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Commentary

Treatment implications of the altered cytokine-insulin axis in neurodegenerative disease

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ABSTRACT

The disappointments of a series of large anti-amyloid trials have brought home the point that until the driving force behind Alzheimer's disease, and the way it causes harm, are firmly established and accepted, researchers will remain ill-equipped to find a way to treat patients successfully. The origin of inflammation in neurodegenerative diseases is still an open question. We champion and expand the argument that a shift in intracellular location of α-synuclein, thereby moving a key methylation enzyme from the nucleus, provides global hypomethylation of patients' cerebral DNA that, through being sensed by TLR9, initiates production of the cytokines that drive these cerebral inflammatory states. After providing a background on the relevant inflammatory cytokines, this commentary then discusses many of the known alternatives to the primary amyloid argument of the pathogenesis of Alzheimer's disease, and the treatment approaches they provide. A key point to appreciate is the weight of evidence that inflammatory cytokines, largely through increasing insulin resistance and thereby reducing the strength of the ubiquitously important signaling mediated by insulin, bring together most of these treatments under development for neurodegenerative disease under the one roof. Moreover, the principles involved apply to a wide range of inflammatory diseases on both sides of the blood brain barrier.

1. Introduction

Research into the cause(s) and mechanism of Alzheimer's disease (AD) continues to make little headway in terms of approved agents for therapeutic use. Despite long-term research into the possible pathogenic relevance of amyloid β (Aβ) in histological sections of afflicted brains, clinical trial outcomes for agents designed to reduce Aβ generation and deposition imply that worthwhile therapeutic targets lie elsewhere. Many alternatives to the Aβarguments have been sidelined for decades by the dominance of amyloid theories, and thus largely untested, can give the impression of being unrelated to each other, adding to the confusion rather than solving it.

In our view most of these neglected alternative targets, and others in the literature, are indeed related, fitting into an inflammatory causation model of AD, albeit not always interpreted as such. In this model, which we have recently discussed [1], a chronic excess of inflammatory cytokines, perhaps directly but much more likely through reductions in insulin sensitivity, lead to this disease. Insofar as space allows, we also discuss the larger picture of how excessive levels of these harmful cytokines might have arisen, how they harm, and how this view of the neurodegenerative states fit into a broader pattern of disease pathogenesis and treatment.

2. Inflammation and disease

The term inflammation, traditionally the preserve of swollen red extremities replete with leukocytes, nowadays encapsulates the clinical consequences of overproduction of the many cytokines that also mediate innate immunity. Yet in lower concentrations probably all of these cytokines – a few are yet to be examined in this light – are essential for homeostasis in normal physiology. When generated in excess these peptides become, as inflammatory cytokines, a general mechanism of disease, the phenotype of which is determined by the precise site and rate of production, and what is present in the mix. Others, with opposing functions, the anti-inflammatory cytokines, can be useful in excess and harmful when lowered. These principles are now accepted to cover a surprisingly

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wide part of the systemic diseases spectrum, rendering inflammation as a therapeutically approachable, and functionally important component of disease pathogenesis in general (Table 1). Evidence is also emerging that these phenotypically diverse conditions are closely linked pathophysiologically, such that they constitute a single conceptual, and therapeutic, challenge. In practice, therefore, a solution to any one of these conditions is likely to be applicable to many (Table 1).

2.1. Inflammation – an early step in the initiation of neurodegenerative diseases

The idea of inflammation preceding amyloid plaque deposition in AD, rather than following it and helping its clearance, effectively began with studies [2] demonstrating that overexpression of interleukin 1 (IL-1), a cytokine generated in concert with tumor necrosis factor (TNF), and functionally overlapping it (Section 8.1), has a role in amyloid plaque formation in AD brains. TNF levels in the CSF from individuals with mild cognitive impairment (MCI), when tracked over a period of time, predicted the likelihood of developing frank AD [3]. Markers of inflammation have since been observed in serum and CSF long before indications of increased Aβ or hyperphosphorylated tau [4,5]. Increased levels of soluble TNF receptors in serum and CSF were also predictive of conversion to clinical AD while circulating clusterin (apolipoprotein J), induced by TNF and IL-1, particularly when both are present [6], was found 10 years earlier than fibrillar Aβ deposition, potentially representing an excellent predictor of onset, progression, and severity of the disease [7]. It was also noted that in contrast to what would be expected if inflammation cleared Aβ, anti-TNF, not TNF, reduce amyloid plaques in transgenic mouse models of AD [8]. The role of tau hyperphosphorylation in an inflammation context has been previously reviewed [1].

The genetic association of a variant *TREMS*, the gene controlling production of the triggering receptor expressed on myeloid cells 2 (TREM2), with AD provided additional evidence that it is fundamentally an inflammatory disease. Two large patient-based studies suggested that loss-of-function mutations in *TREMS* were associated with an increased risk of AD [9,10]. TREM2 is expressed on a number of cells, including those of macrophage lineage, soon after they differentiate. In cultured microglia, knockdown of TREM2 with short hairpin RNA leads to increased inflammatory cytokine transcripts [11] while $TREM2^{-/-}$ mice exhibited greatly increased responses to a number of triggers for TNF production, including CpG oligodeoxynucleotides, where the unmethylated sequences sensed by TLR9 [12] occur.

2.1.1. TLR9 sensing hypomethylated DNA

The cell-surface Toll-like receptors (TLRs), including TLR1, TLR2, TLR4, and TLR6, recognize microbial membrane lipids, whereas TLR3, TLR7, TLR8, and TLR9 recognize, or sense, pathogen-derived DNA in intracellular compartments. TLR sensing is well developed across the primitive phyla, but TLR9 is thought not to have developed until the appearance of vertebrates, in which it was associated with a transition from a mosaic to global pattern of DNA methylation, restricted to CpG-rich regions [13]. Thus TLR9 could detect the presence of pathogens such as bacteria and DNA viruses.

TLR9 is widely distributed in the CNS, as elsewhere, with many little-understood roles in brain development and function, including spatial learning and memory [14]. Once activated, TLR9 responds by releasing the cytokines that constitute the innate immune system [15], yet cause disease in excess, whatever agent triggers their release. This activity of bacterial DNA provides a direct link in logic to present understanding of the pathogenesis of trauma [16], stroke [17], and acute heart failure [18], where mitochondrial DNA, on release from damaged human cells, is sensed by TLR9 as if it were of bacterial origin, thereby triggering the inflammatory cytokine cascade. Mitochondrial DNA thus mimics the action of bacterial DNA, with which, because of their common evolutionary heritage [19], shares a higher degree of hypomethylation than adult mammalian DNA the latter of which is not usually sensed by TLR9 [13]. However, fetal DNA, which is innately hypomethylated, can be recognized by maternal TLR9 in premature birth and pre-eclampsia [20].

2.1.2. Hypomethylated human DNA in neurodegenerative diseases

DNA hypomethylation, an epigenetic change, is central to human aging [21]. It has also been implicated in the pathogenesis of AD, since any inheritance of late-onset AD (LOAD) – by far the most common form of the disease – is non-Mendelian and concordance rates in monozygotic twins are low, and levels of folate and homocysteine in the AD brain are consistent with an abnormal methylation homeostasis [22–25]. These same pathways may also be relevant in Parkinson's disease (PD) [26] and type 2 diabetes mellitus (T2DM) [27].

The literature on the methylation status of mammalian DNA generally relates to the regulation of the promoter activity of individual genes, a key mechanism of learning and memory [28] as well as activity-dependent regulation of the adult nervous system [29]. Nevertheless, well over 90% of human DNA is non-protein-coding. As well as being transcribed to form non-coding RNA (Section 9), the bulk of this "junk" DNA is not only susceptible to hypomethylation, but is said to exist for its epigenetic potential [30]. The possibility of mammalian brain DNA being sufficiently hypomethylated to be sensed by local TLR9 is little discussed. Ample evidence exists that cell-free DNA normally reaches body fluids [31,32]. TLR9s are widespread in the brain, being present on neurons, where they increase with aging [33], and also on astrocytes and microglial cells [34]. All three cell types, when

Table 1
The range of sterile inflammatory disease states currently reported to respond to either of two, or both, different anti-cytokine biologicals and the GLP-1 mimetics, which correct insulin resistance.

	Anti-TNF agent		Anti-P40 agent (anti-IL-12, IL-23, i.e. anti-IL-17)		GLP-1 mimetic	
	Animal models	Patients	Animal models	Patients	Animal models	Patients
Alcohol addiction					[155,171]	
ALS (motor neuron disease)	[156]				[172]	
Alzheimer's disease	[8]	[161]	[165]		[110]	
Crohn's disease	[162]		[166]	[169]		
Parkinson's disease	[157]				[173]	[178]
Post-irradiation brain	[158]				[174]	
Psoriasis			[167]	[170]		[179]
Rheumatoid arthritis	[159]	[163]				
Stroke	[164]		[168]		[173,175]	
Traumatic brain injury	[160]	[164]			[176,177]	

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