

Putative NeuroImmune Mechanisms in Alzheimer's Disease: Modulation by Cholinergic Anti-Inflammatory Reflex (CAIR)

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Abstract

Senile dementia of the Alzheimer type (sDAT) is a progressive disease of the brain that afflicts primarily the elderly. Patients with sDAT lose cognitive, intellectual, functional, and social abilities, and become fully dependent upon their caregiver. As the "baby boomer" generation advances in age, it is predicted that by the end of the decade, over 7.5-10 million people will be diagnosed with sDAT in the US alone. Current interventions for sDAT include acetylcholinesterase inhibitors, indicated for patients with mild to moderate symptoms. Memantine treatment interferes with the glutamate neurotransmitter receptor system, and is the intervention of choice for advanced sDAT. Complementary and alternative medical treatments for sDAT are also widely used, but little is known about the optimal nature and dose of these therapies. We present an innovative interdisciplinary model to characterize more fully the underlying pathophysiological mechanisms in sDAT. The model is based on our current understanding of neuroendocrine-immune interactions, specifically the modulation of the cholinergic anti-inflammatory reflex. We propose that this view could pave the way for new and improved modes of intervention for patients with mild to moderate sDAT.

Keywords: Senile Dementia of the Alzheimer Type, Neuroimmunity, Cholinergic Anti-Inflammatory Reflex.

INTRODUCTION

People often lose mental sharpness with aging. Alois Alzheimer (1864-1915) first described a neuropathological syndrome in 1906 following autopsy on the brain of 56-year-old Augusta D. of Frankfurt. She had died following progressive mental deterioration, increasing confusion, and memory loss. He described an odd disorganization of the neurons in the cerebral cortex. The cells contained clusters suggestive of a rope tied in knots, which he named "neurofibrillary tangles." He also observed unexpected accumulation of cellular debris

around the affected nerves, the "senile plaques." Alzheimer speculated that the nerve tangles and plaques were responsible for the patient's dementia. Additional independent cases confirmed these patterns (Chiappelli *et al.*, 2006a).

At its onset, senile dementia of the Alzheimer's type (sDAT) manifests as simple forgetfulness, and progresses into difficulty in doing things that require planning, decision-making, and judgment. Taking care of self is impaired, and social withdrawal ensues. Simple tasks of daily living become progressively prohibitive. Patients lose interest in personal hygiene and sexual inhibitions. Communication is hindered as memory, thinking, and social behavior are impaired. Personality changes and agitation are not uncommon in the more advanced stages of sDAT, with loss of impulse control, belligerence,

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distrust, increased stubbornness, restlessness, and withdrawal even from close family members. As sDAT reaches its terminal stage, patients become, and often succumb of pneumonia or related complications (Chiappelli *et al.*, 2006a; Chiappelli *et al.*, 2006b).

sDAT is diagnosed with certainty *post mortem*. Examination of brain biopsies reveal that plaques and tangles take over and progressively devastate healthy neurological tissue. Clinical diagnoses obtained before death are reported as "probable" Alzheimer's disease, or as senile dementia of the "Alzheimer type" (hence, sDAT). Diagnostic accuracy approximates 90%, and is strengthened by genetic predisposition observed for sDAT: autosomal dominant presenilin genes are associated with early onset sDAT, as is apolipoprotein (APO) polymorphism (the more copies of its ApoE ϵ 4 allele, the greater the risk for sDAT) (Chiappelli *et al.*, 2006a).

Early diagnosis of sDAT provides a better chance of benefiting from treatment. Staging systems, while artificial benchmarks of a continuous disease process that vary greatly from patient to patient, are useful frames of reference for understanding how the pathology unfolds. The Global Deterioration Scale, one reliable diagnostic instrument we and others have used extensively to outline key symptoms of sDAT, describes seven key stages of the disease, from unimpaired function to very severe cognitive decline (Masera *et al.*, 2002; Chiappelli *et al.*, 2006a).

The neuropathology in sDAT mirrors advancing underlying neuronal degeneration. Nerve cell damage typically begins about the Amyloid plaques, clumps of β -amyloid protein ($A\beta$, 1-42 amino acids) that accumulate outside of cells, and which we can reliably detect in whole saliva (Chiappelli *et al.*, 2006c), and the neurofibrillary tangles, clumps formed by of altered τ (tau) protein inside cells. Neurotoxicity of these structures is clear particularly with respect to the plaques, which consist of extracellular deposits of $A\beta$ peptides, arranged into a β -pleated sheet conformation. Fragments are cleaved from the larger intracellular $A\beta$ precursor protein, which is associated with the plasma membrane. The β - and γ -secretase proteolytic enzymes, encoded by a presenilin gene, cleave the $A\beta$ precursor protein. Larger fragments aggregate more readily

than smaller peptides to form the characteristic plaques (Chiappelli *et al.*, 2006a).

$A\beta$ leads to a local acute phase response. This immune activation is associated with the production and the release of pro-inflammatory cytokines, and a chronic inflammatory response ensues. Activated glial cells (e.g., astrocytes and microglia) secrete interleukin (IL)-1 α , IL-1 β , IL-6 and tumor necrosis factor (TNF)- α , and chemokines that direct invasion of the brain parenchyma by circulating monocytes/macrophages and T lymphocytes. Generated oxidative radicals contribute to neurotoxicity. Cytokine-activated astrocytes further contribute to the production of $A\beta$ through cleavage of the $A\beta$ precursor by β -secretase upon exposure to interferon (IFN)- γ and TNF- α /IL-1 β mixtures. The cytokine S100 β , released by activated astrocytes, promotes the growth of dystrophic neurites in $A\beta$ plaques (Chiappelli *et al.*, 2006a).

The resulting concerted $A\beta$ -induced cellular immune response contributes to severe neuronal damage and apoptosis. Furthermore, neurotoxic effect of $A\beta$ are soon evident systemically, as it induces apoptosis, blunts functional responses of circulating lymphocytes and of monocytes/macrophages, and impairs natural killer activity and response to stimulating hormones, as well as the process of T lymphocyte maturation into memory cells, and psychoneuroendocrine-immune regulation (Prolo *et al.*, 2007; Chiappelli *et al.*, 2006a; Chiappelli *et al.*, 2006c; Masera *et al.*, 2002). It is timely and critical to acquire a novel perspective on sDAT that provides a satisfactory model for explaining these observations, so that improved modes of intervention can be devised and tested for patients with sDAT. Should we devise of an intervention that would blunt the activation of the cellular immune inflammatory processes in sDAT, we could arrest or slow down considerably the progression of sDAT to its more severe later stages.

This is particularly timely and critical because, at present, there is no cure for sDAT. Pharmacologic treatments are available that are aimed at improving or stabilizing symptoms. Atypical anti-psychotics and anti-convulsants with mood-stabilizing properties are commonly used to treat agitation in sDAT. Other FDA-approved drugs for early to moderate sDAT

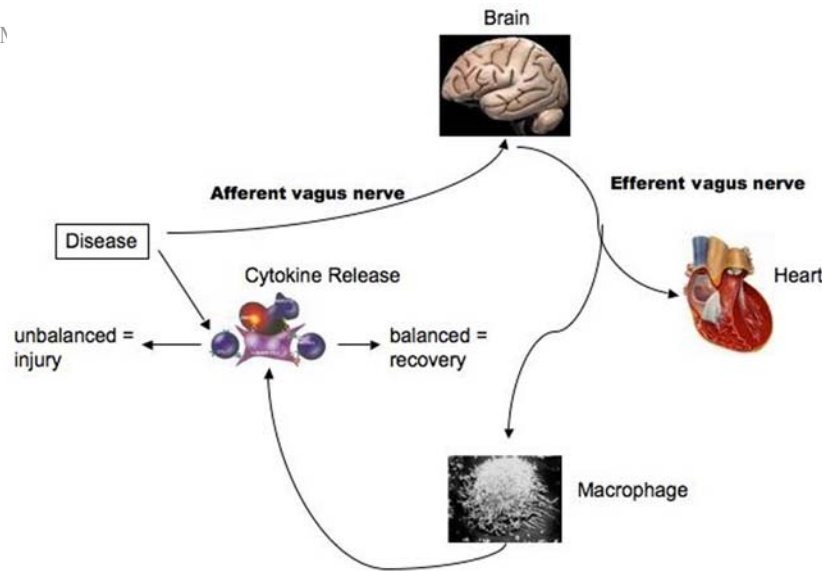


Fig-1: The Cholinergic Anti-Inflammatory Reflex (CAIR). Wiring of the cholinergic antiinflammatory pathway, which balances cytokine production. Pathogens and other forms of disease activate cytokine production, which normally restores health. If the cytokine response is unbalanced, then these same mediators can injure the body. Efferent signals from the vagus nerve, which can be controlled by brain networks, inhibit cytokine production via pathways dependent on the $\alpha 7$ subunit of the AChR on macrophages and other cells. Efferent vagus nerve activity is able to raise heart rate instantaneously. Afferent signals carried in the vagus nerve can inhibit cytokine release. Together, these pathways encompass the cholinergic inflammatory reflex.

include cholinesterase inhibitors (donepezil [Aricept®], rivastigmine [Exelon®], galantamine [Reminyl®], and tacrine [Cognex®]), which inhibit cholinesterase and maintain the neuronal levels of acetylcholine high, even as neurons die. About half of the patients who take cholinesterase inhibitors experience a modest improvement in cognitive symptoms, but serious side effects (e.g., Tacrine), such as liver damage have been reported. Memantine-HCl, a low affinity antagonist for N-methyl-D-aspartate (NMDA) receptor, is recommended for patients with moderate to severe sDAT, which typically exhibit few side-effects (e.g., dizziness, confusion, headache, constipation) (Chiappelli *et al.*, 2006d).

The public perceives these interventions not to be fully satisfactory, and complementary and alternative medical (CAM) interventions are often sought. The literature is mixed with respect to the safety, efficacy and the effectiveness of CAM interventions, although certain herbal remedies appear effective. Ginkgo biloba and polyphenols, derived from green tea or red fruit extracts, are among the most promising of these therapeutic approaches, since they have antioxidant properties. They seem to act by protecting against the $A\beta$ -mediated oxidative stress and inflammatory processes associated with plaque and tangle formation (Chiappelli *et al.*, 2006a).

In brief, the $A\beta$ -mediated processes associated with neuronal toxicity and death, and systemic impairment of the psychoneuroendocrine-immune regulatory system may be important target for effective treatment in early to moderate stages of sDAT. By blocking the inflammatory process, the progression of sDAT may be significantly slowed. To date, epidemiological studies suggest a protective effect of non-steroidal anti-inflammatory drugs against the development of sDAT, but controlled trials have failed to show protective effects (Eikelenboom & van Gool, 2006; Prolo & Chiappelli, 2007). The scientific validity of the following model in protecting against progression of sDAT rests on sound neuroimmunity research. Its clinical validation in the context of sDAT must now be tested in randomized double- blinded clinical trials.

MODEL:

Organisms in the animal kingdom respond to the invasion of foreign antigen by a series of concerted responses, which involve, as early in the evolutionary ladder as mollusks, phagocytic cells and factors that promote the inflammatory process. Vertebrates, from fish to man, seek to restore internal physiological balance (i.e., "homeostasis) following exposure to pathogens by means of a process termed "allostasis". The

allostatic response is complex at the level of interplay, interface and interaction of the psycho-

non-coding DNA in regulating all psycho-neuroendocrine-immune processes and events.

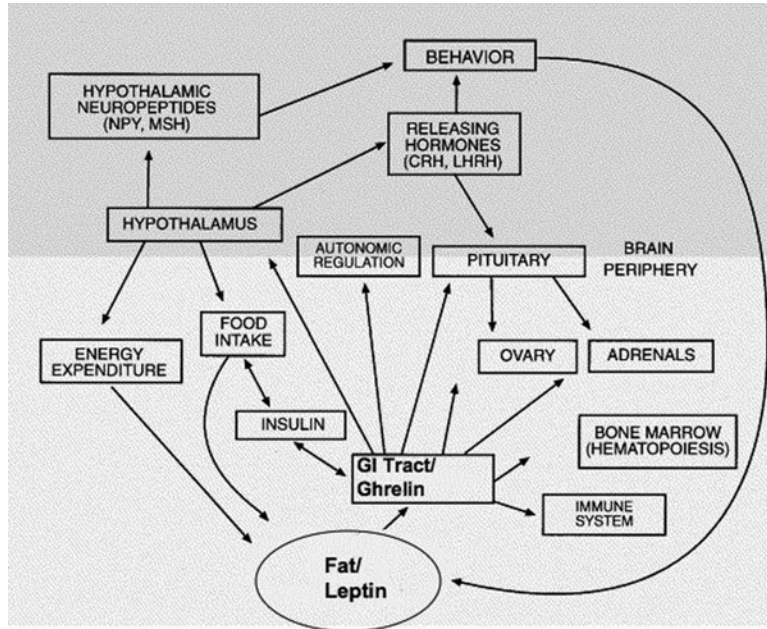


Fig-2: The biological effects of leptin and ghrelin. Fat induces leptin production, ghrelin comes mainly from the gastrointestinal(GI) tract. Both leptin and ghrelin have central and peripheral effects on the hypothalamus, immune system, angiogenesis, autonomic system, adrenals, ovary, endocrine pancreas, pituitary gland, and bone marrow. The hypothalamus is a key mediator of the effects of both hormones on the central nervous system, including neuroendocrine regulation, food intake, and energy expenditure.

cognitive, the nervous, the endocrine and the immune systems. Research has abundantly demonstrated that the physiological intricacies of these responses emerge at the level of cell biology, such that neurons and cells of endocrine glands typically express functional receptors for immune products (e.g., cytokines), and immune cells express functional cholinergic, adrenergic, opioid, steroid and other neuroendocrine receptors. Certain brain cells (e.g., astrocytes) can produce pro-inflammatory cytokines, and cells of the immune system manifest endogenous production of opioid peptides, ACTH and other hormones. The physiological relevance of this brain-hormone-immune crosstalk is evidenced both in the healthy organisms as it follows normal circadian fluxes, and in disease as the nature and role of these intertwined relationships act in concert to promote healing (Prolo & Chiappelli, 2007). It is now clear that these cellular biological events are controlled and regulated at the interactomic, protonomic and genomic levels. Moreover, it will come to no surprise to the experts in this field as research findings in the next decade will show and establish the critical role of post-genomics and

At the physiological levels, the interface between the brain and the immune response is regulated by feedback systems. One such feedback loop, the relevance of which we now propose in the context of sDAT is the cholinergic anti-inflammatory reflex (CAIR). Original descriptions of CAIR indicated that the systemic inflammatory responses to endotoxin could be modulated by the parasympathetic system: specifically acetylcholine (Ach), the principal neurotransmitter of the vagus and other parasympathetic cranial nerves, significantly attenuated the release of pro-inflammatory cytokines (e.g., IL-1 β , IL-6, IL-10, IL-18, TNF- α). Direct electrical stimulation of the vagus in experimental rodents *in vivo* during lethal endotoxaemia blunted the production of these cytokines, the inflammatory and the immune response, and prevented the development of pathophysiological symptoms (Bernik *et al.*, 2002). Later work replicated these observations with different inflammatory triggers, and modes of vagal stimulation (e.g., CNI-1493, a tetravalent guanlylhydrazone) (Tracey, 2002). These observations supported the conclusion that

inflammatory responses are dampened by the cholinergic system, and proposed new opportunities for treating diseases characterized by inflammatory processes through the selective, finely regulated, and reversible 'hard-wired' neural systems, that is CAIR (Tracey, 2002; Romeo *et al.*, 2001) (Fig. 1).

The field has recently expanded to include hormones, such as ghrelin and leptin, which were primarily associated with obesity. Whereas evidence supports the notion that intravenous infusion of ghrelin stimulates appetite in healthy volunteers and cancer patients, a recent study suggests that ghrelin signals are transmitted to the brain via vagal afferent nerves. Central administration of ghrelin stimulates the vagal efferent nerve in anesthetized rats (Wu *et al.*, 2007). Leptin has already revealed a complex regulatory neuroendocrine network (Prolo *et al.*, 1998) (Fig.2). Leptin and ghrelin send complementary, yet antagonistic, signals reflecting acute and chronic changes in energy balance, the effects of which are mediated by hypothalamic neuropeptides, including neuropeptide Y and agouti-related peptide. Endocrine and vagal afferent pathways are involved in these actions of ghrelin and leptin. Since it has been very recently demonstrated that ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve (Wu *et al.*, 2007), pharmacologic stimulation of the vagus nerve may offer a novel approach in all those conditions like neurodegenerative disorders that are characterized by a boost of pro-inflammatory cytokines, such as IL-6, IL-10 and TNF- α .

In summary, CAIR refers to the afferent signals to the brain, transmitted via the cranial nerves (e.g., the vagus) that activate an anti-inflammatory reflex response, which then culminates in efferent vagus nerve signals. This 'hard-wired' connection between the nervous and immune systems can be harnessed therapeutically in animal models of inflammatory disease, via direct electrical stimulation of the vagus nerve, or by means of cholinergic agonists that specifically activate the macrophage $\alpha 7$ subunit of the ACh receptor. Autonomic dysfunction in human inflammatory diseases and the characterization of CAIR contribute to bringing forward promising novel therapeutic strategies.

DISCUSSION

That cholinergic agonists inhibit cytokine synthesis and protect against cytokine-mediated diseases through CAIR, is now well established and supported by over a decade of concerted experimental research. This research has led to pre-clinical testing of stimulation of the vagus nerve to test the effectiveness of CAIR in preventing the damaging effects of cytokine release. The results support the expectations that CAIR works *in vivo*, and that vagal stimulation can substantially decrease the inflammatory response in a variety of conditions, from experimental sepsis, to endotoxemia, ischemia/reperfusion injury, hemorrhagic shock, arthritis, and a variety of other inflammatory syndromes (Romeo *et al.*, 2001). The general nature of this system has led to the proposition that this physiological, functional anatomical mechanism for neurological regulation of cytokine-dependent diseases may define an "immunological homunculus". That it can be traced and identified along the course of the cranial nerves in the brain supports early observations by our laboratory on the role of the glossopharyngeal nerve in the neuroimmune surveillance of the oral cavity (Sjögren *et al.*, 2002).

It is now evident that pro-inflammatory cytokine production by the immune system contributes importantly to both health and disease. The nervous system, via its autonomic arm, and specifically CAIR, regulates, modulates and primarily blunts pro-inflammatory cytokine release. Activation of parasympathetic cranial nerves, of which the vagus is unquestionably the better studied at this time because it has the most widespread systemic implications, decreases the production of cytokines that promote inflammation, thereby preventing tissue injury (Romeo *et al.*, 2001).

Here, we propose that it is possible and even probable that research will soon demonstrate the critical role of CAIR in dampening the inflammatory process in sDAT. We expect that CAIR will be shown to slow down the neuronal damage derived from the inflammatory response to plaques and tangles, and to retard the progression of early to moderate sDAT to the more advanced stages. We argue that the neuroimmunity model of immunophysiology and more specifically its CAIR component provides a critical novel mode of clinical intervention for

preserving patients with sDAT at the early to moderate stages of sDAT. Randomized, double-blind control trials will be constructed to test the putative effectiveness and the efficacy of the efferent neural signaling pathway, the cholinergic antiinflammatory pathway (i.e., CAIR) in modulating the psychoneuroendocrine-immune processes observed in sDAT.

Related studies have already established that vagus nerve stimulation, an established treatment for therapy-refractory epilepsy, leads to improved cognitive abilities in these patients. Use of an implanted of the vagus stimulator (NeuroCybernetic Prosthesis) was shown to lead as well to significant improvements in ADAS-cog and MMSE scores at three and six months of vagal stimulation (which, it is important to note, is well tolerated, and with mild and transient side effects) (Merrill *et al.*, 2006). These observations were replicated, and, moreover, in parallel to the cognitive improvement following 1 year of vagal stimulation (again, well-tolerated), sDAT patients did not show the expected progressive impairment in mood, behavior, or quality of life characteristic of sDAT progression, and that cerebrospinal fluid levels for total τ decreased by 4.8% ($p=0.057$) (Auld *et al.*, 1998).

Overproduction of pro-inflammatory cytokines can cause serious clinical manifestations. In the specific context of this discussion of sDAT, data propose that anti-inflammatory interleukins such as IL-10 regulate β A-mediated microglial inflammatory responses by means of inhibiting cytokines of the pro-inflammatory family (e.g., IL-6). In point of fact, co-occurrence of IL-10_A and IL-6_C polymorphic alleles significantly raised the odds ratio for sDAT (OR 11.2, CI⁹⁵ 1.3-97.3) independently of apolipoprotein E4 (adjusted OR 10.3, CI⁹⁵ 1-108) (Arosio *et al.*, 2004). The findings of genetic polymorphism in sDAT favoring IL-10 was recently confirmed (Bagnoli *et al.*, 2007). Evidence is mounting for a significant role of IL-10 in neuroimmune regulation. As IL-10 predominantly exerts anti-inflammatory actions and suppresses TH1-dependent immune responses, the insulin and insulin-like growth factor I (IGF-I) system appears to ply a central role since it blunts inflammatory and TH1-mediated cellular immune responses through IL-10 (Kooijman *et al.*, 2004). Indeed, the correlation between altered IL-10 levels and production, and altered activity and distribution of immune peripheral

cell subpopulations in patients with sDAT is now well established, and hypothetically points to a mechanistic role for IL-10 and its regulation in the suppressor cell function (e.g., Tregs) in sDAT patients. Taken together, this body of evidence has led to the hypothesis that sDAT pathology may actually derive at its onset from deregulation of the IGF-I signaling complex, from which it might follow that sDAT could termed a "type 3" form of diabetes (Steen *et al.*, 2005). Case in point, the clinical observation that reduced glucose utilization and energy metabolism occur early in the course of sDAT, and correlate with impaired cognition, such that this newly identified form of diabetes mellitus, type 3, shows strong and convincing evidence of progression with advancing severity of neurodegeneration (Rivera *et al.*, 2005). Moreover, this IL-10/IGF-I paradigm is intertwined with the model of CAIR outlined here since, at the protonomic level, choline acetyltransferase expression increases with insulin or IGF-I stimulation in cortical neurons, but is reduced in sDAT (Rivera *et al.*, 2005). At the genomic level, evidence is mounting for an interactomic role of IGF-I regulating the expression of genes such as IL-10 by mechanisms that involve modification of chromatin architecture, such as histone phosphorylation, acetylation, and methylation, and specifically acetylation of histone H3 and H4 (Sun *et al.*, 2006).

The inflammatory response, such as that produced by plaques and tangles in sDAT, pertains to an immunophysiological pathway in which the autonomic nervous system detects the presence of inflammatory stimuli, and modulates cytokine production. Afferent signals to the brain, transmitted via the cranial nerves (e.g., the vagus), activate an anti-inflammatory reflex response (i.e., CAIR) that culminates in efferent vagus nerve signals. This 'hard-wired' connection between the nervous and immune systems can be harnessed therapeutically in animal models of inflammatory disease, via direct electrical stimulation of the vagus nerve, or by means of cholinergic agonists that specifically activate the macrophage $\alpha 7$ subunit of the ACh receptor. Autonomic dysfunction in human inflammatory diseases and the characterization of CAIR contribute to bringing forward promising novel therapeutic strategies for these pathologies (Tracey, 2007; Romeo *et al.*, 2001).

The relevance of CAIR to sDAT is all the more convincing when one recalls the early observation that A β can potently inhibit cholinergic neurotransmitter functions, in addition to its noted neurotoxicity. *In vitro* mechanistic studies have shown that physiologically relevant concentrations of A β -related peptides have acute negative effects on Ach synthesis and release; and that activation of certain cholinergic receptors (e.g., $\alpha 7$ subunit of the ACh receptor) actually impairs the processing of the A β precursor protein and the phosphorylation of τ . Taken together these cellular and molecular findings establish a direct interaction between A β and nicotinic ACh receptors (Kar *et al.*, 2004), and provide a mechanistic explanation for the clinical effects of vagal stimulation of τ levels (Auld *et al.*, 1998). In brief, clinical, animal, cellular and molecular mechanistic studies offer concerted support for a timely experimental pre-clinical test of CAIR in sDAT, which could lead to the development of new promising neuroimmune-based interventions and their assessment by well-designed clinical trials for the benefit of sDAT patients, their caregiver and society at large.

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