EDITORIAL

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Toward a Unifying Hypothesis in the Development of Alzheimer's Disease

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The thesis of the article by Martorana et al. [1] "Beyond the cholinergic hypothesis. Do current drugs work in Alzheimer's disease?" is that despite the observations showing an association between acetylcholine (ACH) deficiency and cognitive decline in Alzheimer's disease (AD) patients, results of clinical trials studying the efficacy of agents which increase the availability of ACH demonstrate only temporary symptomatic relief. A further comment implicates dysfunction of other neurotransmitters as playing a role in the symptom complex observed in AD and recommends further exploration of these disturbed relationships as a future research direction. The identification of neurotransmitter dysfunction has resulted in a major focus on symptom relief, but the discussion should explain the initiation of the disease and account for the large population that develops the illness. The neurotransmitter model is based upon knowledge of specific brain function at specific anatomical sites. This provides a valuable clue in connecting structure to function and implicates basal forebrain nuclei in early deficits of the AD patient. This model does identify vulnerable anatomical sites but falls short in explaining why and how these sites are targeted in AD pathogenesis.

Finding a solution to understanding Alzheimer's disease requires a unifying hypothesis. Up to now, the leading hypotheses have focused on three major themes, neurotransmitter dysfunction, the amyloid cascade, and the role of tau in the formation of neurofibrillary tangles. Perhaps this reflects an over reliance on information derived from studies of genetically associated AD (~5%), leading us to ignore environmental factors that may influence the course of the disease in the much more frequent sporadic form of the illness (~95%). Prevailing thought indicates that amyloid deposition or failure to dispose of excess amyloid leads to dysfunction at the synapse and eventually to tangle formation. However, initiation of this sequence has not been explained and may be the reason effective therapy has not yet been developed despite decades of effort.

The question that must be asked is "what trigger(s) lead to these events?" There is growing interest in the concept which proposes that most sporadic AD is initiated long before evidence of cognitive decline becomes apparent. Under this concept, the complexity of the neurotransmitter dysfunction observed more likely represents individual variation in the response to a chronic process much as one would predict when a stochastic model is applied to the perturbation of an orderly system. Rather than focusing on symptomatic relief, consideration of a broader model that includes mechanisms of disease initiation and progression as well as early identification of high-risk patients supports the structure of a novel unifying hypothesis.

This unified model opens the door to consider new approaches to prevention and therapy. A clue to the localization of damage and the sequence of events may come from the observed functional change in the sense of smell that often precedes loss of cognitive function [2,3]. Documentation of olfactory deficits in AD as well as in Parkinson's disease demonstrates that the deficit precedes classical clinical signs by several years [4]. In fact, recognition that the loss of the sense of smell is an early symptom of AD suggests that the olfactory system may hold the key to the location and origin of triggering events leading to amyloid production/processing/deposition and secondarily to ACH neurotransmitter and other deficits. Experimental evidence in rodents has demonstrated that bulbectomy results in degeneration within the temporal cortex, hippocampus, and raphe nucleus, decreased numbers of cholinergic neurons in the basal forebrain, and increased levels of β-amyloid in limbic structures including the hippocampus [5–7]. ACH-producing cells in the basal nucleus as well as projections from the olfactory system innervate limbic regions of the brain including the amygdala and hippocampal formation demonstrated to have the earliest pathology in AD. Atrophy in the hippocampus and entorhinal cortex as demonstrated by MRI has been shown to correlate with changes in cognition [8]. The proposed model offers a contrast to the presumption that olfactory deficit is a result of disease rather than a precursor to it and furthermore incorporates the chronic nature of the disease process in the sequence of events leading to disease.

Table 1 Proposed sequential steps in the pathogenesis of AD

Susceptibility factors in AD	Infection	Localization of infection	Bacterial response to infection	Cellular response to infection	Pathology at the time of cognitive change
Older Age – infection increased	Exposure in respiratory droplets to infection with *Chlamydia pneumoniae* followed by infection of nasal olfactory neuroepithelia	Tissues: Limbic system including: olfactory bulbs, entorhinal cortex, hippocampus	Organism produces lipopolysaccharide endotoxin and heat shock protein 60; the organism siphons from cells ATP and tryptophan	Prominent proinflammatory – cytokines IL-1β, IL-6, TNFα; generation of reactive oxygen species including superoxide, nitric oxide, hydroxyl radicals, hydrogen peroxide; chronic neuroinflammation with blood brain barrier changes	Amyloid plaques and NFTs in the limbic region including: olfactory bulbs, entorhinal cortex, hippocampus
ApoEε4 genotype– facilitates uptake of organism into cells		Cellular localization: intracellular with growth in: neurons, microglia, astroglia, vascular endothelia, perivascular macrophages		Upregulation of indoleamine 2,3-dioxygenase– results in breakdown of tryptophan– increased kynurenine and quinolinic acid, decreased serotonin and reduced T cells activation resulting in greater tolerance for the infection	Defects in the sense of smell
Decreased mucosal immunity– low IgA allows organism to infect nasal respiratory tract more readily				Increased processing of amyloid–amyloid cascade; activation of lysosomal-phagosomal system; increased autophagy; apoptosis initiated but incomplete; mitochondrial damage; kinase over-activation; accumulation of iron	Neurotransmitter dysregulation, especially of ACH
					Synaptic dysfunction

Note: All of the aspects of localization of infection and cellular changes have been observed in the sporadic late-onset AD brain (for review see Balin et al. [25]) and (for AD review see Herrup [26]).

The model depicted in Table 1, adapted from a figure proposed by Miklossy [9], illustrates the proposed process of AD development depicting the sequence of events and the known aspects of infection. Interestingly there are a surprisingly high number of attributes common between what is known to occur in AD pathogenesis and what is known to occur after infection. Of particular note, this model provides both an explanation of why amyloid production is initiated and of the localization of AD damage to vulnerable regions of the brain. We propose that infection with *Chlamydia pneumoniae* (Cpn) is an event that can trigger this process and act as a unifying factor in the pathogenesis of AD. A large proportion of individuals (as high as 80%) who are at risk for the development of sporadic AD also are prone to infection by the bacterium [10]. This hypothesis, first proposed by Balin et al. [11], has taken on new significance with the discovery that Aβ amyloid has anti-infective properties [12], and may be an integral part of our natural defense response. Aβ amyloid may be generated to block bacterial infection by Cpn. However, because Cpn is an intracellular bacterium, Aβ amyloid may be relatively ineffective in clearing the pathogen. Without proper clearance, products of this chronic infectant such as lipopolysaccharide (LPS) and heat shock protein 60 may actually stimulate neuroinflammation leading to increased cell damage and continual Aβ amyloid production with ever increasing toxicity. Neuroinflammation has been well-recognized in AD in which a prominent proinflammatory cytokine profile has been documented [13]. Similarly, infection with Cpn is known to result in a very prominent proinflammatory response [14]. LPS is found on many gram-negative bacteria and functions as a potent endotoxin. Recognition that the natural defense system is activated by a number of insults permits a broadening of our view of this process and allows an appreciation of the accumulation of Aβ amyloid in other known neurologic infections, such as HIV dementia [15], neurosyphilis, and neuroborreliosis, as well as following brain trauma or other perturbations of brain barriers. This model is consistent with findings in which other members of the natural defense system are known to be toxic upon extended exposure to healthy normal tissues [16, 17].

There is ample evidence supporting the concept of infection as being an initiating event. We and others have observed Cpn to be present in brains of approximately 90% of individuals with sporadic AD including localization of infection in regions associated with olfaction [11, 18]. Cpn is a respiratory Chlamydial bacterium

that is geographically wide-spread [19] and found in large percentages of the adult population [20]. Chronic infection is thought to increase with increasing age of the population [21]. The organism has ready access to the receptors for olfaction in the upper passages of our noses as well as for infection of the lungs proper. As we have previously detected Cpn in the olfactory bulbs of AD afflicted individuals [11] and following intranasal infection of mice with a brain strain of the organism [22], the most prominent route of brain infection in humans may be through the olfactory pathway. Pathology consisting of modified tau proteins and intracellular amyloid deposition has been observed in the olfactory neuroepithelia of AD patients [2]. These observations would help to explain why a change in the sense of smell appears to be a 5-year predictor to a change in cognition [3]. This hypothesis also connects the ApoE-ε4 genetic correlation with AD in that ApoE-ε4 expressing individuals are susceptible to significantly higher Cpn infectivity load than non ApoE-ε4 expressers [10]. Furthermore, amyloid plaques can be induced through nasal infection of normal nontransgenic mice [22], and therapeutic intervention with antibiotics in these animal models is most effective when it is initiated shortly after infection [23], an observation that may inform the design of clinical therapeutic regimens.

In consideration of the failure of alternative models, we urge that this unifying model be given a thorough evaluation in clinical trials. Clinical evaluation of this model requires incorporation of several protocol components to ensure validity. These include: thorough description of a high-risk profile; ability to conduct sensitive cognitive and structural CNS evaluations as early as feasible in the disease process; ability to measure proteins and biomarkers in the CSF; and selection of an appropriate antibiotic at an effective dose and appropriate therapeutic window. In this regard, a previous AD clinical trial demonstrated that doxycycline and rifampin, two antibiotics effective against Cpn, reduced decline in the mini-mental status scores of AD patients at 12 months posttreatment [24]. We propose that individuals at risk for disease, but prior to disease onset (family history, ApoE-ε4, other markers) and perhaps with a defined change in their sense of smell undergo testing for signs of Cpn infection followed by administration of antibiotics such as macrolides, fluoroquinolones, and/or doxycycline, which are effective against Cpn and cross the blood brain barrier, and anti-inflammatory agents like NSAIDS, and then monitored prospectively. Focusing on neurotransmitter-based therapeutic approaches, according to our hypothesis, appears to be too late to impact the disease with any more than a temporary symptomatic improvement. Similarly, in the case of amyloid focused therapies, initiating factors must be considered in the course of their evaluation. This approach will also serve to lower the possibility that the therapy will compromise a likely important natural anti-infective defense mechanism.

Evaluation of this model deserves serious attention, and infected individuals need to be identified as early as possible to prevent the downstream sequelae of the disease process. This approach offers an opportunity to test early detection methods and provide intervention before the neurodegenerative process becomes irreversible. The proposed holistic alternative hypothesis provides a framework for addressing a triggering factor for AD moving beyond the approach of symptomatic relief.

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