Neuroinflammatory Cytokines – The Common Thread in Alzheimer Pathogenesis

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Abstract

This article discusses the potential role of the cytokine cycle and its corollary as drivers of the relentless progression of Alzheimer's neuropathologies, whether they are the result of gene mutations, gene polymorphisms and/or environmental and comorbid conditions. Based on the discovery of cytokine overexpression as an accompaniment to the dementia-related glial activation, the cytokine hypothesis was proposed. This states that in response to the negative impact on neurons of known and unknown risk factors – which include genetic inheritance, comorbid and environmental factors – microglia and astrocytes become activated and produce excess amounts of the immune-modulating cytokine interleukin-1 (IL-1) and the neuritogenic cytokine S100B, respectively. Finding that these glial events occur in foetuses and neonates with Down's syndrome provided the first evidence that productive immune responses by activated glia precede rather than follow overt AD-related pathology. This finding can be added to the demonstration of IL-1 induction of amyloid-β (Aβ) precursor protein and astrocyte activation with excess production of neuritogenic factor S100B. This combination suggests that IL-1 and S100B overexpression would favour the Aβ production and dystrophic neurite growth necessary for laying down neuritic Aβ plaques. This, together with demonstration of IL-1 induction of excessive production of the precursors of other features common in AD prompted a corollary to the cytokine hypothesis. The corollary states that regardless of the primary cause of the neuronal insult, the result will be chronic glial activation, which in turn will result in further neuronal injury, still more glial activation with excess cytokine expression and so on. This article discusses known causes, genetic and environmental risk factors, and comorbid conditions and the potential contribution of glial activation with excessive cytokine expression to each.

Keywords

Alzheimer's disease, amyloid β, apolipoprotein E, β–amyloid precursor protein, cytokines, Down's syndrome, excitotoxicity, glutamate, interleukin-1, S100B, tumour necrosis factor alpha

Alzheimer's disease (AD) is the epitome of a progressive degenerative disease of the brain and culminates in dementia characterised in the beginning stages as a failure to retain new information for appropriate retrieval. As the underlying disease progresses, the patient experiences more profound memory loss and retrieval dysfunctions. These include not only the loss of skills related to activities of daily living, but may also include personality changes associated with inappropriate or psychotic behaviours. These changes in cognitive, behavioural and social functions are now known to be preceded by neuropathological changes in regions of the brain involved in registering, storing and recalling new information in appropriate circumstances and for the appropriate duration. The most striking and diagnostic neuropathological change in the Alzheimer's brain is the presence of amyloid-β (Aβ) plaques lying within the neuronal layers in cortical regions that are important in acquiring, storing and retrieving information. These regions include

temporal lobe structures, such as the hippocampus, as well as frontal and parietal regions. It is the number of Aβ plaques that contain dystrophic neuronal processes (Aβ neuritic plaques) in affected brain regions that provide the neuropathological confirmation of the clinical diagnosis of AD. Many neurons within these plaque-laden cortical layers contain within their cytoplasm fibrillary tangles composed of hyperphosphorylated tau coiled into paired helical filaments, the so-called neurofibrillary tangles.

A progression of these neuropathological events seems apparent. For example, the plethora of Aβ plaques at end stages – from several to many – and an inclusion of neurofibrillary tangles – in a few to numerous neurons – strongly suggests that these neuropathological changes are cumulative. Similarly, progression of plaques from a non-neuritic, diffuse appearance to one of compact deposits is suggested by the appearance of different Aβ plaque types. These

include diffuse Aβ deposits, diffuse neuritic Aβ plaques, dense-core neuritic Aβ plaques and dense core non-neuritic Aβ plaques within a single tissue section.

Neurofibrillary progression is suggested by the formation of 'threads' of paired helical filaments scattered within neuronal cytoplasm, along with other cells in which fully-formed tau tangles push aside the nucleus and acellular tangles that appear to have remained after the death and dissolution of a neuron.

Such progression suggests the presence of driver(s) of these events, which, even without repetition of the causative event(s) or agent(s), result in self-propagation of the neurodegenerative events that culminate in increasing densities of these neuropathological changes. This article discusses the common themes in neuropathogenesis:

- potential causative factors (gene mutations and gene duplication);
- risk-conferring factors (gene polymorphisms, environmental and comorbid conditions and ageing); and
- omnipresent glial activation and excessive expression of putative drivers (these have been found to be factors that promote furtherance of neurodegenerative events).

Causative Factors
Gene Mutations – *APP* and *PSEN1* and *PSEN*2
A small number of families worldwide have a high percentage of individuals who develop AD at an early age, usually well before 65 years of age, suggesting autosomal-dominant inheritance. Glenner's discovery that the so-called senile plaques in the brain of AD patients are largely composed of Aβ highlighted the importance of this peptide in AD pathogenesis.[1,2] This discovery was quickly followed by others demonstrating the structure and size of the Aβ peptide necessary for fibril formation[3,4] and mapping of the amyloid precursor protein (*APP*) gene for the β-amyloid precursor protein (βAPP) to chromosome (Chr) 21.[5–8]

The known occurrence of families with autosomal-dominantly inherited AD led to studies linking mutations in the βAPP coding region to familial AD (FAD).[5,9,10] Although additional family-linked *APP* gene mutations were identified in subsequent searches, they did not account for all the known familial cases. Studies of such families revealed specific mutations in two other gene sequences that are causative for AD: presenilins (*PSEN*) 1 and 2. These genes are located on Chr 1 (*PSEN2*) and Chr 14 (*PSEN1*).[11–14] Inheritance of any one of these autosomal-dominant, highly penetrant missense mutations in the *APP* or *PSEN* genes causes the development of early-onset AD. More recently, early-onset cases have been attributed to promoter mutations that merely elevate the expression of βAPP.[15]

Taken together, mutations in the *APP*, *PSEN1*, and *PSEN2* genes account for the vast majority of familial cases.[16] Of the three genes, the 173 recognised mutations in *PSEN1* are responsible for most cases.[17] Overproduction of longer forms of Aβ is the common thread between these mutations[17] and families with them exhibit the entire array of neuropathological changes characteristic of AD at an accelerated pace, resulting in early onset. It is notable that increased expression of βAPP is characteristic of a number of lifestyle and environmental factors associated with increased risk for later development of AD. Moreover, lifestyle risks and genetic factors are likely additive and, thus, may accelerate onset.[18]

Insights into the mechanistic relationships between these mutations and neuropathology have been afforded through transgenesis of mice. These have had either wild-type[19] or mutated human βAPP sequences,[20,21] mutated PSEN1,[22–25] mutated PSEN1 plus mutated βAPP[26,27] or these plus mutated human tau.[28] Mouse models have produced evidence of glial activation,[29,30] as well as specific receptors and related signaling molecules[31–33] or specific cytokines and other inflammatory factors[34–36] related to Alzheimer's pathogenesis. Such models have also been used to examine broader phenomena and potential interventions, including the protection afforded by exercise[37] and the benefits of non-steroidal anti-inflammatory drugs (NSAIDs).[38–43] Taken together, these findings and the many others in which transgenic mice were used have added greatly to the understanding of molecular biological mechanisms of AD pathogenesis. They have also shown mechanisms, cytokines and other factors that may act as drivers of such mechanisms.

Gene Duplications – Down's Syndrome
A link between Chr-21 genes and Down's syndrome (DS; trisomy 21) was suggested by the finding of Alzheimer's-like clinical and neuropathological changes at middle age in individuals with DS.[44,45] This suggestion was further supported by mapping of the *APP* gene to chromosome 21.[5,6,10,46,47] It is conceivable that any of the other Chr-21 genes could contribute to AD pathology in conventional cases of trisomy, but DS resulting from a partial rearrangement of Chr-21 sequences excluding the *APP* gene was not associated with precocious AD pathology.[48] More recently, duplications of the *APP* locus alone have been identified in French, Dutch and Japanese pedigrees of FAD.[49,50] None of the affected individuals has DS, parsing AD symptomatology with the *APP* gene itself. Duplication of the *APP* gene in either DS or FAD elevates its messenger RNA (mRNA) levels by about 50 %,[51] which is consistent with faithful transcription of the 1.5-fold gene number. The steady-state levels of βAPP protein are greater than expected, however, from the 1.5-fold gene load in trisomy.[5,52] These increases do not occur until advanced age in either DS[53] or mouse models thereof;[54] the increases may even be brain-specific.[55]

These circumstances suggest that the expected increase owing to gene loading is augmented by factors that act to elevate βAPP protein levels, perhaps including activated glia and the cytokines they overexpress in the mature brain.[56,57] This idea, together with previous studies suggesting a role for glia in plaque formation,[58,59] is supported by a series of investigations demonstrating the potential of these cytokines to act as generators and promoters of the neuropathological sequelae of AD, i.e.:

- Aβ plaques;[60–62]
- neurofibrillary tangles;[63–66]
- dysregulation of neurotransmitters;[67]
- growth factor levels;[68] and
- DNA damage and neuron loss.[69–72]

A perhaps more accessible link between AD and DS neuropathologies is afforded by a recently discovered connection between lens pathology in DS and AD, i.e. accumulation of Aβ in the lens and accompanying accumulation in the brain.[73] A great deal of work has been accomplished using mouse models of DS[74] as well as DS fibroblast cell lines.[54,75–78] In particular, work has demonstrated that βAPP fragments, but not Aβ, are responsible for disruptions in the endosomal dysfunction associated with failure to clear unwanted proteins.[78]

The Common Thread – Glial Activation and Cytokine Expression

Familial Alzheimer's Disease

In a neuropathological analysis of cerebellar tissue from FAD (APP Val717 → Ile: V717F), glial activation was consistently and markedly elevated compared to sporadic AD (SAD). This was correlated with a 25 % decline in Purkinje cell numbers.[79] By contrast, Aβ plaque density – whether extracellular, vascular or perivascular – did not differ between FAD and SAD tissues.

Associations have already been made in AD between increased glial activation and excessive cytokine expression and cell death,[70,71,80] and similar relationships have been observed in mouse models.[81] Both human and mouse models, as well as the relationship between cytokines and neurodegeneration, are discussed in a recent review.[82]

Down's Syndrome

DS has been suggested as a human model for studying the progression and potential drivers of AD pathogenesis.[83] This is because:

- the extra copy of Chr 21 in DS is present in the zygote so that all the players, including the 1.5-fold gene loading and its downstream consequences, are in place at conception; and
- the innate and, to date unstoppable, outcome is AD.

Microglia and astrocytes had previously been found expressing excessive amounts of the systemic immune response-generating cytokine interleukin-1 (IL-1) and astrocyte-derived neuritogenic cytokine S100B, respectively, in the AD brain. Considering the relationships between DS and AD, Griffin et al. set out to assess elements of an innate immune response in the DS brain. They wanted to explore the possibility that changes that are nearly universal in the DS brain would render it a suitable system for studying drivers of AD pathogenesis.

Compared with the brains of non-DS individuals of similar ages, IL-1 and S100B were highly overexpressed in activated glia, even in the brains of foetuses, neonates and children with DS.[56] This age distribution is important; neither Aβ plaques nor neurofibrillary tangles were noted in these brains, nor have these AD neuropathological anomalies been reported in brains from DS individuals of these ages.[84,85] Thus, several studies underscore the importance of these cytokines and precocious development of Aβ plaques and neurofibrillary tangles in DS. These show:

- the increase in IL-1 and S100B expression in DS prior to the detection of either Aβ plaques or neurofibrillary tangles;[56,57]
- the capacity of both IL-1[86] and S100B to elevate neuronal βAPP;[87]
- the induction of tau phosphorylation through IL-1-induced activation of mitogen-activated protine kinase p-38;[65] and
- the relation of overexpression of IL-1 and S100B to both Aβ plaque[62,88,89] and neurofibrillary tangle[63,64] development.[57,83]

In addition to these DS-related changes that are accompanied by dramatically increased levels of βAPP,[90] the resulting overexpression of the βAPP cleavage fragment βCTF causes endosomal dysfunction.[78] Griffin et al. propose that this βCTF expression-dependent increase in endosomal dysfunction is indirectly related to the upregulation of IL-1 by excessive DS-related increases in βAPP and the resulting release of another βAPP cleavage fragments secreted (s)APP. Such sAPP induces glial activation and excessive production and release of IL-1[91] and bCTF, which results in endosomal dysfunction and leads to the build-up of unwanted proteins. This induces glial activation and excessive production and release of IL-1[91] and βCTF, which results in endosomal dysfunction and leads to the buildup of unwanted proteins.[92]

These events may create another opportunity for progression through positive feed-forward. The APP intracellular domain, produced by γ-secretase cleavage of βCTF, is capable of promoting AD-related symptoms – including tau pathology and inhibition of neurogenesis – in a manner sensitive to NSAIDs.[93] This may be related to the expanding evidence for an inhibition of neurogenesis by IL-1.[94,95]

Animal Models

The creation of mouse models of FAD through transgenesis with mutations or deletions of *APP*, *PSEN1* and *PSEN2* have greatly aided in the understanding of AD pathogenesis.

Glial activation is a prominent feature in these models from the first FAD-mutant APP (V717F) transgenic mouse model.[20] In more recent studies, astrocyte activation in V717F transgenic mice was shown to be accompanied by microglial activation months before the appearance of Aβ plaques in these animals.

Compared with littermate-controls (non-transgenic mice), V717F mice had elevated numbers of activated astrocytes overexpressing S100B as early as two months of age, while the first Aβ plaques were not found until eight months.[96] The tissue levels of S100B mRNA and protein were elevated at each month from the second to eighth and from that time were correlated with Aβ load in these mice.[96] In addition, the expression of the IL-1β-converting enzyme is greatly increased in these mice, again before plaque formation, and was closely correlated with neuronal DNA damage and cell loss. This is consistent with the idea that increases in active IL-1β contribute to Aβ plaque formation and to neuronal degeneration in mouse models of AD and by analogy to AD.[70] Accompanying this astrocyte activation in V717F transgenic mice, there is microglial activation with overexpression of IL-1 and other inflammation parameters, again prior to Aβ plaque deposition.[97]

Crossing another APP-transgenic mouse (Tg2576) with transgenic mice overexpressing S100B (TghuS100B) exaggerated the glial activation and increased expression of S100B above the level in the TghuS100B mice. It also increased expression of IL-1β, IL-6 and tumour necrosis factor alpha (TNF-α) above the expression level in Tg2576 mice.[34] The increase in glial activation and overexpression of these cytokines was an early phenomenon that foreshadowed an increase in plaque density and Aβ levels.[34]

A study of a mouse model in which PS-1 and PS-2 functions were eliminated (*PSEN1* null mutation with conditional knockout of *PSEN2*) found:[98]

- substantial glial activation;
- neuronal loss in the cortex and hippocampus;
- cortical and hippocampal atrophy; and
- tau hyperphosphorylation with tangle-like intraneuronal inclusions.

All of this occurred with no additional production of Aβ. The activation of glia and the accompanying increase in cytokines, such as S100B

and IL-1, may contribute to this neuronal dysfunction and loss, as demonstrated by Mori et al.[34] and Sheng et al.[70]

Growing evidence has suggested beneficial roles for microglial activation in transgenic mouse models of Aβ deposition. For instance, plaque burdens are increased by deficiencies in the chemokine receptor CCR2[99] or in the complement system.[99] Indeed, Aβ deposition occurs efficiently in the near absence of microglia.[100] Nevertheless, considerable evidence indicates that activated microglia and their products are detrimental in many experimental models and natural disease states. It is likely that microglia could contribute to Aβ removal while simultaneously producing agents that are detrimental to neurological structure/function and conducive to progression of pathology. As evidence, genetic ablation of the microglial fractalkine receptor CX_3CR1 prevents neuronal loss in an APP-transgenic line: 20305648.[37]

Gene Polymorphisms as Risk Factors for Development of Alzheimer's Disease

Genetic anomalies, such as the gene mutations and duplication discussed above, account for very few of the many sporadic AD cases. Inheritance of variants in specific sequences, i.e. polymorphisms, of a relatively small number of genes may contribute to these sporadic cases. Several such polymorphisms have been implicated in modifying the risk for development of AD.

In this article, the discussion includes the most important polymorphism, the ε4 variant of the apolipoprotein E (*APOE*) gene, which is moderately penetrant and confers high risk for development of AD. For the purpose of establishing a link between neuroinflammatory glial activation and excessive expression of proinflammatory cytokines, the genetic links to just two of these cytokines, TNFα and IL-1, are reviewed here. This exclusivity leaves further discussion of other important factors involved in AD pathogenesis to another review. These include components of the complement system and many other factors[101–103] that doubtless contribute to such pathogenesis and are deserving of a more sharply focused discussion.

Apolipoprotein E Gene

The gene polymorphism that is most important in AD risk is a single nucleotide substitution in the coding region of the *APOE* for apolipoprotein E (ApoE) on Chr 19, creating what is termed the ε4 allele. The resulting gene product – ApoE4 – has an arginine–cysteine substitution at position 112 of its amino acid sequence. This alters the interaction of the protein with its receptor and increases AD risk.[104,105] Individuals with two copies of the ε4 allele of APOE have a 50–90 % chance of developing AD by the age of 85 and those with one copy have about a 45 % chance.

The mechanisms underlying the genetic influence of *APOE* ε4 on AD risk are unknown, but hypotheses include an interaction with Aβ as plaques develop.[106] Inheritance of *APOE* ε4 is associated with increased Aβ plaque density[107] as well as neuronal pathologies.[108] In addition to this, *ApoE* is important for the molecular trafficking[109] and induction of neuronal expression of βAPP, perhaps in particular with regard to specific genotypes of *APOE*. This is because the *APOE* ε3 variant is more effective at βAPP induction than APOE ε4.[110]

A good deal of evidence indicates an impact of *APOE* genotype on inflammatory sequelae. *APOE* ε4 was found to produce a dose-dependent increase in microglial activation and this effect was independent of Aβ deposition.[111] The protective effect of NSAID use is much stronger in *APOE* ε4 carriers;[112–116] indeed, some of these studies have restricted a significant NSAID effect to ε4 carriers alone. This may explain the failure of several prospective trials with NSAIDs.[117–119] The only trial showing a benefit was the only one powered to detect an effect of *APOE* genotype.[120] Mechanisms to explain the interaction between *APOE* genotype and inflammatory events are suggested by studies showing that microglia are more robustly activated in the presence of human ApoE4.[91,121–123] This may involve differential inhibition of inflammatory agents, such as sAPP by ApoE3 and ApoE4.[91]

Reports from the first phase of the multicenter AD Neuroimaging Initiative (ADNI) are making major contributions to knowledge concerning correlations between neuroimaging and cerebrospinal fluid (CSF) analytes and AD progression. They are also helping discern the role of environmental, comorbid conditions and specific gene polymorphisms in increased risk or perhaps precocious development of AD neuropathological changes. For instance in a new report from ADNI, genotypes, including *APOE* and two other 'promising genes' contactin 5 (*CNTN5*) and bridging integrator 1 (*BIN1*), were associated with a number of neuroimaging parameters.[124] More specifically, the inheritance of *APOE* ε4 and a maternal history of the disease were linked to hippocampal atrophy.[125]

With regard to comorbid conditions, obesity is linked to lower brain volumes in AD and in mild cognitive impairment (MCI), a condition likely to be prodromal AD.[126] Importantly, there were indications of increased plaque burden by 3-D imaging with the 'Pittsburgh compound' PiB correlate with hippocampal atrophy[127] as well as with progression from MCI to AD.[128] Similar correlations hold with CSF measurements of Aβ and tau. Worldwide ADNI efforts will aid in establishing the progression from early stages, i.e. MCI to AD. Such efforts will then be useful in defining the mechanisms involved in driving AD neuropathology as well as developing and analysing rational strategies for therapeutic interventions.[129]

Tumour Necrosis Factor Alpha

In a study to identify polymorphisms in *TNF*, the gene encoding the proinflammatory cytokine TNFα, a polymorphism at -850 C/T in *TNF* was found to increase the risk for AD.[130] Specific polymorphisms in promoter regions of inflammatory cytokine genes, such as TNFα and IL-6, are associated with late-onset sporadic AD.[131] For instance, heterozygosity for a polymorphism at -1082 of the IL-10 gene increased the odds ratio of AD two-fold. When combined with a polymorphism at -308 in *TNF*, the odds ratio was increased 6.5 times.

Interleukin-1

Both *IL1A* and *IL1B*, which encode proteins IL-1α and IL-1β, map to Chr 2. Polymorphisms in each of these genes have been associated with AD. Many, but not all, case-control studies of individuals who died with AD have shown that polymorphisms in either of these IL-1 genes are associated with increased odds for AD. The principal risk-conferring polymorphism in *IL1A* is located in the gene promoter region (-889 C/T), while that in *IL1B* is located either in the promoter region (-511 C/T) or in the coding region (+3954). In a number of studies, inheritance of a polymorphism at either -889 of *IL1A* or +3954 of *IL1B* was shown to increase the odds of AD by

two- to four-fold.[132–137] Importantly, in another of study of living AD patients, a -889 polymorphism in *IL1A* was associated not only with increased an risk for AD (4.5 times) but also with earlier disease onset (seven to nine years earlier).[134] Although the number of studies supporting such an association is increasing, a few studies have failed to substantiate this.[138,139] In one study, *IL1B* at -511 increased the odds for AD but the *IL1A* -889 polymorphism did not.[140] A strong association was shown between AD and *IL1B* +3954, but not -511 in another study.[141] Other studies have found no relation between *IL1B*-511 and AD.[142,143] Meta-analyses have supported an association for at least the -889 polymorphism in the *IL1A* gene.[144–146] In the large Honolulu-Asia Aging Study, the authors concluded that certain loci within the IL-1 gene cluster were associated with AD.[147]

There are likely multiple reasons for these conflicting reports of the link between IL-1 gene polymorphisms and AD risk. These might include the disparate population groups studied, sample size and contributions by other risk-modifying genes. Importantly toward this point, in an extremely rigorous analysis of AD-related polymorphisms,[148] a polymorphism at -889 in *IL1A* was suggested as one that approached significance. It may have failed to show linkage only owing to deficiencies in replication and the spectrum of populations studied. In a recent study of the importance of polymorphisms in cytokine genes, specific polymorphisms in both *IL1A* and *IL1B* were positively associated with AD. Polymorphisms in the genes for anti-inflammatory factors, such as IL-4 and IL-10, were strongly associated with a reduced risk for AD.[149]

As with all risk-conferring factors – whether related to inheritance or environmental events – downstream outcomes may at least be additive, or at worst multiplied. The multigene and additive nature of quantitative-trait loci is in contrast to high-penetrance single-gene mutations, which cause rather than alter risk for disease. A case in point with regard to the inheritance of specific gene combinations, or haplotypes, and the additive nature of risks on outcomes is the dramatic increase in risk with inheritance of both *IL1A* and *IL1B* polymorphisms. Inheritance of the polymorphisms in both *IL1A* and *IL1B* is associated with a much greater risk (~10 times) of developing AD.[132,133,150,151]

Age-related Alzheimer's Disease Neuropathological Changes
Advancing Age
Ageing provides the backdrop for the progression of degenerative events in general, including the development of the clinical symptoms and neuropathological changes characteristic of AD. Only with ageing do the cognitive and functional declines, as well as the elevated densities of neuritic Aβ plaques in neuronal layers and neurofibrillary tangles within neurons, manifest themselves. The importance of ageing in neuropathogenesis is readily apparent. Even individuals who have causative factors – specific genetic mutations or DS, both of which provide virtual assurance of development of AD – reach adulthood or even middle-to-late age before overt signs appear. In addition to this, with advancing age cognitively normal individuals often have an abundance of neurofibrillary tangles and neuritic Aβ plaques that may approach densities consistent with a neuropathological diagnosis of AD (for a review, see Mrak and Griffin[152]). As it is uncommon for the densities of these anomalies to reach those necessary for a definitive diagnosis of AD, Mackenzie and colleagues[153] suggest that cognitive function is related to plaque

density and that ageing *per se* is not synonymous with Aβ deposition and cognitive failure.

Until recently, little could be discerned regarding the relevance of age-related accumulations of Aβ plaques and neurofibrillary tangles, especially as to whether they were portents of later development of AD. One study used *in vivo* Aβ labeling together with functional magnetic resonance imaging to test cognitively intact elderly individuals. Sperling and her colleagues found a relationship between Aβ deposit density and changes in a specific memory mode previously noted in AD patients.[154] These findings suggest that events during supposedly normal ageing may presage memory loss and neural dysfunctions related to learning and memory. In this way, they may contribute to later development of AD, with greater memory dysfunction and neuropathological change. The changes highlight the importance of defining seminal events and triggers of the progressive acquisition of such Aβ deposits. This will help toward the development of rational strategies to halt or impede such acquisition. In this way it may be possible to prevent or delay consequent neural dysfunction and dementia.

Some cognitively intact individuals have large numbers of non-neuritic Aβ deposits, but interestingly these deposits are not associated with activated microglia. This suggests that microglial activation contributes to the progression of Aβ deposits into the more mature neuritic Aβ plaques diagnostic of AD.[155] Even in individuals who are cognitively normal and who have few Aβ deposits or plaques, the density and activation state of astrocytes and microglia increase with advancing age. The levels of S100B expression gradually increase,[57,156] while the levels of IL-1 remain stable until the sixth decade.[157] The latter finding suggests that in normal ageing, IL-1 levels are maintained at lower levels in order to delay neurodegenerative consequences.

Environmental Events that Contribute to Precocious (Age-inappropriate) Development of Alzheimer's Disease Neuropathological Changes
In addition to genetic mutation, gene duplication, gene polymorphisms and the wear and tear of time with ageing, a number of lifestyle or environmental risk factors have been associated with the precocious development of AD. Given the fact that epilepsy is often one of the consequences of head injury, it is likely that these problems exacerbate and/or hasten the precocious development of neuropathological changes of AD.

Indeed, this is the case when either epilepsy or head injury is assessed with regard to the influence of inheritance of an APOE ε4 allele; such inheritance adds to the risk for a poor outcome in both epilepsy and head injury.[158,159] Conditions associated with an increased risk for AD are as wide ranging as:

- heart disease;[160]
- AIDS;[161,162]
- obesity and diabetes;[126,163–168] and
- hypertension and heart disease.[165,168–170]

As with head injury and epilepsy, the risks conferred are increased in carriers of one or more APOE ε4 alleles.[164,170,171] In addition, head injury, epilepsy and AIDS include early overexpression of βAPP and increases in neuroinflammatory cytokines, such as IL-1 and S100B.[82,172–177] In several of the conditions mentioned above, glial activation has been

associated with neuropathological changes similar to those in AD; glial activation is sometimes present long before frank neurodegeneration is noted. Conditions involving glial activation include:

- DS;[56,57]
- head injury;[172,175]
- epilepsy;[176,177]
- obesity and diabetes;[126,166,167] and
- AIDS.[178]

Summary

The pervasive appearance of glial activation and proinflammatory cytokine overexpression prior to and accompanying the neuropathological changes of AD suggests their importance in its development. The known capacity of at least one of these cytokines – IL-1 – to induce expression of other cytokines and the hallmarks of AD neuropathology highlights cytokine importance in Alzheimer's pathogenesis.

Discovery of proinflammatory activity exhibited by sAPP provided the missing link between the two universal responses of neural systems to injury: increased neuronal expression of APP and glial activation with excess cytokine expression. Namely, injury-induced overproduction of sAPP provides for a progressive feed-forward cycle whereby the resultant microglial activation and release of IL-1 leads to further βAPP elevation.

Knowledge of the mechanisms involved in this link has provided a framework for understanding the way in which neuronal-glial interactions culminate in AD. Moreover, this also explains, at least in part, why neuronal trauma sets a cycle in motion. Neuronal trauma can be induced by untoward genetic inheritance, direct injury, comorbid conditions or as-yet unknown factors. The self-propagating cycle, properly called the cytokine cycle, promotes chronic production of glial cytokines, precursors of Aβ plaques, neurofibrillary tangles, Lewy bodies, the buildup of unwanted proteins and cognitive decline. ■

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