risk factor, the prevalence of these pathologies in an aging society is presently a major medical puzzle and a major challenge for science.

Among many postulates for the pathogenesis of AD, the more plausible explanation is based on the findings that tau hyperphosphorylations constitute a common final pathway of altered signaling mechanisms in degenerating neurons. However, this does not account for all the sequence of events after the early triggering factors that lead to neuronal degeneration. AD is a multifactorial disease. Several risk factors as well as the contribution of genetic vulnerability and polymorphisms among certain groups of subjects are involved in its pathogenesis. Strong evidence has been cumulated on the role of neuroinflammation in AD pathogenesis. Neuropathological studies in human brains demonstrating the activation of glial cells[3] has been corroborated by *in vitro* studies in which amyloid Aβ-exposed and activated glial cells overproduce pro-inflammatory cytokines, which in turn trigger a neurodegenerative cascade in living neurons.[4–6] Studies with transgenic animal models of AD have also demonstrated that brain inflammation appears to be a key component of AD pathogenesis.[7] Moreover, epidemiological data show that individuals consuming nonsteroidal anti-inflammatory drugs (NSAIDs) have a lower risk of AD.[8] In fact, inflammation is associated with brain lesions in AD while a sparing effect of NSAIDs has been shown; these NSAIDs are also protective in animal models of AD.[7] In fact, patients receiving systemic NSAIDs developed significantly less AD manifestations, suggesting that ameliorating inflammation in the brain helps to prevent or slow down the onset of AD.[3,9] However, this effect may not apply to all NSAIDs equally; a more recent study failed to demonstrate such an effect for certain drugs, such as naproxen and celecoxib.[10]

How Does Neuroinflammation Participate in AD Pathogenesis?

Neuroinflammation is characterized by the generation of a set of pro-inflammatory mediators locally produced by host cells, indicating the engagement of the innate immune system. In this context, AD can be considered an autotoxic disease in which an innate immune system uses local phagocytes, such as microglia, as an effector. Activated complement fragments and inflammatory cytokines have been identified in lesions (Figs. 1 and 2). AD exhibits marked inflammatory phenomena from the inherent toxicity of aggregates of Aβ oligomers (much earlier than the other lesion, the senile plaques) and small aggregates of the hyperphosphorylated tau protein. Thus, the AD phenotype is a result of the convergence of multiple risk factors that activate one or more danger/alarm signal detectors, with microglial activation, production of nuclear factor (NF)-κB, and inducing multiple degeneration-promoting signals, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, that converge to produce an abnormal processing of tau protein (Fig. 2). These anomalous signals lead to an overactivation of some cell cycle enzymes, such as cdk5 and the neuronal glycogen synthase kinase, with the consequent tau hyperphosphorylations.[11,12] Increasing evidence has accumulated on the substantial toxicity of abnormally phosphorylated tau variants contributing to the neurodegenerative cascade.[13–15] Once this pathway is triggered, the expression of clinicopathological disorders cannot be stopped.

Several lines of evidence indicate that under certain experimental conditions, damage/alarm signals, such as oxidative stress, exposure to toxins, hypoxia, oxidized low-density lipoproteins, or mechanical damage promote neuronal degeneration.[16–18] The resulting inflammatory cytokines in all of these situations can play a dual role either promoting neurodegeneration or assisting neuroprotection.[5,6,9] Thus, if pro-inflammatory mediators were simply protective, one should expect that

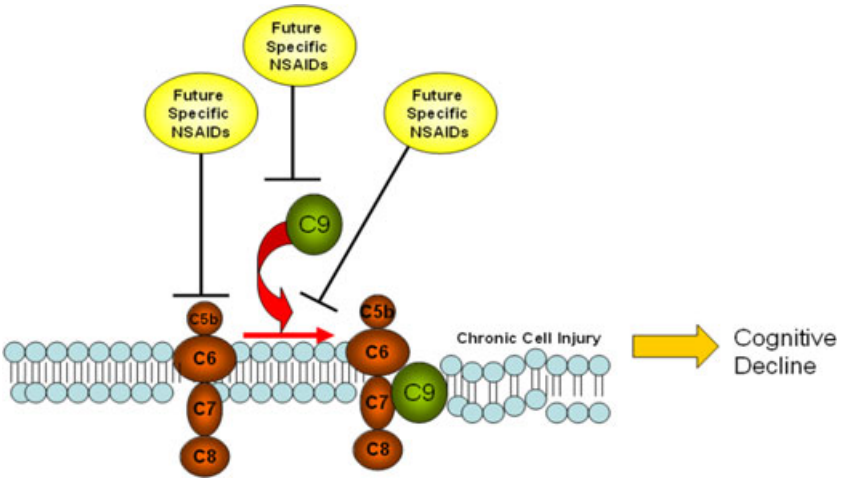

Figure 1. Formation of the membrane attack complex (MAC) on the surface of brain cells as a result of the activation of the complement system and potential targets for future specific nonsteroidal anti-inflammatory drugs NSAIDs against Alzheimer's disease (AD) during early stages of brain damage. (In color in *Annals* online.)

individuals receiving NSAIDs would be at a higher risk of AD, which appears not to be the case.[3,9] In fact, the evidence indicates that only a few pro-inflammatory molecules, such as TNF-α,[6] exert neuroprotective effects.

Innate Immunity in Alzheimers's Disease

Neuronal damage in AD occurs long before the clinical onset of the disease, a decade or even longer time intervals,[1] as a consequence of the permanent action for years of the various exogenous or endogenous risk factors. Among the major risk factors are chronic stress, blood lipid disorders,[17,19–22] repeated mechanical head trauma, oxidative stress,[23,24] brain iron overload,[25] folic acid deficiency, K^+ efflux, and several others, such as genetic polymorphisms.[26] All these conditions, or the convergence of several of these, are sufficient to trigger danger/alarm signals[27] that, through activation of innate immunity, modify the normal activity of microglial cell receptors, such as toll-like receptors (TLRs), receptors for highly glycosylated end products (RAGEs), and other sensitive receptors on the glial cell surface. Thus, activated glial cells respond with an overproduction of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which are found to be considerable increased in AD.[9,28–30] In this context, we have proposed a novel unifying hypothesis that the release of endogenous damage/alarm signals,[16] in response to converging and accumulated cell distress (e.g., dyslipidemia, vascular insults, head injury, oxidative stress, folate deficiency), is the earliest triggering event in AD pathogenesis, which then leads to the activation of innate immunity[3,9] and, subsequently, an inflammatory cascade (Figs. 1 and 2). In this hypothesis we consider the risk factors that have been analyzed separately and in different contexts, with microglial activation, tau hyperphosphorylation in the affected neurons, and the resulting neuronal damage. The protracted progression of the disease, with slowly increasing damage in brain parenchyma preceding the onset of symptoms, suggests that moderate tissue distress triggering damage/alarm signals drives an escalating inflammatory process until tissue damage causes progressive eventually irreversible pathology. This hypothesis is based on known facts about AD and experimental models of AD as well as our own reviews of these complex sets of factors.[5,9,14,16]

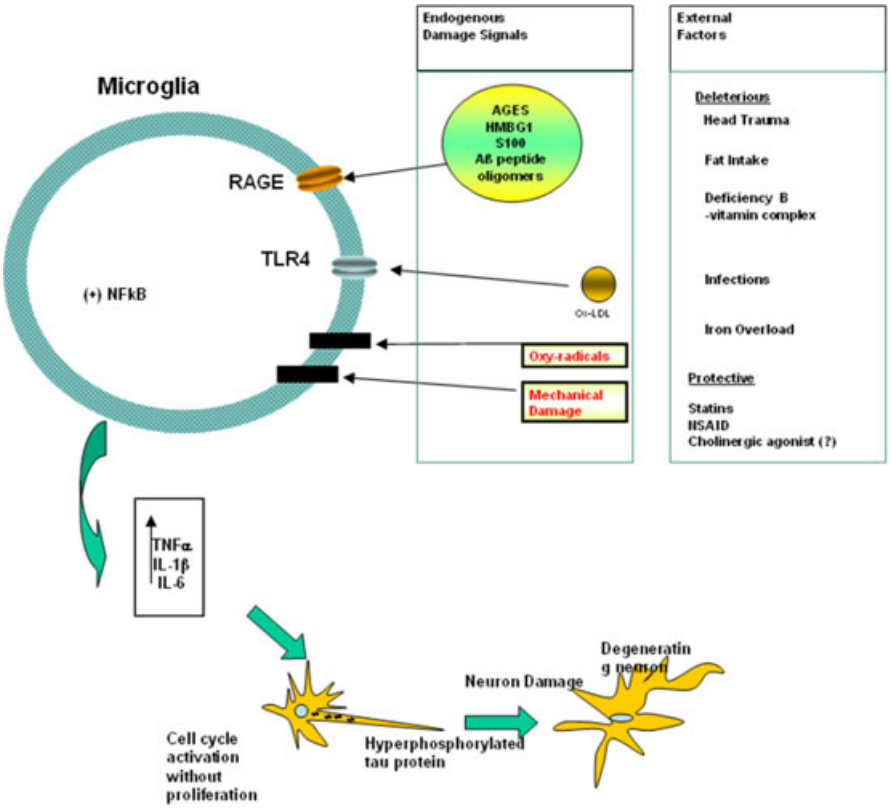

Figure 2. Schematic representation of the roles of endogenous danger/alarm signals in the innate immune system at the early stages of Alzheimer's disease (AD) pathogenesis. Danger signals include advanced glycation end products (AGES), HMBG1 (high-mobility box group 1), S-100 proteins, and amyloid -β (Aβ) peptide oligomers (but not β-pleated fibrillar aggregates). These activate microglia through the AGES or receptors for highly glycosylated end products (RAGE), shown here as a transmembrane protein. Oxidized low-density lipoproteins (oxLDL) activate toll-like receptors (TLRs), particularly TLR4. Additional danger signals are trauma and oxyradical damage, possibly acting on separate receptors (black boxes inserted in the membrane) as well as by inducing the production of additional Aβ peptide oligomers, AGES, and S-100 protein that contribute to this process. These danger signals trigger innate immune system alarm mechanisms resulting in long-term overproduction of tumor necrosis factor alpha (TNF-α), interleukin 1-beta (IL-1β), and IL-6. These signals would then mediate neuronal damage that is reflected in alterations, such as tau hyperphosphorylation, which eventually result in neuronal degeneration and progressively more severe clinical manifestations of cognitive and behavioral decline unrelated to the amyloid aggregation process. (In color in *Annals* online.)

Future Avenues for Rational Development of New Anti-inflammatory Agents against AD

Activated complement fragments as well as inflammatory cytokines have been identified in association with the histologically evident lesions in the brain of patients affected with AD.[31,32] The activation of the complement system induces formation of the membrane attack complex (MAC) on the surface of brain cells[33] (Fig. 1). Thus, a slow, chronic, asymptomatic, and autotoxic process leads to progressive neurodegeneration stages and to cognitive impairment in Alzheimer patients. From the point

of view of an integrated analysis of this evidence, we propose potential targets for future specific NSAIDs against early stages of brain damage. Considering that Aβ extracellular deposits and neurofibrillary tangles are present during early preclinical stages of AD until the terminal stages, their ability to strongly activate complement system provides a mechanism for initiating and sustaining chronic, low-level, inflammatory responses that may accumulate over the disease course.[34] This supports the idea that the complement system cascade intervention can be a pharmacological approach to AD in the near future. Besides Aβ effects on the complement system, amyloid oligomers also signal through RAGE receptors, resulting in the sustained production of NF-kB, which is involved in the inflammatory cascade (Fig. 2).

Innate Immunity in AD and Other Degenerative Diseases

The notion that endogenous signals of damage trigger the earliest events of AD pathogenesis finds further support in the natural history of the inherited forms of early onset AD in which mutations in the affected genes are expressed as an increased production of pro-inflammatory Aβ peptide oligomers; this needs decades to cause pathology and correlates with a more precocious onset of the familial forms of this disease compared to the most prevalent sporadic forms of the disease. Along the same line of evidence, persons with Down's syndrome, a condition associated with a high risk of AD, exhibit increased serum levels of Aβ in childhood and adolescence and rapidly accumulating senile plaques and neurofibrillary tangles thereafter. Intriguingly, plasma Aβ levels in these subjects correlate inversely with age,[35] a key observation that contradicts the highly prevalent amyloid hypothesis. In summary, we propose that the disease phenotype/neurological condition that has so far been called "Alzheimer's disease" is not a result of a single "cause" but to the convergence of multiple risk factors that coalesce in the activation of one or more damage/danger signal detectors (Fig. 1). The long-term effect of these triggering factors results in microglial activation and the protracted production of NF-κB, inducing multiple predominantly degeneration-promoting signals, such as the inflammatory cytokines. All of these converge to produce abnormal processing of tau protein, which acts as a final common pathway.[1,16] Recent reports describe the roles of IL-6 and TNF-α on AD pathogenesis. We reported that IL-6 induces tau hyperphosphorylation and neuronal cell death, both mediated by deregulation of the cdk5/p35 complex.[4] We have also reported that TNF-α can decrease cdk5 activity and prevent hippocampal neuron death induced by $A\beta_{1-42}$ peptide *in vitro*.[6] Once this pathway has been triggered by multiple mediators of inflammation, the full expression of the clinicopathological disorder probably cannot be stopped. In this hypothetical scheme, interstitial amyloid deposits, senile plaques, neurofibrillary tangles, neuronal degeneration, and, of course, clinical manifestations occur subsequently. The key element of this proposal, which is experimentally testable, is that the danger/alarm signals must activate the sensors of the innate immune system in the brain. It remains to be determined precisely how this hypothetical chain of events is unique to what we conceptualize today as "Alzheimer's disease," as there are phenotypically distinct disorders that are also associated with inflammatory phenomena but that do not result in the clinical and histopathological manifestations considered to define AD. Among the possible explanations for this conundrum, we propose that the location where these phenomena are triggered (e.g., medial aspect of the temporal lobe in AD versus lateral aspect of the temporal and/or frontal lobe in the so-called frontotemporal atrophies versus midbrain and diencephalon in progressive supranuclear palsy) may alter the time course, topographic distribution, lesion array, and, ultimately, clinical manifestations of the ensuing disorder. As a complementary (and not

necessarily mutually exclusive) explanation, there is also an emerging molecular basis for the phenotypic diversity among neurodegenerative disorders that exhibit inflammatory phenomena. For example, there is a differential activation of TLRs in animal models of AD versus models of other disorders. In fact, TLR2 is activated in models of AD but not in models of other degenerative disorders. Furthermore, it is plausible that other mediators of innate immunity may also be expressed differentially in distinct neurodegenerative disorders, which is another important avenue for further experimental assessment.

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Conflicts of Interest

The authors declare no conflicts of interest.

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