Medical Hypotheses 85 (2015) 779–790

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Review of Amyotrophic Lateral Sclerosis, Parkinson's and Alzheimer's diseases helps further define pathology of the novel paradigm for Alzheimer's with heavy metals as primary disease cause

Franco Cavaleri *

Faculty of Medicine, Department of Experimental Medicine, Brain Research Center, UBC Hospital, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada
Biologic Nutrigenomic Health Research Corp., 688-2397 King George Hwy, White Rock, BC V4A 7E9, Canada

ARTICLE INFO

Article history:
Received 29 July 2015
Accepted 11 October 2015

ABSTRACT

Pathologies of neurological diseases are increasingly recognized to have common structural and molecular events that can fit, sometimes loosely, into a central pathological theme. A better understanding of the genetic, proteomic and metabolic similarities between three common neurodegenerative diseases – Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD) – and how these similarities relate to their unique pathological features may shed more light on the underlying pathology of each. These are complex multigenic neuroinflammatory diseases caused by a combined action by multiple genetic mutations, lifestyle factors and environmental elements including a proposed contribution by transition metals. This comprehensive dynamic makes disease decoding and treatment difficult.

One case of ALS, for example, can manifest from a very different pool of genetic mutations than another. In the case of ALS multiple genes in addition to SOD1 are implicated in the pathogenesis of both sporadic and familial variants of the disease. These genes play different roles in the processing and trafficking of signalling, metabolic and structural proteins. However, many of these genetic mutations or the cellular machinery they regulate can play a role in one form or another in PD and AD as well. In addition, the more recent understanding of how TREM-2 mutations factor into inflammatory response has shed new light on how chronic inflammatory activity can escalate to uncontrolled systemic levels in a variety of inflammatory diseases from neurodegenerative, auto-inflammatory and autoimmune diseases. TREM-2 mutations represent yet another complicating element in these multigenic disease pathologies.

This review takes us one step back to discuss basic pathological features of these neurodegenerative diseases known to us for some time. However, the objective is to discuss the possibility of related or linked mechanisms that may exist through these basic disease hallmarks that we often classify as absolute signatures of one disease. These new perspectives will be discussed in the context of a new paradigm for Alzheimer's disease that implicates heavy metals as a primary cause. Plausible links between these distinctly different pathologies are presented showing intersections of their distinct pathologies that hinge on metal interactions.

Introduction

Neurodegenerative diseases with distinctly different clinical presentations share many pathological features at a subcellular level. These dissimilarities often involve disease-specific proteins that are signatures of a disease, for instance, that nevertheless partake in similar subcellular and even systemic events in other disease pathologies.

Cell biology from prokaryote to eukaryote is full of repeated or common systems. Identifying and decoding these repeated models and attempting to overlap them on to pathological pathways of diseases we know less about might help us expand our understanding of the less defined pathology. In this meta-analysis of the literature the pathologies of Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD) are compared and contrasted to further define and better understand genetic and metabolic similarities as well as highlight how distinct signatures define them from one another. This strategy is applied

* Address: Faculty of Medicine, Department of Experimental Medicine, Brain Research Center, UBC Hospital, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada. Tel.: +1 855 518 8858, mobile: +1 604 307 6348.
E-mail address: franco.c@biologicnr.com

http://dx.doi.org/10.1016/j.mehy.2015.10.009
0306-9877/

here to help expand the new AD paradigm that centers on transition metals as a major cause of sporadic AD [1].

At a subcellular level it is clear that processing, trafficking and removal of misfolded or otherwise aberrant proteins play central roles in the pathogenesis of many debilitating neurodegenerative diseases [2–4]. In AD, for instance, we find misfolded β-amyloid peptide which accumulates in the endoplasmic reticulum (ER) to contribute to ER stress [5]; it is found in the cytosol and plasma and other membranes; with variants even found to partake in transcriptional regulation at a nuclear level [6]. The β-amyloid peptide aggregates extracellularly as amyloid plaque also referred to as neuritic plaques [7]. In addition to these plaques, AD is also characterized by hyperphosphorylated TAU aggregated in the cytosol to form neurofibrillary tangles (NFTs) [8].

Presenilin mutations are linked to early onset familial AD (FAD) [9]. Presenilin 1 and presenilin 2 mutations result in the alteration of β-amyloid peptide processing from amyloid β precursor protein (APP) [10]. These mutations lead to increased and altered γ-secretase enzyme expression to yield abnormally elevated levels of the toxic β-amyloid peptide [11–13].

Although the neuritic plaques and neurofibrillary tangles are characteristics more commonly associated with AD, PD displays similar pathological features, however, centered on different peptide aberrations. In PD, the α-synuclein gene is at the center of the disease pathology. Mutations can result in a gain-of-function mechanism the outcome of which is an abnormally high cytoplasmic accumulation of α-synuclein forming Lewy bodies [14]. These aggregates can also include ubiquitin and syniphilin-1; proteins involved in facilitation of proteasomal degradation [15].

This indicates an attempt by the preclinical PD cell to eliminate the aberrant α-synuclein via ubiquitination and subsequent proteasomal degradation. However, degradation failure by the proteasome pathway results in oligomer accumulation and the characteristic Lewy body deposits located in the neuron cell bodies, axons and even synapses [10,16,8]. Accumulation of the aberrant α-synuclein is thought to contribute to degeneration of dopaminergic neurons [17].

At an intracellular level, oxidation and the related localized inflammation play central roles in the pathology of each AD and PD. Glutathione (GSH) depletion and the associated oxidative stress are shown to be pathological features that induce modifications of TDP-43 [18]. Lewy bodies with TDP-43 inclusions are closely associated with AD, PD and ALS [19]; an aberrant protein that is common to all three diseases. TARDBP (TDP-43) mutation is thought to be a major cause of ALS resulting in a toxic gain-of-function and cytosolic accumulation of the protein which eventually leads to induction of apoptosis [20].

Metal toxicity and the consequential oxidative load will be shown to be a common factor in our comparative study of these three neurodegenerative diseases. Oxidative insults on the α-synuclein peptide, aberrations of which are signatory of PD, are shown to induce its oligomerization. Metal catalyzed oxidation of α-synuclein, in fact, is shown to inhibit filament formation and promote α-synuclein oligomerization via cross-linking [21]. Both AD and PD are characterized by α-synuclein inclusions in senile (amyloid) plaques and Lewy body formations respectively [22].

Both wild type and mutated α-synuclein possess an aggregation propensity [23,14] and it is proposed herein that the common factor between the two is the oxidation-induced cross-linking that transition metals like copper can catalyze. Rasia et al., for example, show that Cu(II) binds to the α-synuclein peptide at the N-terminus utilizing His-50 as the anchoring amino acid [24]. In AD, as will be demonstrated in greater detail in a hypothetical model, Cu(II) is implicated as a factor that induces β-amyloid peptide aggregation as well [25].

If we look at the AD model with a focus on transition metal involvement we find more evidence to support this oxidative source as a probable starting point or as an upstream facilitator along the disease evolution as outlined in the recently published variation of the newly proposed AD paradigm [1]. Post-mortem investigation of AD brain tissue has revealed elevated mercury levels [26]. Interneuron amyloid plaques of AD and non-AD brains are associated with aluminum, iron, copper and zinc [27,28]. This presents more evidence indicating a potential catalytic influence by toxic levels of uncontrolled (free) metals in neurological tissue. It also shows these metals to be highly associated with β-amyloid.

Related studies also show that copper level reduction in tissues may contribute directly to lower APP gene expression [29] indicating a correlation between APP demand and free copper. β-Amyloid protein 42 is shown to be a more effective reductant than β-amyloid protein 40; and it is also shown that β-amyloid protein 42 has a higher affinity for metal chelation than 40 [5]. Is β-amyloid protein 42's known incremental toxicity associated with its greater propensity for metal chelation? These are questions that must be posed and investigated further in new research objectives.

If we go back to PD, one of the main hallmarks of the disease is degeneration of the dopaminergic neurons in the substantia nigra. This is a distinct feature of PD and not the other two, AD or ALS, or of other neurodegenerative diseases for that matter. However, here too, copper as well as iron are implicated in the transformation of the α-synuclein peptide to form aggregates [30–34].

SOD1 mutations are central to ALS. SOD1 is also a metallopeptide; an apoprotein that depends on copper and zinc to carry out its antioxidant activity. Magnesium is another metal central to mitochondrial SOD activity. The peptide plays a central role in protection of the cell from oxidative stress [35,36]. However, this protective feature can shift to a pro-oxidative activity in the case of misfolded SOD1 where the metal is misplaced and may serve as a facilitator of oxidation in this exposed position. In fact, research does support the notion that misfolded SOD1 will induce oxidation rather than serve as a quencher of oxidation [37]. Research also indicates that misfolded SOD1 can induce generation of the hydroxyl radical. It is also shown that the peptide can release its copper so the highly reactive free metal can induce intracellular oxidative damage via the Fenton reaction [38].

Over-expression of SOD1 is shown to protect neurons from oxidative injury due to ischemic events [39]. The antioxidant role of the peptide may inspire an intuitive appreciation for this potential, however, overexpression is also associated with a higher likelihood of SOD1 misfolding [40,41]. This is likely due to the pro-oxidative state that an unnaturally elevated intracellular SOD1 density might create. SOD1 mutations have also been associated with excito-toxicity [42]. This might be a function of interrupted trafficking and processing of vesicles central to neurotransmitter release and NMDA and AMPA receptor management. It may also be directly associated with inflammatory activity; a pro-oxidative state of SOD1 can spark an oxidative and subsequently, an inflammatory cascade.

It is documented and reported by Bolton et al. that inflammation is shown to have a glutamate-promoting influence on glutamate-regulating enzymes. The dysregulation of the glutamate synthetic enzyme, glutamate synthetase and the glutamate neutralizing enzyme, glutamine dehydrogenase results in over-secreted glutamate and unnaturally extended survival of the AMPA and NMDA agonist (glutamate) to over-stimulate neurons [43] and possibly play a role in apoptosis.

Suppression of mutated SOD1 in motor neurons and glia via virus therapy that encodes shRNA reduces mutated SOD1 transcription [44]. This shows promise as a possible way by which the progressive neurodegenerative effects of SOD1 mutation can be countered. It also sheds light on how the mutation might induce

and advance clinical symptoms of ALS. However, if this misfolded SOD1 results in misplacement of the metal to turn this antioxidant molecule into a pro-oxidant, this oxidative overload might be the method by which functional healthy SOD1 is subsequently misfolded as it comes into play to neutralize the misfolded SOD1 with exposed transition metal activity.

If the cell is not able to process the aberrant peptide by way of the Unfolded Protein Response (UPR) or other degradation pathways the pro-oxidative activity can transfer from cell-to-cell. Functional wild type SOD1 can aggregate with misfolded SOD1. The aggregates can also readily exit the cell and enter new ones through macropinocytosis to introduce a pathogenic cycle in healthy cells [45–47].

This pro-oxidative SOD1 hypothesis may align well with the hypothesis posed here explaining molecular, metabolic and structural events in AD. In particular, this may relate to chelation of metals by the AD β-amyloid peptide as described in previous articles [1]; and briefly summarized in these pages to come.

Common model of sequential events

Metal mismanagement, oxidation, inflammation and apoptosis

Each of the three neurodegenerative diseases presented here – AD, ALS and PD – are proteinopathies characterized by aberrant proteins that are specific and distinct to each of the diseases. However, it must be highlighted as a central point of this review that each protein, β-amyloid, SOD1 and α-synuclein peptides respectively, is intimately associated with metal ion chelation or interaction. Furthermore, it must be noted that these transition metals, copper playing a frequent role, are all divalent and facilitative of oxidative activity via the Fenton reaction if free and uncontrolled.

AD research indicates that APP exhibits antioxidant properties [48] and the β-amyloid peptide exhibits potent metal chelating and therefore indirect antioxidant properties [49]. The proposed new AD paradigm expanded herein states that these antioxidant properties may, in fact, be the primary roles for each protein. This is generally accepted for SOD1 but is proposed herein in reference to the β-amyloid peptide species, of which, multiples exist.

In particular, the new paradigm states β-amyloid peptide has a high affinity for copper, aluminum, iron and zinc chelation and upon chelation it sequesters and prevents the metal from generating reactive oxygen species (ROS) [50]. Free iron, for example, resulting from brain injury expands free radical development from ROS to produce H_2O_2 and on to produce other oxidants [51]. In accordance with the hypothesis, iron chelation may prove to be disease mitigating in post-traumatic brain injury (TBI) therapy. β-Amyloid peptide oligomer formation, itself, is shown to spontaneously generate H_2O_2 as a function of the reaction; and iron is shown to enhance this reaction [52].

TBI is characterized by accumulation of amyloid aggregate and lipid peroxidation [53,54]. Uryu et al. also reveal that a single TBI results in low levels of iron deposition while cases of multiple TBI in their mouse models results in incremental iron deposition. Amyloid aggregate is found to contain iron (Fe II) amongst the metals regularly associated with it [55,56]. Amyloid plaque formation in cases of TBI may be a response to free iron accumulation; an attempt to sequester and discard the catalytic metal.

In fact, it has been shown that copper and zinc are also enriched in extracellular amyloid plaque deposits of the Alzheimer's brain [25,57]. Wirths et al. show via intracellular β-amyloid protein staining that extracellular plaque deposition is long preceded by intracellular β-amyloid protein accumulation in the hippocampus neuron [58]. This falls in line with the proposed preclinical strategy by the cell to remove toxic intracellular metals and prevent extracellular metals from entering the cell to avoid pathological consequences.

β-Amyloid peptide is also implicated in the initialization of lipid peroxidation, a critical step toward apoptotic events and neuronal loss [59]. It is proposed herein, that the chelated metal by the β-amyloid peptide is likely to play a central role in lipid peroxidation as β-amyloid protein with its metal payload is translocated to the exterior of the cell from its intracellular toxic existence. In fact, Petersen et al. show that neuroblastoma cells can internalize externally applied β-amyloid peptides and co-localize the peptides to the mitochondrial cristae. The peptide can move in both directions – into and outside the cell [60–62].

When we speak to the different species of β-amyloid protein and their involvement in the disease pathology, for example, it must be understood that the in vivo state is such that the β-amyloid protein pool is heterogeneous. This pool includes but is not limited to the β-amyloid variants 33-43 abundantly co-existing with the β-amyloid 40, 42 and 43 species; the latter of which (40, 42 and 43) are reported in the literature and previously cited to be the more toxic forms. However, these relatively more toxic species of β-amyloid are also present in healthy brains although usually at lower levels. The diseased state is characterized by a change in the proportion of these β-amyloid species in the heterogeneous pool to include higher levels within the pool and not the exclusive existence of the more toxic forms [63].

The metal-induced oxidative load fits as a theme in the ALS model as well. Copper in misfolded SOD1 may be mis-positioned to promote an oxidative influence instead of conferring to the peptide an antioxidant activity [47]. SOD1 aggregation is likely the result of an attempt by the cell to isolate the toxic peptide in a less invasive form if autophagy and proteasome degradation fail. Misfolded SOD1 can transfer from cell to cell in cells that lack direct contact even [45]. Just as in AD, soluble β-amyloid aggregates to form the insoluble β structure [64], misfolded SOD1 aggregates to form insoluble inclusions that are toxic [35].

In addition to migrating into the cell from cell to cell like misfolded SOD1 can, the β-amyloid peptide in AD is known, as previously mentioned, to penetrate the mitochondrial membrane. This infiltration of the mitochondria is another critical event promoting apoptosis through the escalation of oxidation, interference with ATP synthesis and/or breach of the mitochondrial membrane to release cytochrome C – another key trigger and signature for apoptosis [65]. However, here again, whether the cause of this mitochondrial distress is the β-amyloid peptide penetrating without a mission and merely a function of its uncontrolled state; or it is the metallo-β-amyloid chelate that has not completed its trafficking objective to export the toxic β-amyloid chelate into the interneuronal space will be debated in more detail later in the context of the hypothesized new paradigm.

The new AD paradigm proposes that the preclinical AD neuron relies on APP and properly processed β-amyloid protein for normal cell function; protection from oxidation to preserve neurons and cognition with age. The brain utilizes more oxygen per gram of tissue than other tissues of the body and with this metabolic activity and exposure to oxygen it must be expected that these cells will be equipped with multiple features that protect them from the consequential incremental oxidative stress. The common endogenous antioxidant enzymes: catalase (CAT), SOD, glutathione peroxidase (GSH), heme oxygenase-1, NADPH quinone oxidoreductase and glutamate-cysteine ligase are known to play central roles in oxidative control. These endogenous antioxidants are regulated by the transcription factor Nrf2 [66–69].

However, it is proposed herein that APP is another of these endogenous antioxidants providing redundant but cumulative protection; that β-amyloid protein is part of this design and its role is to protect the oxygen-vulnerable neuron from metal toxicity. The

oxygen-rich environment is one to be more vulnerable to transition metal exposure and it must be expected that multiple protective countermeasures are built into this system. β-Amyloid protein's biological role is proposed to chelate and sequester free metals that could exacerbate oxidative activity via the Fenton reaction.

β-Amyloid protein of variable types migrate to the mitochondrial membrane with the proposed purpose of sequestering free copper, iron and other metals including mercury. These transition metals can otherwise exacerbate oxidative state of the mitochondria where ROS production can easily reach uncontrolled levels. BACE1-cleaved APP yields the β-amyloid variants 40 and 42 cleaved on the extracellular side of the transmembrane APP. This β-amyloid peptide variant is proposed to have a greater affinity for transition metal chelation; a design to sequester extracellular metal before it enters the cell where it can induce intracellular oxidative and inflammatory activity. The fact that the BACE1-PSEN-AβPP system is shown to be a highly conserved ancient system [70] amongst species also sheds more light on a likely chelation role which at the very least is not refuted by current literature.

A simplistic look at the amino acid profile of the typical BACE1-cleaved peptide gives us: (N-terminal) DAEFRHDSGYEVHHQK **LVFFA**EDVGSNKGAIIGLMVGG**VVIA** (C-terminal) [63]. We see an abundance of residues at the N-terminal end, as underlined, that are charged – K, D, R and E. In the context of this new paradigm the BACE1 cleaved peptide has a higher affinity for transition metal chelation due to the peptide's N-terminal charged amino acid sequence but also due to the cluster of histidine (H) residues. Histidine 50, for example, plays a significant role in α-synuclein cross-linking in PD pathology via copper–histidine interaction [71,32]. Generation of this extended β-amyloid peptide by BACE1 is inspired by a sequence of events that is in line with the proposed paradigm. Oxidative load which can be exacerbated by transition metal toxicity results in a proportionally activated NF-kappa-B signalling [72,73]. NF-kappa-B signalling upregulates BACE1 activity [74,75] a commonly elevated sequence in AD pathology [76].

This β-amyloid peptide is proposed to primarily function in the extracellular medium where upon chelating free metals it aggregates to form the notorious fibrils and neuritic plaques. Aggregation of the peptide is known to be a function of the hydrophobic regions shown in bold – **LVFFA** as well as **VVIA** at the C-terminal end [77]. However, research also shows such aggregation to be facilitated by metal induced cross-linking as seen in copper induced cross linking of α-synuclein in PD [71]. Aluminum, iron and zinc are shown to promote β-amyloid aggregation and toxicity [78,79]. Interesting to note is the fact that the histidine residues are also located in the BACE1 cleaved peptide exclusively, where metal chelation in the context of the new paradigm is said to be its primary role. The α-secretase cleaved peptide which is not inspired by NF-kappa-B signalling voids the charged amino acid sequence as well as the histidine rich cluster: LVFFAEDVGSNKGAIIGLMVGGVVIA [63]. Research also shows that the affinity for transition metals by the amino acids found in this shorter peptide is as such G > A > L [80]. It is possible that Isoleucine (I) will work in this manner relating to metal affinity much like leucine (L). Although this activity by the amino acids will differ in peptide form versus free form these activities can be easily tested. In other words, the new paradigm acknowledges chelation activity built into the α-secretase cleaved peptide albeit much lower than that of the BACE1 cleaved alternative.

As evidenced in a previous article introducing this new AD paradigm, exposure to toxic transition metals is shown to increase APP transcription and BACE1 activity [81]. This protective BACE1 action sequesters the toxic metals by chelation and can do so for a preclinical phase of AD that may precede clinical symptoms for as long as twenty years. Anatomical changes in the brain have, in fact, been mapped and shown to be a function of these metabolic changes [82].

The number of plaques that accumulate as a result of this health-preserving countermeasure, however, can be incremental in the case of AD patients over those accumulated in healthy brains [83]. Nevertheless, amyloid plaque development does not correlate to cognitive deficit also indicating [84,85], in support of the newly proposed AD paradigm and in accordance with the mechanics of this model, that this plaque development is not necessarily harmful to the neuron. It may, as proposed here, otherwise be safeguarding the cell by sequestering the deleterious metal within the plaque for microglia to remove.

Microglia are equipped with multiple classes of receptors that respond to β-amyloid peptide. These receptors include TLRs, Compliment Receptors, Scavenger Receptors, Receptor of Advanced Glycation End-product, FPRL1 and others including TREM2 [86]. Under healthy conditions the microglia manage the interneuronal environment including prevention of plaque accumulation. Stefani et al. report in their review that mature amyloid plaques present far less toxicity than their precursor prefibrillar aggregates [87]. This is explainable in the context of the new paradigm; the amyloid plaques are simply neutralized disposal sites. However, it is also demonstrated that this plaque can be the source of soluble toxic β-amyloid peptide that can dissociate from the plaque due to interactions with lipids and other biochemicals [88]. Hence, microglial management of plaque concentration also plays an important role in minimizing long term risk especially if metal toxicity is persistent over time.

Iron, amongst other heavy metals (zinc and aluminum), for example, is also shown by Mantyh et al. to induce β-amyloid protein synthesis and aggregation [78]. The literature also shows that β-amyloid protein accumulates as a function of mercury cytotoxicity [89]. Metal exposure is shown, over and over again, to induce generation of the AD hallmarks: Incremental TAU phosphorylation and release of β-amyloid protein 40 and 42 [89]. Research shows irrefutably that ROS and other free radical species that can escalate due to transition metal reactivity induce IL-1β increment and ultimately IL-1 [90]. IL-1's activity is shown to be linked to hyperphosphorylation of TAU [91].

TAU hyperphosphorylation alters the protein's interactive capacity for the microtubule system of the cell. In fact, phosphorylation of TAU at serine 262, threonine 231 and serine 235 inhibits TAU microtubule binding by 35%, 25% and 10% respectively [92] resulting in TAU dissociation from the cytoskeletal microtubule system. TAU in normal healthy brains is found to have approximately 2–3 moles of phosphate per mole of TAU while in AD brains TAU can be associated with as much as four times more phosphorylation [93].

In the case of AD, this TAU modification results in disruption of cell trafficking and in the context of the newly proposed AD paradigm it contributes to interruption of β-amyloid protein transport. The consequence of interrupting trafficking is that free metal and β-amyloid protein-chelated metal accumulate in the cell to apoptotic levels; TAU hyperphosphorylation is thought to lead to aggregation and neurofibrillary tangle (NFT) formation [94,95]. NFT's, a hallmark of AD, are expected to be factors that impose more neuron impairment than the β-amyloid peptide can induce in AD pathology. This is due to the microtubule impediment which leads to cell distress and apoptosis [96]. However, TAU inclusions in neuron disease are not exclusive to AD.

It is also shown that cognitive impairment in ALS patients is associated with abnormal TAU metabolism found as components of neuronal and glial inclusions [97–99]. This may be a function of the misfolded SOD1-induced oxidation and subsequent

inflammatory activity carried forward by IL-1 induction leading to the same outcome that is found in AD – TAU hyperphosphorylation and NFT generation. In fact, research by Masters et al. shows that increases in mutant SOD1 parallels inflammasome activity and subsequent increases in IL-1β [100].

In the case of ALS, however, the primary stressor in relation to the proposed paradigm, is misfolded SOD1 and not toxicity by intolerable levels of a free divalent metal. Nevertheless, misfolded SOD1 is expected to pose as an oxidative stressor through, possibly, its misplacement of copper. Although copper is essential its redox reactivity makes it a dangerous element if mismanaged [101].

In fact, in vitro applications of exogenous antioxidants such as even N-acetylcysteine (NAC) helps return cellular functions back to normal countering mutant (G93A) SOD1-induced mitochondrial dysfunction, ATP decrease and ROS increase [102]. SOD1 aggregation is also reduced upon ROS sequestering, however, in this model upon inhibition of proteasome activity, (G93A) misfolded SOD1 aggregation is found to once again escalate. This may be exemplary of how cells might tolerate one misfolding mutation or toxic levels of metals like copper, iron or others to result in subclinical levels of disease. This can escalate to clinical manifestation in the case of multigenic disease development where trafficking or proteasome activity are concurrently altered whether by co-inheritance or via independent somatic events.

It is possible that the cell disposal systems such as proteasome degradation and Unfolded Protein Response (UPR) function as viable compensatory mechanisms for the SOD1 aberration in a preclinical phase of ALS. In this case misfolded SOD1 concentration is relatively controlled. In AD it is proposed here that the BACE1 compensatory system functions at designed capacity to counter metal toxicity by chelating the metal with the β-amyloid peptide and disposing of the chelate to accumulate as the relatively benign extracellular amyloid plaque. In PD, metal toxicity, in particular copper (Cu II) and iron (Fe II) are implicated in the induction of oxidative cross-linking of α-synuclein [87,31–34].

Furthermore, it is shown that amyloid plaques of AD patients can contain α-synuclein [103] and that α-synuclein-containing Lewy body-like inclusions are found in PD patients with dementia [104]. Cerebral spinal fluid evaluation of PD patients with dementia reveals an underlying AD pathology in PD patients with dementia – high TAU, and phospho-TAU and a conditionally elevated β-amyloid protein [105]. These PD characteristics lead to the expansion of the newly proposed AD paradigm to include a proposed linked activity in early stages of PD.

A study using transgenic mouse models of PD overexpressing α-synuclein shows a progressive increase in phosphorylated-TAU similar to that formed in AD [106] as well. Another study by Lee et al. shows the possibility of a mechanistic link between TAU and α-synuclein contributing further to the overlap in pathological and clinical features of AD and PD [107]. These findings do not necessarily provide conclusive support for the central hypothesis proposed herein describing the BACE1/β-amyloid protein as the countermeasure to metal toxicity but it does provide insight on how metal toxicity such as copper may be linked in the PD and AD models.

In a theoretical case of the proposed new AD paradigm the BACE1/β-amyloid protein countermeasure may have saved the cell from copper-induced α-synuclein cross-linking thus thwarting the pathology from taking the path to α-synuclein aberration and phenotypic manifestation as PD. However, during the course of executing the successful BACE1/β-amyloid protein countermeasure in this theoretical case, for years and even decades, extraneuronal amyloid plaque accumulation occurs; with the signatures of α-synuclein-containing Lewy body structures as found by Suh and Checler [103].

In due time, after a long preclinical phase that could last as long as two decades, additional exogenous or other endogenous factors in this theoretical case could contribute compounding oxidative strain or sporadic mutations in trafficking or degradation processes might reach critical states and interrupt cell trafficking. An additive oxidative force that is environmental or an age-related decline of endogenous antioxidants may add to the cell's oxidative load to induce IL-1 elevation, TAU hyperphosphorylation and eventual microtubule derailment. In this theoretical scenario the BACE1/β-amyloid protein countermeasure will ultimately fail due to trafficking impediment and AD may rapidly progress from this point on to clinical levels. However, PD was averted decades before and kept at bay because of this BACE1/countermeasure engagement.

The eventual failure may result alternatively from microglial failure to phagocytose amyloid plaque aggregates in the interneuronal space. As previously described microglia are equipped with scavenger receptors, compliment receptors, TLRs and other β-amyloid protein-recognizing receptors such as FPRL1 and they play important roles in removal of debris including β-amyloid protein. FPRL1, for example, is a G-Protein coupled receptor. β-Amyloid protein is a strong ligand for this receptor and upon being triggered the receptor plays a positive role in β-amyloid protein clearance by microglia which internalize and discard the accumulating β-amyloid protein [108]. However, it is also demonstrated that this receptor can confer neurotoxicity and inflammation if the response is not controlled. Microglia are also shown to secret enzymes designed to degrade β-amyloid peptides – an enzyme very much similar to Insulin Degrading Enzyme [109].

Mismanagement of the consequential inflammatory cytochemicals rising out of this β-amyloid scavenging is likely a key feature of the diseased state. Targeting this inflammatory response by microglia with inhibitive drugs is then a likely way to limit bystander neuron damage in AD pathology. In addition, it has already been shown in the literature that prolonged inflammatory activity can lead to glutamate mismanagement and overstimulation of neurons by the agonist [43].

The failure that may be contributing to the AD pathology, according to the 'new paradigm', may be microglial in part or in whole and have less to do with β-amyloid protein synthesis; and more to do with chelated β-amyloid protein mismanagement. As described, β-amyloid protein is proposed to be necessary for removal and neutralization of metal toxicity; microglial discarding of the accumulated β-amyloid debris is a crucial requirement for normal brain function and failure by the microglia could be more central to the pathology of AD than β-amyloid generation.

In accordance with the newly proposed AD paradigm postmortem brain tissue analysis from this theoretical AD patient would exhibit Lewy body structures with α-synuclein inclusions but also copper-containing amyloid plaque formations that co-present α-synuclein. This is, in fact, shown in the literature; amyloid plaques of AD patients can contain α-synuclein [103].

FAD presents a different initialization mechanism than sporadic AD. In FAD, APP processing is dramatically increased beyond what is typical. The result is early onset symptoms of disease [110]. Presenilin 1 mutations are a cause of autosomal dominant Alzheimer's disease. Presenilin 1 is the catalytic subunit of the γ-secretase enzyme and mutation interferes with cleavage of APP [111]. This cleavage can lead to the production of various β-amyloid peptide variants including β-amyloid peptide 40 and β-amyloid peptide 42. In the context of this proposed new paradigm this α-secretase-cleaved peptide is proposed to have an affinity for divalent metal chelation but a propensity that is much lower than that of the BACE1-cleaved β-amyloid peptide for reasons relating to the amino acid sequence as described earlier and again later.

Aberrant production of this α-secretase-cleaved peptide eventually also leads to pro-oxidative accumulation of the β-amyloid peptide 40, 42 and 43 species. The result as proposed in the context

of the new paradigm, is a pro-oxidative accumulation of the metallo-chelated protein which due to the sheer volume of these deleterious species in the heterogeneous mixture of amyloid protein pool results in cell disruption. It is proposed that in this familial case of disease, however, the incremental α-secretase-cleaved protein begins to compete intracellularly with the endogenous antioxidants SOD, CAT and GSH for the essential cofactor metals Mn, Cu, Zn and Fe (and in the case of GSH, selenium).

This creates a pro-oxidative environment that leaves a rate limiting availability of the metals for the endogenous antioxidants. This is a different starting point for the disease than the sporadic variation. In sporadic AD, the accumulation of toxic metal can initiate an incremental BACE1 activity through NF-kappa-B activation – a function of a genetically built in compensatory system (depicted in Fig. 1 p. 16) designed to perform in opposition to or as compensation for metal or other oxidative toxicity. Oxidation or cell stress initiate NF-kappa-B family protein activity [112–115,74]. As previously cited, NF-Kappa-B transactivation is intimately associated with increased BACE1 activity. This relationship is even shown to be direct up-regulation of BACE1 by NF-kappa-B [76].

Oxidation by transition metal Fenton reactivity (reduction) as is proposed in this new Cavaleri AD Paradigm could play a central role in upregulated oxidative and general cell stress and start in motion the AD pathology. BACE1 cleavage of APP followed by γ-secretase results in the β-amyloid protein variants 40, 42 and 43; species of the protein with greater affinity for divalent metal chelation due as previously mentioned to an incremental segment of the peptide that bolsters a highly charged residue sequence as shown in the underlined segment of the APP fragment.

AP Peptide. . ..EEISEVKM **β** DAEFRHDSGYEVHHQK $^{\alpha}$**LVFF**AED**VG**SNK**GA**IIG**LMV**GG**VVIA**T$^{\gamma}$.

The charged amino acids in the underlined segment are more likely to partake in chelation of metals. These include the basic amino acids Arginine (R), Histidine (H), Lysine (K) and acidic amino acids Aspartic Acid (D) and Glutamic Acid (E). This BACE1 (β-secretase) cleaves APP on the extracellular side of the plasma membrane as does α-secretase. BACE1 (β-secretase) cleavage results in the following sequence: DAEFRHDSGYEVHHQK **LVFF**AED**VG**SNK**GA**IIG**LMV**GG**VVIA**T. Cleavage by α-secretase results in a sequence with significantly fewer charged amino acids: **LVFF**AED**VG**SNK**GA**IIG**LMV**GG**VVIA**T.

Gamma-secretase cleaves APP on the intracellular side of the plasma membrane at various points of the C terminal end of the peptide as shown in the above peptide:

(N-terminal) **LVFF**AED**VG**SNK**GA**IIG**LMV**GG**VVIA**T (C-terminal).

In accordance with the proposed evolutionary design of this system and the proposed 'new paradigm', the BACE1 action is designed to inhibit the entry of exogenous toxic metals by chelating them and forming inter-neuronal fibril (β-Sheet) and subsequently amyloid aggregates. However, research already cited also shows β-amyloid protein can migrate into the cell efficiently to continue its pursuit and sequestering of uncontrolled free divalent

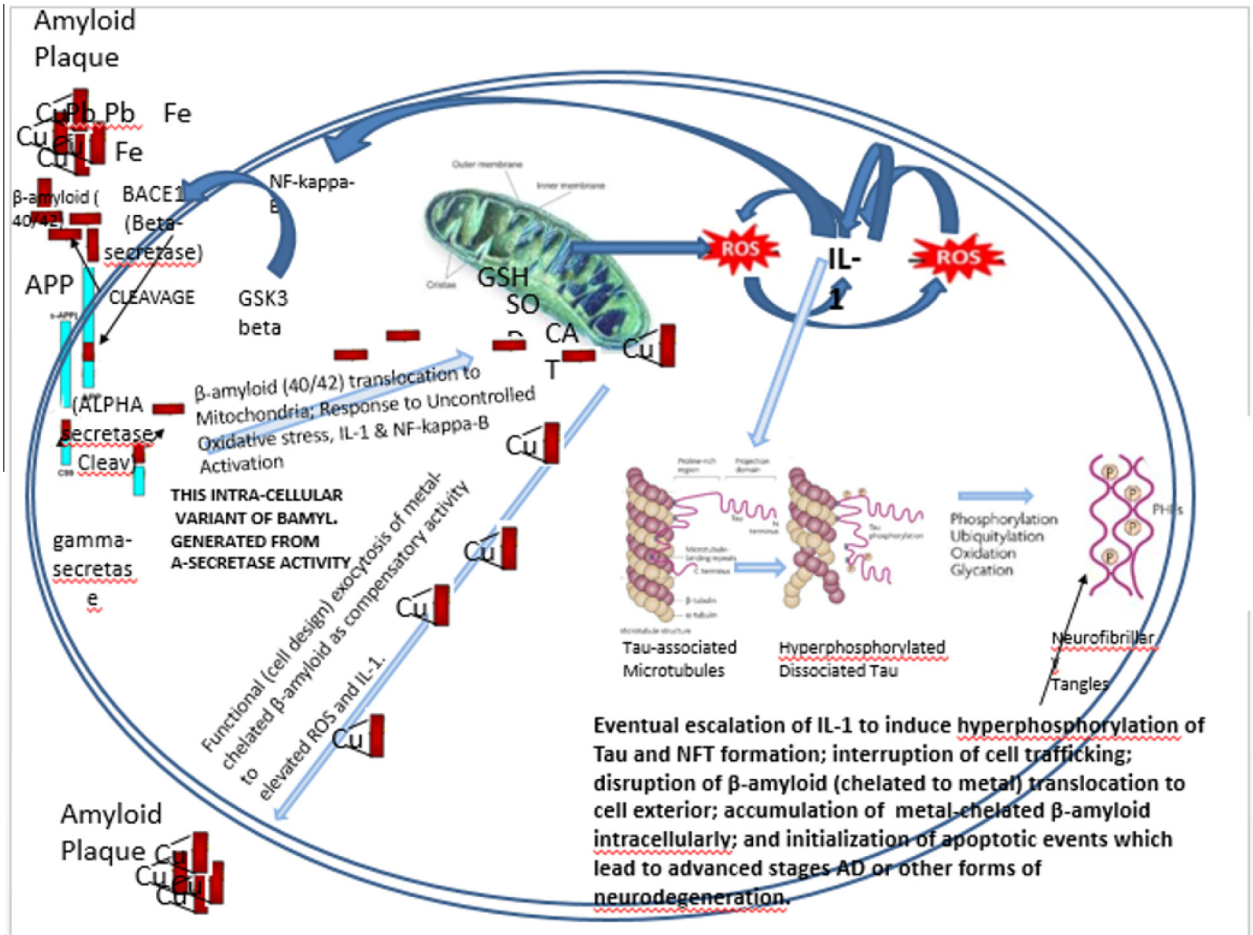

Fig. 1. Presents a schematic of the newly proposed Alzheimer's disease paradigm with transition metals as a primary causal factor. Presents formation of extra-neuronal neuritic plaque comprised of metal-chelated β-amyloid peptides configured into fibrils (β-sheet) and subsequently into amyloid plaque. Also depicts β-amyloid protein intracellular migration patterns when functional trafficking activity is intact.

metals and subsequently it is proposed it moves as the chelate to the extracellular space.

Incremental BACE1 activity can accompany presenilin-1 mutation in FAD once FAD exacerbates the oxidative load in the neuron earlier in the pathology of the disease evolution than it does in the ROS (and heavy metal ion) escalated model that underlies sporadic AD (model shown at Fig. 1 p.16). This theory assigns functionality for the different peptide lengths produced by these different secretase enzymes and aligns the NF-kappa-B transcription factor as an activator of the BACE1 countermeasure upstream of BACE1 as reported in the literature [76]. This BACE1-cleaved variant of the β-amyloid protein is produced as a function of oxidation and inflammation facilitated by NF-kappa-B transcription; and is equipped with amino acids having a higher affinity for metal chelation.

Presenilin mutations result in incremental amyloid peptide production that differs in residue sequence from the BACE1-cleaved peptide and may have a lower affinity for divalent metals and as such a lower toxicity level. Synthesis of these variants is not initiated by a cell under the stress of oxidative activity. However, the volume of the amyloid protein produced in the presenilin mutation-induced pathology is proposed as a component of the new AD paradigm to eventually outcompete endogenous antioxidants CAT, GSH and SOD for the intracellular metal cofactors essential to their antioxidant activity. The resulting oxidative activity from this route eventually initializes NF-kappa-B and subsequently BACE1 engagement as the countermeasure even in FAD.

Neuroinflammation

The central nervous system (CNS) is uniquely situated in the context of the immune system. The blood brain barrier (BBB) is designed to protect the CNS from inadvertent exposure to circulating toxins, metabolites and even peripheral immune cells. Microglia are innate immune cells that serve as the CNS resident macrophages protecting the delicate oxygen-rich environment from invading pathogens, aberrant proteins and other toxic factors. It is proposed, as part of the new AD paradigm that the innate immune system plays a central role in the AD pathology. The fact that these cells are primary immune effectors in the brain is already well known [116].

Dysregulated microglial transformation can result in cytokine release and major histocompatibility complex facilitation to engage the peripheral immune system [117–119]. At this point systemic immune system engagement takes place and neurological disease crosses to advanced auto-inflammatory stages that can include autoimmunity. Cell aberrations including amyloid deposits as we've seen, can induce microglial transformation to active phagocytic cells that scavenge amyloid [120–124].

It has been understood for some time that microglial cell surface receptors specific for β-amyloid protein mediate microglial transformation to facilitate clearance of these potentially toxic peptides [125] and the inflammation associated with these protective activities is not necessarily pathologic. In fact, under the right conditions that result in the protective phenotype (M2-like), microglia will produce and secrete IL-10 [126] to cycle back and inhibit inflammation [127]. Intracranial administration of β-amyloid protein as a potentiator of amyloid scavenging has been investigated in transgenic mice and found to successfully induce microglia to remove compact deposits of endogenous amyloid in APP transgenic mice [119].

If we look at the PD model the literature reveals similar activity. Alpha-synuclein aggregate transforms microglia into phagocytic cells in a dose-dependent manner [128]. Even overexpression of wild type α-synuclein leads to the same microglia activity and subsequent inflammatory response [129,130].

In ALS models, mutant SOD1 accumulation is shown to parallel an increase in localized IL-1β [100] from innate immune cells just as we have seen IL-1β and IL-1 involvement in AD [131] and PD. Interleukin-1 plays a role in early stage localized inflammation and polarization of microglia but due to its potential to autopropagate, left unchecked it can escalate inflammatory activity to systemic engagement. IL-1 potentiates IL-6 and other cytokines in endothelial cells to relay immune recruitment to systemic levels [132]. Inhibition of localized IL-1 has been shown to slow and even inhibit systemic inflammatory activity [133] and is an important target early on, as proposed herein, in the prevention of advanced neurologic disease. We have already established that through one mechanism or another even glutamate survival is extended by the local inflammatory activity to compound neuron distress.

Inflammatory activity is modulated at various points in the cascade at a cellular and subcellular level as well as later in the escalation to systemic activity. At a cellular level a recently discovered modulation feature in myeloid cells sheds light on a modality by which chronic inflammatory activity including that related to auto-inflammation and later in the cycle in autoimmunity might be generated – TREM-2. This sets research sites on a new drug target.

Triggering Receptor Expressed on Myeloid cells (TREM-2) partakes in a regulatory process of the inflammatory cascade. In TREM-2 knockdown cells TLR-induced TNFα production is increased over wild type. TREM-2 is responsible for inhibition of DAP12 associated cytokine production [134]. DAP12 is an ITAM-containing adapter. Ultimately one of the identified roles for TREM-2 is that of an attenuator of macrophage activation [135]. TREM-2 is expressed on macrophages permeating tissues from systemic circulation and it plays a significant role in microglial activity. Research supports that β-amyloid peptide stimulates microglial secretion of cytotoxic inflammatory mediators [136,137] to set the stage for mismanaged glutamate and neuron distress and TREM-2 mutations can play a role in this.

TREM-2 is shown to be up-regulated in early inflammatory activity in healthy cells in order to down-regulate inflammation. Blockade of TREM-2 in early effector phase of experimental autoimmune encephalomyelitis, as an example, results in disease exacerbation [138]. TREM receptor activity potentiates phagocytic activity. Reduced TREM-2 function is shown to compromise phagocytosis and therefore impair microglial removal of apoptotic and other debris [139]. This theoretically includes compromised removal of aberrant or chelated β-amyloid protein, α-synuclein and aggregates of SOD1.

TREM receptors are shown to be expressed in many cells from monocytes, macrophages, microglia and dendritic cells to osteoclasts and platelets [140]. They are implicated in many chronic inflammatory diseases from multiple sclerosis, ALS [141] and inflammatory bowel disease [142–144]. Interesting is the finding that sex steroids such as estradiol and progesterone are shown to up-regulate TREM-2 and IL-10 and it may be that this is one way (through TREM-2 activation and IL-10 elevation) the sex steroids support neuron health [141]. TREM-2 mutation is also implicated in bone disease and linked to osteocyte control by RANK-RANKL-OPG signalling [145].

Inflammatory induction in the form of IL-1 and TNFα play roles in advancing osteoclastogenesis and bone resorption and dysregulated/mutated TREM-2 may play a role [146]. Osteoclasts are ultimately transformed macrophages. TREM-2 mutation offset may be the pathway by which sex hormones help regulate bone metabolism and if this is so and the neuron protection or support by sex hormones is via the same modality, the question must be asked: Can the sex hormones play a role in mitigating neurodegenerative disease in TREM-2 facilitated cases despite a TREM-2 mutation?

There is, in fact, evidence suggesting a role for the sex steroid hormones in neuron health as will be further elaborated. Dysregulation of TREM-2 has also been implicated as a risk factor in neurodegenerative diseases like FTD, AD and PD [147,148,135,149] and more recently in ALS [150].

Summary

It has been suggested here that the neuron's protective counteraction to compounded oxidative stress is, in fact, incremental synthesis of APP and the processing yield from BACE1's catalytic actions on this protein – various β-amyloid peptide species. The incremental synthesis of APP and β-amyloid peptide resulting from oxidative load are proposed, as part of this new paradigm, to be added redundant and compounding compensatory support to the more common endogenous antioxidants SOD, CAT and GSH. However, in this APP/β-amyloid peptide we also have an added protective feature – a free metal countermeasure. APP is known to carry antioxidant potential [48] and the various β-amyloid peptides produced from APP are known to chelate free metal [49].

This β-amyloid peptide synthesis is proposed here to be an intended design. As an interesting parallel, the very endogenous antioxidants SOD, CAT and GSH, themselves, depend on transition metals to exhibit their antioxidant properties – copper/zinc/manganese, manganese and selenium respectively. Shifting the perspective of their roles slightly allows us to consider a compounding benefit by these endogenous antioxidants associated with transition metal management. Transactivation to synthesize these endogenous antioxidants by nuclear Nrf2 [68,151] results in the sequestering of intracellular metals (indirectly) that themselves may be contributing to elevated oxidation. It also results in a concurrent production of antioxidant peptides (SOD, CAT and GSH) that depend on the metal cofactor; thus a compounding protective value.

This perspective that APP and β-amyloid peptide may be playing related roles and/or additive functions to those of SOD, CAT and GSH may at first appear to be counterintuitive based on currently studied AD and PD models. However, when we look at the signalling sequence summarized in Fig. 1 (p. 16), the incremental ROS and the NF-kappa-B it induces is shown to induce BACE1 [152]. From here it is evident that BACE1 recruitment is inspired as a function of ROS and NF-kappa-B activation and in accordance with the proposed new AD paradigm is positioned in the sequence as a reaction to oxidation and inflammation – a protective countermeasure. β-Amyloid peptide, itself, can cycle back to also upregulate BACE1 activity creating and exponential forward signalling [153]. This is proposed, here, as a function of the new AD paradigm to simply be propelled by metal chelation by this β-amyloid peptide and the consequential oxidation resulting in the forward loop.

In the case of ALS, copper is already sequestered by SOD1 as a functional component of the antioxidant peptide and this differs from the pathology of AD. It takes on a different pathology that involves oxidative load induced by misfolded SOD1. Deleterious oxidation induced by this pathologic SOD1 might be a function of misfolding which exposes the metal as previously described. Transmission of the misfolding is conveyed based on healthy SOD1's affinity to quench the oxidative load imposed upon the cell by the misfolded SOD1. The metal is already sequestered in the SOD1 peptide and subsequently in the aggresome therefore the signalling for the synthesis of the countering APP and β-amyloid peptide is not warranted in ALS. This is aligned with the fact that ALS is not an amyloidogenic disease.

In all cases – ALS, PD and AD – trafficking and proteasome activity serve as a second level of compensation designed to remove the toxic insoluble proteins. However, if the cell is faced with additional aberrations associated with trafficking such as TAU hyperphosphorylation as in AD, removal of the misfolded chelated β-amyloid peptide fails. In such a case oxidative load increases and the inflammatory pathway begins its escalatory phase. Cases of the disease that produce abnormally elevated levels of the aberrant protein that are not manageable by functional countermeasures of the cell will also succumb to symptoms of disease pathology.

At any point in the progression of these events such as even a mild escalation of β-amyloid peptide and α-synuclein, microglia can be triggered by the aggregates to induce their transformation [154]. A TREM-2 mutation in the resident microglia in addition to trafficking aberrations or independent of these trafficking- or degradation-associated mutations could trigger an exacerbated inflammatory response [155]. This could occur even to low molecular weight aggregates made up of misfolded SOD1, β-amyloid peptide or α-synuclein.

Most diseases including our three, AD, ALS and PD, are multigenic. The multigenic model of disease involves multiples of aberrations and stressors which most often include mutations of the cell's compensatory systems. Multigenic diseases will require multi-target approaches to treat them effectively. In the case of AD, this proposed model points to preventive measures that must be applied early in the preclinical phase to prevent neuron damage and apoptosis. In the context of the proposed new AD paradigm this cannot involve inhibition of BACE1 (as currently targeted) to thwart the very system that is protecting the cell. Research already shows that BACE1 inhibition can result in neurological deficits [156]. In addition, this preclinical phase can be as lengthy as twenty years as cited. This provides an expansive window where long term persistence with the right intervention protocol might prove fruitful. Metal chelation may be a central feature of the treatment.

In the case of AD, ALS and PD we may have some common nodes to consider such as TREM-2 restoration or compensation if TREM-2 mutation is evident; NF-kappa-B inhibition to inhibit IL-1 or to compensate for TREM-2 mutation; and/or direct IL-1 inhibition. We can also consider a chelation approach to sequester free transition metals if these toxic metals exist in concentrations that are contributing to pathogenesis. Research using animal models can be designed to test these targets as a combined therapy. In vitro and subsequent in vivo research has already been designed at our lab to test the proposed new Cavaleri AD paradigm.

Drugs like Anakinra are shown to improve symptoms rising out of auto-inflammatory mechanisms [157,133]. Inhibition of IL-1 by Anakinra is also shown to inhibit systemic evolution of disease pathology via indirect IL-6 inhibition [158]. Such strategies may not resolve the genetic source of the inflammatory response or the underlying pathogenesis of the neurodegenerative disease but it may prove to down-regulate inflammasome dysregulation that can arise from poorly regulated inflammation due to mutations in TREM-2 or poorly managed intracellular oxidation.

Inhibition of NF-kappa-B can serve well to mitigate escalation of disease at the point of inflammasome assembly and innate immune system initialization either instead of or in combination with direct IL-1 inhibition. A drug in current study at UBC's Brain Research Center has shown promise as a role player in such preventive and even therapeutic programs. This is due to the drug's pleiotropic pharmacology including a propensity to mitigate NF-kappa-B transactivation; chelate transition metals; serve as a potent antioxidant (reducing agent); and activate endogenous antioxidant generation through Nrf2 transactivation. We are currently testing these activities in the context of our proposed new AD paradigm.

This current research (unpublished) is also pointing to cell-type specific activity by this potentially therapeutic agent not before identified; activity conducive to preserving non-macrophage cell

function by down-regulating genomic events related to prolonged inflammation that can be deleterious to the neuron. However, the pharmacology is shown to concurrently support basal NF-kappa-B activity and inflammatory events leading to macrophage (including microglia) polarization; supportive of effective immune response for debridement, scavenging and tissue protection and restoration. This pharmacology can play a significant role in anti-inflammatory applications including targeting amyloidogenic, neuroinflammatory and neurodegenerative diseases like AD.

Sex-hormones as we have seen, have also shown promise in the up-regulation of TREM-2 and IL-10 and this may serve the therapeutic model as part of the multidrug program to reduce the rate in which these multigenic diseases might advance. Research does, in fact, indicate a lower risk for AD with hormone (estrogen) replacement therapy (HRT) in older women [159]. Research also indicates maintenance in hippocampal volume in women applying HRT but whether this translates into preserved cognitive capacity is uncertain [160]. Research shows estrogens to confer neuroprotection by astrocyte regulation [161] in models of multiple sclerosis. Estrogens are shown to directly suppress β-amyloid signalling into the nucleus and in this way may be playing a positive role in amyloid management [6]. Estrogens are also shown to decrease the risk for AD and it may likely be due to facilitation of microglia phagocytosis and degradation of amyloid protein [162].

However, it must be noted, on the other hand, other studies point to a weak conclusion that estrogens possibly contribute deleterious effects due to the fact that autoimmune diseases manifest with higher frequency in women. In multiple sclerosis, for instance, where autoimmunity is definitively involved in the pathology it is shown that testosterone therapy may play a role in slowing down progression. Pilot studies are revealing promising results although it's too early to make conclusions about neuropathologies that are as complex as these [163,164].

However, it is commonly understood that neurons have androgen receptors that play roles in neuron development; specifically in axon and white matter development [165]. Testosterone is shown to have neuroprotective effects in motorneurons [166]. This protection may be a function of its capacity to preserve excitatory transmission and myelin integrity [167]. Studies targeting the neuron androgen receptor as a treatment strategy for demyelinating diseases such as multiple sclerosis have shown promising results [168].

It's possible that the increased prevalence of multiple sclerosis in women predisposed due to other factors of the multigenic disease is a function of protective estrogen deficits as menopause approaches and ensues. Estrogen deficits associated with menstrual cycle changes are also shown to exacerbate symptoms of multiple sclerosis [169]. Sex hormone reduction in males takes on a very different pattern. Nevertheless, estrogen with progestin therapy may be a viable inclusion in the treatment of neurological diseases for women with testosterone therapy accompanied by dehydrotestosterone inhibition to play a role as part of the male therapeutic arsenal.

Applied in the early stages (early in the preclinical phase of the disease), such regulation of inflammatory cytokines and endocrine restoration may slow the transformation of microglia from their disease propagating state; and empower them to function as designed. The treatment of multigenic diseases such as these three will require case specific evaluation to determine precise genetic factors contributing to the pathology and the establishment of gene-specific therapies or mutation-specific treatment with multiple drugs that address the multigenic condition.

IL-1 is implicated as a mediator of dopaminergic cell death in PD [170]; as we've seen it plays a role in AD; and it is generally understood to be a factor in localized inflammation that starts in motion systemic acute phase response. Anakinra is a potent IL-1 receptor antagonist [171] that crosses the BBB to reach cerebrovascular and spinal fluid concentrations that are therapeutic [172–174]. It is understood that steroid hormones readily cross the BBB via protein-bound or protein-mediated transport [175].

The therapeutic agent (NF-kappa-B inhibitor) we are currently investigating at the Brain Research Center may fit as a component of this common therapeutic foundation as well. Its full potential to cross the BBB is also in the process of being confirmed while we confirm other properties that are conducive to its role in this common therapeutic foundation.

Based on our review and the expansion of the new AD paradigm, it will likely be found that future treatment strategies for these three diseases – AD, ALS and PD and even other neurodegenerative diseases – may be comprised of a group of drugs that form a common treatment foundation. These may even include our three described – sex-steroid hormones, IL-1 inhibition and NF-kappa-B inhibition. It may include transition metal chelation as part of this common therapeutic foundation and for PD and AD an added component that transactivates Nrf2. The investigation of this and other common therapeutic drug foundations and their potential in AD, ALS and PD will be furthered in our own work one drug at a time. Our primary work is currently being executed in the context of the new AD paradigm to further define the disease model and the potential of a drug therapy.

Conflicts of interest statement

The author hypothesis is not inspired by third party influence; financial or other. The proposed paradigm shift and the treatment protocol in this review have not yet been studied in the context of Alzheimer's disease. A research grant will be pursued from a federal agency in order to avoid conflict of interest.

References

[1] Cavaleri F. Paradigm shift redefining molecular, metabolic and structural events in Alzheimer's disease involves a proposed contribution by transition metals. Defined lengthy preclinical stage provides new hope to circumvent advancement of disease-and age-related neurodegeneration. Med Hypotheses 2015.

[2] Ebneth A et al. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. J Cell Biol 1998;143(3):777–94.

[3] Cohen FE, Kelly JW. Therapeutic approaches to protein-misfolding diseases. Nature 2003;426(6968):905–9.

[4] Devi L, Anandatheerthavarada HK. Mitochondrial trafficking of APP and alpha synuclein: relevance to mitochondrial dysfunction in Alzheimer's and Parkinson's diseases. Biochim Biophys Acta 2010;1802(1):11–9.

[5] Jhoo JH et al. β-Amyloid (1–42)-induced learning and memory deficits in mice: involvement of oxidative burdens in the hippocampus and cerebral cortex. Behav Brain Res 2004;155(2):185–96.

[6] Bao J et al. Suppression of β-amyloid precursor protein signaling into the nucleus by estrogens mediated through complex formation between the estrogen receptor and Fe65. Mol Cell Biol 2007;27(4):1321–33.

[7] Chen X-H et al. Long-term accumulation of amyloid-β, β-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol 2004;165(2):357–71.

[8] Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med 2004.

[9] Houlden H et al. A novel presenilin mutation (M233V) causing very early onset Alzheimer's disease with Lewy bodies. Neurosci Lett 2001;313(1):93–5.

[10] Selkoe DJ. Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. Nat Cell Biol 2004;6(11):1054–61.

[11] Duff K et al. Increased amyloid-β42 (43) in brains of mice expressing mutant presenilin 1. Nature 1996;383(6602):710–3.

[12] Citron M et al. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. Nat Med 1997;3(1):67–72.

[13] Giliberto L et al. Mutant presenilin 1 increases the expression and activity of BACE1. J Biol Chem 2009;284(14):9027–38.

[14] Singleton A et al. α-Synuclein locus triplication causes Parkinson's disease. Science 2003;302(5646):841.

[15] Dawson TM, Dawson VL. Rare genetic mutations shed light on the pathogenesis of Parkinson disease. J Clin Investig 2003;111(2):145–51.

[16] Hashimoto M et al. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. NeuroMol Med 2003;4(1–2):21–35.
[17] Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. Science 2003;302(5646):819–22.
[18] Iguchi Y et al. Oxidative stress induced by glutathione depletion reproduces pathological modifications of TDP-43 linked to TDP-43 proteinopathies. Neurobiol Dis 2012;45(3):862–70.
[19] Nakashima-Yasuda H et al. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol 2007;114(3):221–9.
[20] Rutherford NJ et al. Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. PLoS Genet 2008;4(9):e1000193.
[21] Cole NB et al. Metal-catalyzed oxidation of α-synuclein helping to define the relationship between oligomers, protofibrils, and filaments. J Biol Chem 2005;280(10):9678–90.
[22] Paik SR, Shin H-J, Lee J-H. Metal-catalyzed oxidation of α-synuclein in the presence of copper (II) and hydrogen peroxide. Arch Biochem Biophys 2000;378(2):269–77.
[23] Polymeropoulos MH et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 1997;276(5321):2045–7.
[24] Rasia RM et al. Structural characterization of copper (II) binding to α-synuclein: insights into the bioinorganic chemistry of Parkinson's disease. Proc Natl Acad Sci USA 2005;102(12):4294–9.
[25] Atwood CS et al. Dramatic aggregation of Alzheimer Aβ by Cu (II) is induced by conditions representing physiological acidosis. J Biol Chem 1998;273 (21):12817–26.
[26] Cornett C et al. Trace elements in Alzheimer's disease pituitary glands. Biol Trace Elem Res 1998;62(1–2):107–14.
[27] Syme CD et al. Copper binding to the amyloid-β (Aβ) peptide associated with Alzheimer's disease folding, coordination geometry, pH dependence, stoichiometry, and affinity of Aβ-(1–28): insights from a range of complementary spectroscopic techniques. J Biol Chem 2004;279 (18):18169–77.
[28] Atwood CS et al. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-β. Biochemistry 2004;43(2):560–8.
[29] Bellingham SA et al. Copper depletion down-regulates expression of the Alzheimer's disease amyloid-β precursor protein gene. J Biol Chem 2004;279 (19):20378–86.
[30] Hashimoto M et al. Oxidative stress induces amyloid-like aggregate formation of NACP/α-synuclein in vitro. NeuroReport 1999;10(4):717–21.
[31] Binolfi A et al. Site-specific interactions of Cu (II) with α and β-synuclein: bridging the molecular gap between metal binding and aggregation. J Am Chem Soc 2008;130(35):11801–12.
[32] Bush AI. Metals and neuroscience. Curr Opin Chem Biol 2000;4(2):184–91.
[33] Jomova K et al. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 2010;345(1–2):91–104.
[34] Hashimoto M et al. Human recombinant NACP/α-synuclein is aggregated and fibrillated in vitro: relevance for Lewy body disease. Brain Res 1998;799 (2):301–6.
[35] Valentine JS, Doucette PA, Zittin Potter S. Copper–zinc superoxide dismutase and amyotrophic lateral sclerosis. Annu Rev Biochem 2005;74:563–93.
[36] Hwang C-S et al. Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of Candida albicans against oxidative stresses and the expression of its full virulence. Microbiology 2002;148 (11):3705–13.
[37] Yim MB et al. A gain-of-function of an amyotrophic lateral sclerosis-associated Cu, Zn-superoxide dismutase mutant: an enhancement of free radical formation due to a decrease in Km for hydrogen peroxide. Proc Natl Acad Sci 1996;93(12):5709–14.
[38] Eum WS, Kang JH. Release of copper ions from the familial amyotrophic lateral sclerosis-associated Cu. Zn-superoxide dismutase mutants. Mol Cell 1999;9(1):110–4.
[39] Chan PH. Oxygen radicals in focal cerebral ischemia. Brain Pathol 1994;4 (1):59–65.
[40] Jaarsma D et al. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol Dis 2000;7(6):623–43.
[41] Grad LI et al. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc Natl Acad Sci 2011;108(39):16398–403.
[42] Stieber A et al. Disruption of the structure of the Golgi apparatus and the function of the secretory pathway by mutants G93A and G85R of Cu, Zn superoxide dismutase (SOD1) of familial amyotrophic lateral sclerosis. J Neurol Sci 2004;219(1):45–53.
[43] Bolton C, Paul C. Glutamate receptors in neuroinflammatory demyelinating disease. Mediators Inflamm 2006;2006.
[44] Foust KD et al. Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS. Mol Ther 2013.
[45] Münch C, O'Brien J, Bertolotti A. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc Natl Acad Sci 2011;108 (9):3548–53.
[46] Urushitani M et al. The endoplasmic reticulum-Golgi pathway is a target for translocation and aggregation of mutant superoxide dismutase linked to ALS. FASEB J 2008;22(7):2476–87.
[47] Bosco DA et al. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci 2010;13 (11):1396–403.
[48] Goodman Y, Mattson MP. Secreted forms of β-amyloid precursor protein protect hippocampal neurons against amyloid β-peptide-induced oxidative injury. Exp Neurol 1994;128(1):1–12.
[49] Kontush A. Amyloid-β: an antioxidant that becomes a pro-oxidant and critically contributes to Alzheimer's disease. Free Radical Biol Med 2001;31 (9):1120–31.
[50] Halliwell B. Reactive oxygen species and the central nervous system, in Free Radicals in the Brain. Springer; 1992. p. 21–40.
[51] Liu D, Liu J, Wen J. Elevation of hydrogen peroxide after spinal cord injury detected by using the Fenton reaction. Free Radical Biol Med 1999;27 (3):478–82.
[52] Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci 2008;31(9):454–63.
[53] Johnson VE, Stewart W, Smith DH. Traumatic brain injury and amyloid-β pathology: a link to Alzheimer's disease? Nat Rev Neurosci 2010;11 (5):361–70.
[54] Uryu K et al. Repetitive mild brain trauma accelerates Aβ deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. J Neurosci 2002;22(2):446–54.
[55] Bush AI, Tanzi RE. The galvanization of β-amyloid in Alzheimer's disease. Proc Natl Acad Sci 2002;99(11):7317–9.
[56] Bousejra-ElGarah F et al. Iron (II) binding to amyloid-β, the Alzheimer's peptide. Inorg Chem 2011;50(18):9024–30.
[57] Cherny RA et al. Treatment with a copper–zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 2001;30(3):665–76.
[58] Wirths O et al. Intraneuronal Aβ accumulation precedes plaque formation in β-amyloid precursor protein and presenilin-1 double-transgenic mice. Neurosci Lett 2001;306(1):116–20.
[59] Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress. Free Radical Biol Med 2002;32(11):1050–60.
[60] Frautschy SA, Baird A, Cole GM. Effects of injected Alzheimer beta-amyloid cores in rat brain. Proc Natl Acad Sci 1991;88(19):8362–6.
[61] Clifford PM et al. Aβ peptides can enter the brain through a defective blood–brain barrier and bind selectively to neurons. Brain Res 2007;1142:223–36.
[62] Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol 2007;8 (2):101–12.
[63] Benilova I, Karran E, De Strooper B. The toxic A [beta] oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 2012;15 (3):349–57.
[64] Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 2006;75:333–66.
[65] Goldstein JC et al. The coordinate release of cytochrome c during apoptosis is rapid, complete and kinetically invariant. Nat Cell Biol 2000;2(3):156–62.
[66] de Vries HE et al. Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? Free Radical Biol Med 2008;45(10):1375–83.
[67] Dumont M, Beal MF. Neuroprotective strategies involving ROS in Alzheimer disease. Free Radical Biol Med 2011;51(5):1014–26.
[68] Bryan HK et al. The Nrf2 cell defence pathway: Keap1-dependent and-independent mechanisms of regulation. Biochem Pharmacol 2013;85 (6):705–17.
[69] Nguyen T, Huang H, Pickett CB. Transcriptional regulation of the antioxidant response element Activation by Nrf2 and repression by MafK. J Biol Chem 2000;275(20):15466–73.
[70] Nik Moussavi. S.H., et al., The BACE1-PSEN-AβPP regulatory axis has an ancient role in response to low oxygen/oxidative stress. J Alzheimer's Dis 2012;28(3):515–30.
[71] Wright JA, Brown DR. Alpha-synuclein and its role in metal binding: relevance to Parkinson's disease. J Neurosci Res 2008;86(3):496–503.
[72] Flohé L et al. Redox regulation of NF-kappa B activation. Free Radical Biol Med 1997;22(6):1115–26.
[73] García-Ruiz C et al. Role of oxidative stress generated from the mitochondrial electron transport chain and mitochondrial glutathione status in loss of mitochondrial function and activation of transcription factor nuclear factor-kappa B: studies with isolated mitochondria and rat hepatocytes. Mol Pharmacol 1995;48(5):825–34.
[74] Bourne KZ et al. Differential regulation of BACE1 promoter activity by nuclear factor-κB in neurons and glia upon exposure to β-amyloid peptides. J Neurosci Res 2007;85(6):1194–204.
[75] Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12(9):1005–15.
[76] Chen C-H et al. Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. Int J Neuropsychopharmacol 2012;15(1):77–90.
[77] Murphy RM. Peptide aggregation in neurodegenerative disease. Annu Rev Biomed Eng 2002;4(1):155–74.
[78] Mantyh PW et al. Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of β-amyloid peptide. J Neurochem 1993;61 (3):1171–4.

[79] Rottkamp CA et al. Redox-active iron mediates amyloid-β toxicity. Free Radical Biol Med 2001;30(4):447–50.
[80] Amrallah AH, Abdalla NA, El-Haty EY. Mixed ligand complexes of benzimidazole and pyrimidine hydroxy azo dyes with some transition metals and glycine, DL-alanine or DL-leucine. Talanta 1998;46(4):491–500.
[81] Cavaleri F. Paradigm shift redefining molecular, metabolic and structural events in Alzheimer's disease involves a proposed contribution by transition metals. Defined lengthy preclinical stage provides new hope to circumvent advancement of disease-and age-related neurodegeneration. Med Hypotheses 2015;84(5):460–9.
[82] Elias MF et al. The preclinical phase of Alzheimer disease: a 22-year prospective study of the Framingham Cohort. Arch Neurol 2000;57 (6):808–13.
[83] Praticò D et al. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci 2001;21(12):4183–7.
[84] Terry RD et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30(4):572–80.
[85] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002;298 (5594):789–91.
[86] Doens D, Fernández PL. Microglia receptors and their implications in the response to amyloid beta for Alzheimer's disease pathogenesis. J Neuroinflam 2014;11(1):48.
[87] Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med 2003;81(11):678–99.
[88] Martins IC et al. Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice. EMBO J 2008;27(1):224–33.
[89] Olivieri G et al. Mercury induces cell cytotoxicity and oxidative stress and increases β-amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. J Neurochem 2000;74(1):231–6.
[90] Martinon F. Signaling by ROS drives inflammasome activation. Eur J Immunol 2010;40(3):616–9.
[91] Salminen A et al. ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. J Neuroinflam 2009;6(1):41.
[92] Sengupta A et al. Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules. Arch Biochem Biophys 1998;357(2):299–309.
[93] Gong C-X, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. Curr Med Chem 2008;15(23):2321.
[94] Morrissette DA et al. Relevance of transgenic mouse models to human Alzheimer disease. J Biol Chem 2009;284(10):6033–7.
[95] Blurton-Jones M, LaFerla FM. Pathways by which Aβ facilitates tau pathology. Curr Alzheimer Res 2006;3(5):437–48.
[96] Dickson DW. Apoptotic mechanisms in Alzheimer neurofibrillary degeneration: cause or effect? J Clin Investig 2004;114(1):23–7.
[97] Yang W et al. Microtubule-associated tau protein positive neuronal and glial inclusions in ALS. Neurology 2003;61(12):1766–73.
[98] Strong M et al. Tau protein hyperphosphorylation in sporadic ALS with cognitive impairment. Neurology 2006;66(11):1770–1.
[99] Buée-Scherrer V et al. Neurofibrillary degeneration in amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam. Immunochemical characterization of tau proteins. Am J Pathol 1995;146(4):924.
[100] Masters SL, O'Neill LA. Disease-associated amyloid and misfolded protein aggregates activate the inflammasome. Trends Mol Med 2011;17(5):276–82.
[101] Rotilio G, Rossi L, Ciriolo M. Copper-dependent oxidative stress and neurodegeneration. IUBMB Life 2000;50(4–5):309–14.
[102] Beretta S et al. Mitochondrial dysfunction due to mutant copper/zinc superoxide dismutase associated with amyotrophic lateral sclerosis is reversed by *N*-acetylcysteine. Neurobiol Dis 2003;13(3):213–21.
[103] Suh Y-H, Checler F. Amyloid precursor protein, presenilins, and α-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. Pharmacol Rev 2002;54(3):469–525.
[104] Hurtig H et al. Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. Neurology 2000;54(10):1916–21.
[105] Compta Y et al. Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in Parkinson's disease. Mov Disord 2009;24 (15):2203–10.
[106] Haggerty T et al. Hyperphosphorylated Tau in an α-synuclein-overexpressing transgenic model of Parkinson's disease. Eur J Neurosci 2011;33 (9):1598–610.
[107] Lee VM, Giasson BI, Trojanowski JQ. More than just two peas in a pod: common amyloidogenic properties of tau and α-synuclein in neurodegenerative diseases. Trends Neurosci 2004;27(3):129–34.
[108] Iribarren P et al. Role of formyl peptide receptor-like 1 (FPRL1/FPR2) in mononuclear phagocyte responses in Alzheimer disease. Immunol Res 2005;31(3):165–76.
[109] Vekrellis K et al. Neurons regulate extracellular levels of amyloid β-protein via proteolysis by insulin-degrading enzyme. J Neurosci 2000;20 (5):1657–65.
[110] Citron M. β-Secretase as a target for the treatment of Alzheimer's disease. J Neurosci Res 2002;70(3):373–9.
[111] Takasugi N et al. The role of presenilin cofactors in the γ-secretase complex. Nature 2003;422(6930):438–41.
[112] Kefaloyianni E, Gaitanaki C, Beis I. ERK1/2 and p38-MAPK signalling pathways, through MSK1, are involved in NF-κB transactivation during oxidative stress in skeletal myoblasts. Cell Signal 2006;18(12):2238–51.
[113] Schenk H et al. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappa B and AP-1. Proc Natl Acad Sci 1994;91(5):1672–6.
[114] Wang T, Zhang X, Li JJ. The role of NF-κB in the regulation of cell stress responses. Int Immunopharmacol 2002;2(11):1509–20.
[115] Baeuerle PA, Henkel T. Function and activation of NF-kappaB in the immune system. Annu Rev Immunol 1994;12(1):141–79.
[116] Lee CD, Landreth GE. The role of microglia in amyloid clearance from the AD brain. J Neural Transm 2010;117(8):949–60.
[117] Schenk D, Yednock T. The role of microglia in Alzheimer's disease: friend or foe? Neurobiol Aging 2002;23(5):677–9.
[118] Butovsky O et al. Activation of microglia by aggregated β-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-γ and IL-4 render them protective. Mol Cell Neurosci 2005;29 (3):381–93.
[119] Wilcock DM et al. Intracranially administered anti-Aβ antibodies reduce β-amyloid deposition by mechanisms both independent of and associated with microglial activation. J Neurosci 2003;23(9):3745–51.
[120] El Khoury J et al. Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils. Nature 1996;382(6593):716–9.
[121] Paresce DM, Ghosh RN, Maxfield FR. Microglial cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor. Neuron 1996;17(3):553–65.
[122] Kakimura J-I et al. Microglial activation and amyloid-β clearance induced by exogenous heat-shock proteins. FASEB J 2002;16(6):601–3.
[123] Simard AR et al. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 2006;49 (4):489–502.
[124] Coraci IS et al. CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to β-amyloid fibrils. Am J Pathol 2002;160(1):101–12.
[125] Bamberger ME et al. A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation. J Neurosci 2003;23(7):2665–74.
[126] Jack CS et al. TLR signaling tailors innate immune responses in human microglia and astrocytes. J Immunol 2005;175(7):4320–30.
[127] Mizuno T et al. Production of interleukin-10 by mouse glial cells in culture. Biochem Biophys Res Commun 1994;205(3):1907–15.
[128] Zhang W et al. Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J 2005;19(6):533–42.
[129] Su X et al. Synuclein activates microglia in a model of Parkinson's disease. Neurobiol Aging 2008;29(11):1690–701.
[130] Alvarez-Erviti L et al. Alpha-synuclein release by neurons activates the inflammatory response in a microglial cell line. Neurosci Res 2011;69 (4):337–42.
[131] Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. Nature 1997;388 (6645):878–81.
[132] Sironi M et al. IL-1 stimulates IL-6 production in endothelial cells. J Immunol 1989;142(2):549–53.
[133] Dinarello CA. Blocking IL-1 in systemic inflammation. J Exp Med 2005;201 (9):1355–9.
[134] Hamerman JA et al. Cutting edge: inhibition of TLR and FcR responses in macrophages by triggering receptor expressed on myeloid cells (TREM)-2 and DAP12. J Immunol 2006;177(4):2051–5.
[135] Turnbull IR et al. Cutting edge: TREM-2 attenuates macrophage activation. J Immunol 2006;177(6):3520–4.
[136] Rogers J et al. Microglia and inflammatory mechanisms in the clearance of amyloid β peptide. Glia 2002;40(2):260–9.
[137] Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. J Neuroinflam 2004;1(1):14.
[138] Piccio L et al. Blockade of TREM-2 exacerbates experimental autoimmune encephalomyelitis. Eur J Immunol 2007;37(5):1290–301.
[139] Klesney-Tait J, Turnbull IR, Colonna M. The TREM receptor family and signal integration. Nat Immunol 2006;7(12):1266–73.
[140] Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. Curr Opin Immunol 2009;21(1):38–46.
[141] Habib P et al. Sex steroid hormone-mediated functional regulation of microglia-like BV-2 cells during hypoxia. J Steroid Biochem Mol Biol 2013;138:195–205.
[142] Schenk M et al. TREM-1-expressing intestinal macrophages crucially amplify chronic inflammation in experimental colitis and inflammatory bowel diseases. J Clin Investig 2007;117(10):3097–106.
[143] C. Correale et al. Bacterial sensor triggering receptor expressed on myeloid cells-2 regulates the mucosal inflammatory response. Gastroenterology 2013;144(2):346–56. e3.
[144] Derive M, Massin F, Gibot S. Triggering receptor expressed on myeloid cells-1 as a new therapeutic target during inflammatory diseases. Self Nonself 2010;1(3):225–30.
[145] Wada T et al. RANKL–RANK signaling in osteoclastogenesis and bone disease. Trends Mol Med 2006;12(1):17–25.
[146] Leibbrandt A, Penninger JM, RANKL/RANK as key factors for osteoclast development and bone loss in arthropathies, in: Molecular Mechanisms of Spondyloarthropathies. Springer. 2009. p. 100–13.

[147] Guerreiro R et al. TREM2 variants in Alzheimer's disease. N Engl J Med 2013;368(2):117–27.
[148] Daws MR et al. Cloning and characterization of a novel mouse myeloid DAP12-associated receptor family. Eur J Immunol 2001;31(3):783–91.
[149] Neumann H, Daly MJ. Variant TREM2 as risk factor for Alzheimer's disease. N Engl J Med 2013;368(2):182–4.
[150] Cady J et al. TREM2 variant p. R47H as a risk factor for sporadic amyotrophic lateral sclerosis. JAMA Neurol 2014;71:449–53.
[151] Jin W et al. Role of Nrf2 in protection against traumatic brain injury in mice. J Neurotrauma 2009;26(1):131–9.
[152] Chen C-H et al. Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. Int J Neuropsychopharmacol 2012;15(01):77–90.
[153] Read J, Suphioglu C. Dropping the BACE: beta secretase (BACE1) as an Alzheimer's disease intervention target. Neurodegenerative diseases. Croatia: InTech; 2013.
[154] Fu R et al. Phagocytosis of microglia in the central nervous system diseases. Mol Neurobiol 2014;49(3):1422–34.
[155] Stefano L et al. The surface-exposed chaperone, Hsp60, is an agonist of the microglial TREM2 receptor. J Neurochem 2009;110(1):284–94.
[156] Harrison SM et al. BACE1 (β-secretase) transgenic and knockout mice: identification of neurochemical deficits and behavioral changes. Mol Cell Neurosci 2003;24(3):646–55.
[157] So A et al. A pilot study of IL-1 inhibition by anakinra in acute gout. Arthritis Res Ther 2007;9(2):R28.
[158] Ikonomidis I et al. Inhibition of interleukin-1 by anakinra improves vascular and left ventricular function in patients with rheumatoid arthritis. Circulation 2008;117(20):2662–9.
[159] Zandi PP et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 2002;288 (17):2123–9.
[160] Erickson MC. Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. Compr Rev Food Sci Food Saf 2010;9(6):602–19.
[161] Spence RD et al. Estrogen mediates neuroprotection and anti-inflammatory effects during EAE through ERα signaling on astrocytes but not through ERβ signaling on astrocytes or neurons. J Neurosci 2013;33(26):10924–33.
[162] Li R et al. Estrogen enhances uptake of amyloid β-protein by microglia derived from the human cortex. J Neurochem 2000;75(4):1447–54.
[163] Sicotte NL et al. Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol 2007;64(5):683–8.
[164] Gold SM, Voskuhl RR. Estrogen and testosterone therapies in multiple sclerosis. Prog Brain Res 2009;175:239–51.
[165] Perrin JS et al. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J Neurosci 2008;28(38):9519–24.
[166] Tehranipour M, Moghimi A. Neuroprotective effects of testosterone on regenerating spinal cord motoneurons in rats. J Mot Behav 2010;42 (3):151–5.
[167] Ziehn MO et al. Therapeutic testosterone administration preserves excitatory synaptic transmission in the hippocampus during autoimmune demyelinating disease. J Neurosci 2012;32(36):12312–24.
[168] Hussain R et al. The neural androgen receptor: a therapeutic target for myelin repair in chronic demyelination. Brain 2013;136(1):132–46.
[169] Smith R, Studd J. A pilot study of the effect upon multiple sclerosis of the menopause, hormone replacement therapy and the menstrual cycle. J R Soc Med 1992;85(10):612–3.
[170] Koprich JB et al. Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J Neuroinflam 2008;5(1):8.
[171] Vounotrypidis P et al. Refractory relapsing polychondritis: rapid and sustained response in the treatment with an IL-1 receptor antagonist (anakinra). Rheumatology 2006;45(4):491–2.
[172] Clark SR et al. Interleukin-1 receptor antagonist penetrates human brain at experimentally therapeutic concentrations. J Cereb Blood Flow Metab 2007;28(2):387–94.
[173] Galea J et al. Intravenous anakinra can achieve experimentally effective concentrations in the central nervous system within a therapeutic time window: results of a dose-ranging study. J Cereb Blood Flow Metab 2010;31 (2):439–47.
[174] Baamonde A et al. Antihyperalgesic effects induced by the IL-1 receptor antagonist anakinra and increased IL-1β levels in inflamed and osteosarcoma-bearing mice. Life Sci 2007;81(8):673–82.
[175] Pardridge W. Transport of nutrients and hormones through the blood–brain barrier. Diabetologia 1981;20(3):246–54.