

Hypothesis

The Damage Signals Hypothesis of Alzheimer's Disease Pathogenesis

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Abstract. Virtually none of the hypotheses on Alzheimer's disease (AD) pathogenesis address the earliest events that trigger the molecular alterations that precede cerebral degeneration and account for the diversity of risk factors that converge on a well-defined disease phenotype. We propose that long-term activation of the innate immune system by an individual array of risk factors constitutes a unifying mechanism leading to the triggering of an inflammatory cascade that converges in cytoskeletal alterations (tau aggregation, paired helical filament formation) as a previously hypothesized final common pathway in AD. The key pathogenic phenomena consist in the long-term, maladaptive activation of innate immunity-triggering receptors – such as the toll-like and advanced glycation end-products receptors, and others located in the microglial membrane – by seemingly heterogeneous risk factors such as hyperlipidemia, hyperglycemia, oxidative stress, head injury, amyloid oligomers, etc. Our hypothesis provides a unifying mechanism that explains both the diversity of risk factors acting over long periods of time and the individual response to such insults. This formulation is amenable to both empirical testing and implementation into therapeutic strategies that may lead to effective prevention of AD as well as other disorders in which impaired regulation of the innate immunity is the unifying cause of the condition.

Keywords: Alzheimer's disease, danger signals, glial cells, inflammatory cascades, innate immunity, neuronal cells, tau protein

NEUROIMMUNOMODULATION HYPOTHESIS

Among the many hypotheses on the pathogenesis of Alzheimer's disease (AD) being tested, virtually none provide a unifying insight on the seemingly diverse early events that trigger the putative metabolic and cellular alterations that precede neuronal degeneration [3,8]. In fact, virtually none of them provide a well articulated,

cogent reason why factors as diverse as head injury, high fat intake, B vitamin deficiency [4,7], central nervous system infections [6], alterations in cholesterol homeostasis [13,15] and many others, increase the risk of developing the disorder, whereas none of these risk factors seem to act as true causative factors. For example, the most commonly held amyloid hypothesis rests on the concept that the amyloid- β peptide, $A\beta_{1-42}$, self-polymerizes over years to eventually form senile plaques, which are thus putatively responsible for the entire array of subsequent brain lesions. In its original version, this hypothesis did not account for the diversity of risk factors, nor did it explain precisely what caused the amyloid protein to polymerize. Furthermore, rel-

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atively recent findings [8] point to oligomers of unpleated $A\beta$ as the major culprit for synaptic impairment in the pathway to eventual neuronal degeneration in AD [5], and interstitial amyloid deposits thus appear to be a much later phenomenon in a chain of multiple events affecting progressively more severe neuronal and neuropil alterations [3]. Unfortunately, this revised version of the amyloid hypothesis still cannot account for the diversity of deleterious and protective factors. Thus, a substantially more plausible explanation is derived from the observation that tau hyperphosphorylation constitutes a 'final common pathway' for a host of upstream altered signaling mechanisms leading to neuronal degeneration [8]. This raises the question as to precisely what triggers the pathological phosphorylation and how such triggering mechanisms can be set off by widely diverse risk factors, which we address below after considering the key evidence for inflammation in AD pathophysiology.

For over a decade, converging lines of work reveal the involvement of inflammatory processes in the development of AD [9,10,16]. In one set of such studies, patients receiving systemic non-steroidal anti-inflammatory drugs (NSAIDs) developed significantly less AD manifestations, suggesting that ameliorating inflammation in the brain helps to prevent or slow down the onset of AD [9]. However, this effect may not apply to all NSAIDs equally, since a more recent study failed to demonstrate such an effect for naproxen and celecoxib [1]. While design and methodological challenges cannot be ruled out in the interpretation of clinical trials, it is intriguing that in animal models of AD inflammation in the brain appears to be a key component of disease pathogenesis [19]. Furthermore, several lines of evidence indicate that under certain experimental conditions, damage/alarm signals such as oxidative stress, exposure to toxins, hypoxia, or mechanical damage promote neuronal degeneration [8,16]. The resulting inflammatory cytokines in all of these situations can play a dual role, either promoting neurodegeneration or assisting neuroprotection [12]. Thus, if pro-inflammatory mediators were simply protective, one should expect that individuals receiving NSAIDs would be at higher risk of AD, which appears not to be the case [7,16]. In fact, the evidence indicates that only few proinflammatory molecules, such as tumor necrosis factor- α (TNF- α) [16], exert neuroprotective effects.

Several lines of evidence indicate that the neuronal damage in AD occurs long before the clinical onset of the disease [16]. Putative risk factors such as blood

lipid disorders or repeated mechanical head trauma can be expressed as augmented oxidative stress, shear stress, mechanical cell damage, K^+ efflux, and membrane permeabilization [16]. These conditions are sufficient to trigger innate danger/alarm signals [2] and to activate microglial cells, to release pro-inflammatory cytokines such as, TNF- α , IL-1 β and IL-6, which are activated in AD [1]. Here we propose a novel, unifying hypothesis in that the release of endogenous damage/alarm signals [2], in response to converging and accumulated cell distress (dyslipidemia, vascular insults, head injury, oxidative stress, folate deficiency, etc.), is the earliest triggering event in AD pathogenesis, which then leads to the activation of innate immunity [17] and, subsequently, an inflammatory cascade (Figure 1). In this hypothesis, we integrate the risk factors that have been analyzed separately and in different contexts, with microglial activation and the resulting neuronal damage. The protracted progression of the disease, with a 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 factors [8], but has not been enunciated or published elsewhere.

Activated complement fragments, including membrane attack complex, as well as inflammatory cytokines have been identified in association with the histologically evident lesions [10]. Since brain lesions are not completely removed by inflammatory phagocytes, they can strongly activate antigen-presenting cells (APCs). AD exhibits marked inflammatory phenomena due to the inherent toxicity of the extracellular aggregates of $A\beta$ oligomers (i.e., a much earlier stage of amyloid protein alterations than that in which senile plaques appear). Intriguingly, $A\beta$ oligomers are a potent damage/alarm signal by activating APCs via the advanced glycation end products receptor (RAGE) to produce proinflammatory cytokines and — through a complex pathway (Fig. 1) — neurofibrillary tangles that will eventually be released to the extracellular space ('tombstone' tangles) and are a hallmark of advanced brain damage which correlates with increasing impairment in cognitive function [8,19].

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 affect-

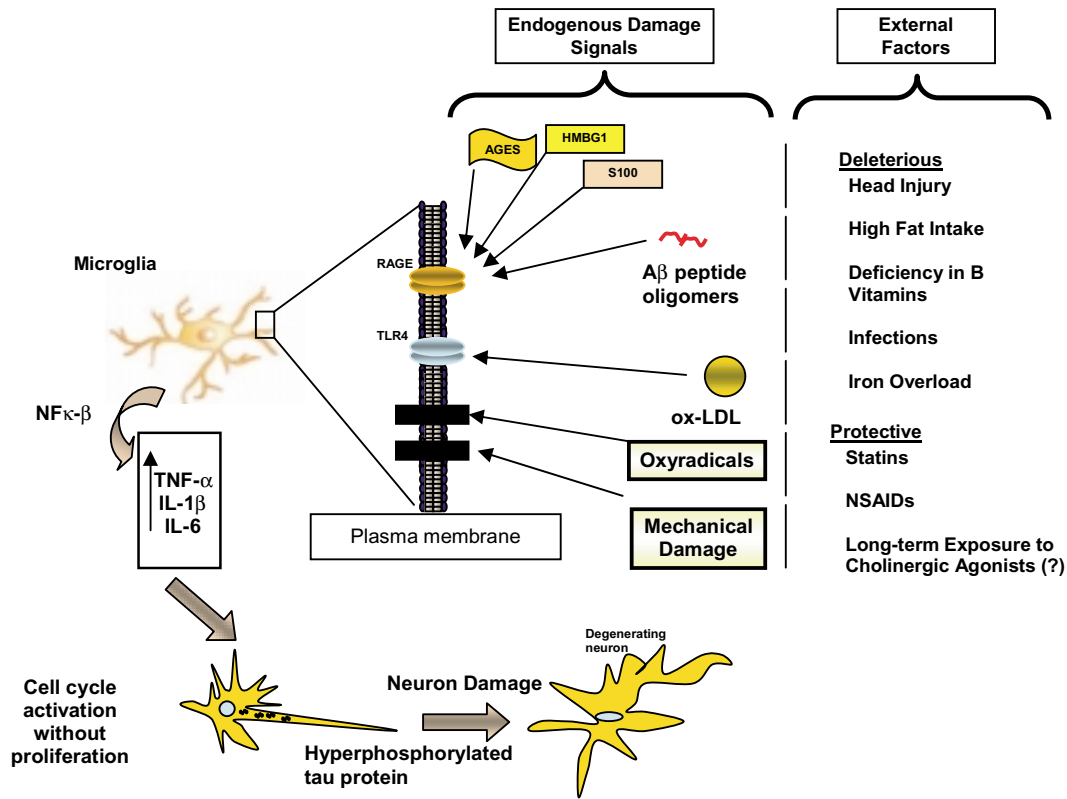


Fig. 1. Schematic representation of the hypothetical roles of endogenous danger/alarms signals built into the innate immune system in the early stages of the pathogenesis of AD. Danger signals such as advanced glycation end products (AGEs), HMBG1 (high mobility box group 1), S-100 proteins, and A β peptide oligomers (but not β -pleated fibrillar aggregates), activate microglia through the AGE receptor (RAGE; shown here as a transmembrane protein in a cartoon of a lipid bilayer). Separately, oxidized low-density lipoproteins (oxLDL) activate toll-like receptors (TLRs), and, in particular, TLR4. Additional danger signals, such as trauma and oxyradical damage, possibly acting on separate receptors (black boxes inserted in the membrane) as well as by inducing the production of additional A β peptide oligomers, AGE and S-100 protein could also contribute to this process. Separately and in various combinations (as it may apply to different individuals), these danger signals would trigger innate immune system alarm mechanisms resulting in the production of tumor necrosis factor alpha (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6). These signals would then mediate neuronal damage directly, reflected in alterations such as tau protein hyperphosphorylation and paired helical filaments formation, which eventually result in neuronal degeneration A β peptide aggregation in a β -pleated configuration, and progressively more severe clinical manifestations of cognitive and behavioral decline unrelated to amyloid aggregation.

ed genes are expressed as increased production of pro-inflammatory A β , which necessitates decades to cause pathology, and correlates with a more precocious onset of the disease compared with sporadic forms of AD. 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 A β in childhood and adolescence and rapidly accumulating senile plaques and neurofibrillary tangles thereafter. Intriguingly, A β levels in these subjects correlate inversely with age [11], 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 due to a single "cause" but to the convergence of multiple risk factors that coalesce in the activation of

one or more damage/danger signals detectors (Fig. 1), and the long term effect of these triggering factors results in microglial activation and the protracted production of NF κ -B, inducing multiple predominantly degeneration, promoting signals such as TNF- α , IL-1 β , IL-3 and IL-6. All of these converge to produce abnormal processing of tau protein, which acts as a final common pathway [8]. Recent reports describe the roles of IL-6 and TNF- α in the pathogenesis of AD. We reported that IL-6 induces tau hyperphosphorylation and neuronal cell death, both mediated by deregulation of the CDK5/p35 complex [14]. We have also reported that TNF- α can decrease CDK5 activity and prevent hippocampal neurons death induced by A β_{1-42} peptide *in vitro* [12]. Once this pathway has been triggered by multiple mediators of inflammation, the full expression

of the clinico-pathological 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. An approach to test this hypothesis, among several others, would be to assess the induction of AD-like pathology in 'knock-out' mice lacking receptors such as TLR4 and RAGE. However, possible results have to be taken cautiously because of the limitations of studies in animal models with respect to the human brain, as mentioned above. It remains to be determined precisely how this hypothetical chain of events is unique to what we conceptualize today as "Alzheimer's disease," since 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 at which these phenomena are triggered (e.g., medial aspect of the temporal lobe in AD versus lateral aspect of the temporal lobe and/or frontal lobe in the so-called fronto-temporal 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 Toll-like receptors in animal models of AD versus models of other disorders. In fact, Toll-like receptor 2 (TLR2) is activated in models of AD, but not in models of other 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.

This evolving concept of the pathogenesis of AD is also consistent with emerging evidence that immunomodulation may be beneficial not only as a palliative therapy, but also in the prevention of this condition [18], since such interventions are felt to affect the rate of A β aggregation and disaggregation, cell-mediated clearance and its passive redistribution in brain parenchyma, cerebrospinal fluid and plasma, among other mechanisms that are not primarily in the

immune/inflammatory domain, but that result from a relatively broad array of manipulations of the immune system. Finally, it has not escaped our attention that this model also predicts that most treatments directed against a single factor among the multiple converging vulnerability versus protective mechanisms will be either ineffective or weakly effective, except in the presumably rare cases in which only one factor is the most strongly affected. Therefore, the most effective means of treatment and prevention will necessitate individualizing the therapy by prior determination of which among the various possible converging factors are involved in a specific patient, including means to avoid overwhelming the protective factors involved in that patient.

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