Lymphocytes in Alzheimer's disease pathology: Altered signaling pathways

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder marked by progressive impairment of cognitive ability. Patients with AD display neuropathological lesions including plaques, neurofibrillary tangles, and neuronal loss in brain regions linked to cognitive functions. Despite progress in uncovering many of the factors that contribute to the etiology of this disease, the cause of neuronal death is largely unknown. Neuroinflammation seems to play a critical role in the pathogenesis of AD. Inflammatory processes in the brain are mainly mediated by the intrinsic innate immune system consisting of astrocytes and microglial cells, and cytokine, chemokine, and growth factor signaling molecules. However mounting evidence suggest that the Central Nervous System (CNS) is accessible to lymphocytes and monocytes from the blood stream, indicating that there is an intense crosstalk between the immune and the CN systems. On the other hand some AD-specific brain-derived proteins or metabolites may enter the plasma through a deficient blood-brain barrier, and exert some measurable signaling properties in peripheral cells. The goals of this review are: 1) to explore the evidences of changes in signaling pathways that could mediate both central and peripheral manifestations of AD, and 2) to explore whether changes in immune cells, particularly lymphocytes, could contribute to AD pathogenesis.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder with a prevalence of 11% in people age 65 and older and represents 60% of all cases of dementia [1]. It begins with a decline in cognition followed by a number of other changes in brain functioning, including impairments in language and visual-spatial skills, and disorientation. This impairment in cognitive functions is due to anatomical atrophy of AD brains, which correlates with severe neuronal loss. Neuropathological hallmarks are extracellular deposits of misfolded amyloid-β (Aβ) protein, so-called senile plaques and intracellular accumulations of hyperphosphorylated microtubule-associated tau (neurofibrillary tangles, NFT). Although the molecular mechanisms of AD are still being unraveled, neuroinflammation has been reported to contribute to the pathophysiology of AD and late-onset neurodegenerative diseases [2]. Local immune responses involving glial cells and the complement system are activated in the AD brain [3; 4]. The cells responsible for the inflammatory reaction are microglia, astrocytes and neurons. These activated cells produce high levels of inflammatory mediators such as proinflammatory cytokines and chemokines, prostaglandins, leukotrienes, thromboxanes, coagulation factors, free radicals as reactive oxygen species and nitric oxide, complement factors, proteases and protease inhibitors, and C-reactive protein [5]. In addition, there is now strong evidence suggesting the involvement of a systemic immune response in AD [6]. Both increased concentrations of proinflammatory cytokines and changes in lymphocyte subsets, with an augmented percentage of immune cells expressing activation markers, are described in AD [7-9]. Thus, it seems that the brain and immune system are intricately connected and involved in significant crosstalk to maintain homeostasis.

The communication pathways from peripheral sites of inflammation to the brain have been investigated in animal models [10]. It was reported that systemically generated inflammatory mediators signal to the brain via both neural and humoral routes in the blood [11; 12]. Cytokines such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor (TNF) circulate in the blood and signal to the CNS via the circumventricular organs, which lack a patent blood-brain barrier (BBB) [13] or signal across the BBB via receptors expressed on the endothelium [14]. The presence of multiple pathways from the peripheral immune system to the brain highlights the importance of this signaling process. On the other hand, some AD-specific brain-derived proteins or metabolites may enter in the plasma through a deficient BBB, and exert some measurable signaling properties [15]. Indeed, significant changes in gene expression or at posttranscriptional level have been reported in freshly isolated peripheral blood mononuclear cells (PBMCs) from AD patients and non-demented controls [16-18]. Whether the systemic alterations represent a cause or consequence of the neurodegenerative process is still a controversial issue.

In this review we will summarize current knowledge of changes in signaling pathways in lymphocytes from AD patients and discuss the significance of changes in peripheral cells in AD pathogenesis.

Altered signaling pathways in AD lymphocytes

The concept of PBMCs as a “window” into the CNS was first proposed by Percy et al., in their comprehensive review of peripheral manifestations of AD [19]. Lymphocytes represent a useful material, easily accessible to study the biochemistry and molecular biology of the CNS and for investigating possible systemic derangements in neurodegenerative disorders. Based on this assumption, PBMCs have been widely used by several laboratories as an experimental model to investigate receptor signal

transduction alterations searching for emerging biomarkers at peripheral level to implement the strategies for diagnostic and/or therapeutic approach in AD.

Expression profiling of PBMCs has disclosed dysfunction of pathways subserving signal transduction, lipid metabolism, mitochondrial bioenergetics, intracellular trafficking, proteasomal activity, and cell survival [16; 17; 20]. Among others, abnormal amyloid precursor protein (APP) expression, altered levels of antioxidant enzymes, oxidative damage to DNA, RNA and protein, deregulated cytokine secretion and augmented rates of apoptosis are features shared by AD brain and lymphocytes [18]. Moreover, lymphocytes express N-methyl-D-aspartate (NMDA), dopamine and acetylcholine receptors [21-23] thought to function primarily in the CNS. Therefore, in addition to classical immunological stimuli such as antigens, cytokines, chemokines and growth factors, they can be activated by neurotransmitters.

Here, we will focus on altered control of cell cycle and apoptosis, mitochondrial dysfunction, and changes in the proteasome activity as peripheral markers for detection of AD in lymphocytes.

Cell cycle control failure in AD lymphocytes

Increasing evidence suggest that neuronal cell cycle events underlie neurodegeneration. It is thought that in AD susceptible neurons re-enter an aberrant cell cycle, but since they cannot complete the cell cycle, they die [24-27]. Cell cycle re-entry appears to represent an early and critical event in AD, leading to the development of AD-related pathology such as hyperphosphorylation of tau and Aβ deposition and ultimately inducing neuronal cell death [28; 29]. Several factors, including many of the identified risk factors for Alzheimer's disease, such as elevated plasma homocysteine levels, ageing, menopause, low thyroid levels, low level prolonged oxidative stress or head injury, can either represent mitogenic signaling for neurons or facilitate cell cycle re-

entry in vulnerable neuronal populations [30]. On the other hand, the APP and Aβ have mitogenic properties in vitro, and accumulation of growth factors have been found in diffuse amyloid deposits [31]. The rapid response of microglia and astrocytes to any disturbance in the CNS microenvironment results in an increased expression of specific cell surface receptors, and in the release of growth factors and cytokines, which may be protective acutely but, if not resolved, may contribute to cell cycle activation in vulnerable neurons. In addition and because of failure of BBB in AD [32], growth factors and cytokines can eventually activate the immune cells. Indeed cell cycle deregulation has been found in peripheral cells from AD patients such as lymphocytes and fibroblasts [33-39]. Similar to that described in AD brain, the G_1/S transition control mechanism fail in lymphocytes from AD subjects. In a early work by Nagy group, aimed specifically at the detection of any defects of the G_1/S transition control, [35] was reported a significant reduction on the relative lengthening of the G_1 phase in response to cell cycle inhibitors in AD lymphocytes. Interestingly, the relative lengthening of the G_1 phase distinguish lymphocytes from sporadic or familial AD patients [40]. In consonance with the idea that cell cycle disturbances are early pathogenic events in AD brain, the G_1/S control failure has been also detected in lymphocytes from Mild Cognitive Impairment (MCI) patients, a prodromal stage of AD [35; 38].

During the beginning of the cell cycle at the G_1 phase, a variety of growth signals induce changes in cell cycle regulatory proteins. The G_1/S transition is regulated by cyclin D, and E proteins, cyclin-dependent kinases (CDKs) CDK4/6, and cyclin-dependent kinases inhibitors (CDKIs) [41]. Cyclin D forms an active complex with its catalytic subunits CDK4/6-CDKs resulting in the phosphorylation and activation of the retinoblastoma protein (pRb) complexes to E2F1-DP1. Transcription factor E2F1 is

almost exclusively localized in the nucleus and when co-expressed with its DNA-binding partner, DP1, it relocates from the cytoplasm to the nucleus forming activated E2F1-DP1 complex [42]. Protein complex pRb-E2F1-DP1 is a major regulator of the G_0/G_1-to-S-Phase transition. Initial phosphorylation of pRb-E2F1-DP1 facilitated by complex cyclin D/CDK4/6 results in gene transcription of cyclin E. Cyclin E then binds to its catalytic subunit CDK2 forming active complex cyclin E/CDK2 which hyperphosphorylates pRb in the pRb-E2F1-DP1 complex. Hyperphosphorylation of pRb causes disassociation and full activation of E2F1-DP1, thus allowing binding to and activation of E2F response elements in promoters of S-Phase cell cycle proteins. In addition CDKIs either from the CIP/KIP1 (p21 and p27) or the INK families (p16 and p18) play a predominant role in controlling the G_1/S transition [43]. Evidences for changes in the expression levels of these cell cycle regulatory proteins in AD brain have been long recognized [24; 44]. Enhanced levels of phosphorylated pRb were found in AD [45; 46], and altered subcellular localization of the transcription factor E2F-1 was reported in AD brain [45; 47]. Several groups have described similar changes in cell cycle regulatory proteins in lymphocytes from late-onset AD. They are summarized in Table 1.

TABLE 1

Changes in cell cycle regulatory proteins in lymphocytes from late-onset Alzheimer's disease patients.

Protein	Role	Change	Reference
Cyclin D	G_0/G_1 late G_1/S	↑	[40]
Cyclin E	G_1 to G_1/S	↑	[39]
CDK2	Late G_1/S	↑	[39]
CDK4	G_1/S	↑	[17]
p21	Multi cyclin/CDK inhibitor	↑↓	[33; 40; 48]
p27	Cyclin D and E/CDK inhibitor	↓	[34; 39; 49]
p16	CDK4/CDK6 inhibitor	↑	[17]
pRb family of proteins	CDK2/4/6 check point	↑	[34; 39; 50]
E2F	Transcriptional activator of S-phase specific genes	↑	[39; 50]
NF-κB	Transcriptional activator of cyclin D1	↑↓	[50; 51]
p53	Transcriptional activator of p21	↑	[38; 40; 52]

While it is clear that failure of regulation of cell cycle occurs in neurons and peripheral cells in AD, the signaling pathways that trigger these events are not well defined. The same or similar mitogenic signals in both brain and lymphocytes might alter cell cycle activity by activation of growth factor receptors or their signaling molecules (kinases or transcription factors).

Previous work from this laboratory aimed at studying the underlying signaling pathways involved in the enhanced proliferation of immortalized lymphocytes from AD patients [34; 49; 53; 54]. We investigated the effects of perturbing receptor signaling pathways by using inhibitors of protein-kinases or calmodulin (CaM), as well as pertussis toxin, on the proliferation of normal and AD lymphoblasts. We demonstrated that Ca^{2+}/CaM signaling was overactivated in AD cells, as CaM antagonist were able to restore normal rates of cell proliferation [34; 54]. Increased activity of the Na^{+}/H^{+} exchange and transcriptional alterations of E2F and NF-κB (nuclear factor kappa-light-

chain-enhancer of activated B cells) found in AD cells were also sensitive to CaM antagonists [50; 54]. Increased proliferation of AD lymphoblasts was causally linked to decreased levels of the CDK inhibitor p27 and enhanced phosphorylation of pRb protein [34; 49; 53]. Moreover, we reported that Ca^{2+}/CaM-dependent overactivation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt signaling cascade in AD cells, plays an important role in regulating p27 abundance by increasing its degradation in the ubiquitin-proteasome pathway [53]. Half-life of p27 protein was markedly reduced in lymphoblasts from AD patients compared with that in control cells. Our results indicated that the increased phosphorylation of p27 at Thr187, rather than changes in the 26S proteasome activity, is likely responsible for the enhanced degradation of p27 in AD cells. It was suggested that overactivation of PI3K/Akt in AD cells somehow facilitates phosphorylation of Thr187 by the cyclin E/CDK2 complex. In addition, PI3K/Akt phosphorylates other p27 residues (Thr157, Thr159), which are important in controlling the nucleo-cytoplasmic traffic of p27 [55]. The exclusion of p27 from the nucleus would then facilitate its degradation by the proteasome [53; 56] and thus, relieving cyclin E/CDK2 kinase activity from p27 inhibition.

The importance of PI3K/Akt signaling pathway in AD brain was put forward by previous reports that linked this cascade with amyloid-β, neurofibrillary tangles and neuronal loss in AD brain. Increased phospho-Akt (Ser473) has been detected in AD temporal cortex neurons [57]. Furthermore, overactivation of Akt in AD brains is accompanied by increased levels of phosphorylation of Akt substrates such as glycogen synthase kinase 3β (GSK3β), mammalian target of rapamycin (mTOR), tau and lower levels of p27 [57; 58].

On the other hand, Ca^{2+}/CaM overactivation of PI3K/Akt in AD lymphoblasts was associated with higher CaM content than in control cells [59]. It was suggested that the

increased CaM levels in AD cells synergize with serum to overactivate PI3K/Akt pathway. In agreement with previous reports [60; 61], we found that CaM is able to bind to the 85 KDa regulatory subunit of PI3K (p85). Moreover it was observed a significant higher binding of CaM to p85 in AD lymphoblasts compared to control cells, thereby resulting in enhanced Akt phosphorylation.

The up-regulation of CaM levels in AD lymphoblasts is not the consequence of altered expression of any of the three different genes that encode CaM, but rather the result of decreased rates of CaM degradation [59]. It was shown that intracellular Ca^{2+} levels and reactive oxygen species (ROS) seem to control CaM degradation [59]. In AD cells, reducing the rate of degradation of CaM could be the cellular response to Ca^{2+} overload, since it is well documented higher levels of cytosolic free Ca^{2+} in lymphocytes from sporadic as well as familial AD patients [62-66].

A close association between alterations in Ca^{2+} homeostasis and cell cycle activity in AD cells was also found by other groups [67; 68]. In addition, Ca^{2+} dysfunction has been linked to changes in other cell survival signaling transduction processes. AD cells were shown to exhibit changes in the functioning of G proteins [69], activity of PKC [70; 71], and increased activity of IP3 receptor activity was reported in lymphocytes derived from familial AD patients [62; 72].

Signaling molecules downstream PI3K/Akt such mTOR or GSK3β, are altered in lymphocytes derived from AD patients [35; 73; 74]. mTOR is a highly conserved serine-threonine kinase that is essential for the co-ordination of intra and extra-cellular signals concerning cell growth, division and differentiation [75]. In the central nervous system, the mTOR pathway is known to play a key role in regulating synaptic remodeling [76], as well as modulating autophagy activity in neurons [77]. The first evidence that mTOR signaling was altered in peripheral cells from AD patients was

reported by the Nagy's laboratory [35]. They found that AD lymphocytes showed a reduced response to rapamycin when compared with control cells. Later on it was demonstrated that alterations in mTOR signaling were associated with cognitive decline in AD [78]. The examination of the functional integrity of mTOR signaling in AD lymphocytes by gene expression analysis revealed that the up- or down- regulated mTOR genes were significantly enriched for 25 molecular and cellular functions including cellular growth and proliferation, cell cycle, and the main metabolic pathways [79]. Interestingly it was suggested that the assessment of the functional integrity of the downstream signaling cascade of mTOR from lymphocytes may reflect susceptibility to develop AD rather than provide an assessment of disease state, and therefore could be exploited diagnostically [79].

GSK3β the key enzyme in the control of glycogen synthesis is also involved in the regulation of critical intracellular signaling pathways, including cell cycle, gene expression and apoptosis [80-82]. The disruption of GSK3β homeostasis has been linked with the development of several diseases including AD. GSK3β activity is increased within the AD brain, favoring the hyperphosphorylation of microtubule-associated protein tau and the formation of neurofibrillary tangles [83]. Moreover, the activation of GSK3β inhibits the cleavage of the APP protein, increasing the production of the amyloid-β (Aβ42) peptide [84] and leads to memory impairment in animal models [85; 86]. Therefore, the deregulation of GSK3β activity has major effects in key pathological features of AD and therefore is widely considered a therapeutic target of interest. Altered regulation of GSK3β was also found in white blood cells and platelets from AD patients and MCI [73; 87]. These observations suggested that the peripheral determination of GSK3β activity might be a useful diagnostic biomarker for early AD and a surrogate marker of early pathophysiological changes.

GSK3β activity is also modulated by other relevant extracellular signaling pathways besides the PI3K/Akt; they are the insulin/insulin-like growth factor I (IGF-I), or the canonical Wnt pathways [88].

There is widening recognition that AD is closely linked to a state of relative insulin resistance in the brain [89; 90]. Levels of IGF-I, insulin and cognate receptors are deregulated in AD brain [91]. In normal brain, IGF-I and insulin promote glucose utilization, energy metabolism and neuronal survival [92], largely through PI3K/Akt/GSK3β signaling [93]. Insulin receptors populate neuronal synapses and astrocytes in memory-processing brain regions [94]. Acute insulin treatment improved memory in both humans and rodents [95-97]. On the other hand, increased Aβ production prompts the onset of glucose intolerance and insulin resistance [98]. The insulin-induced activation of the PI3K/Akt/GSK3β is also blunted in peripheral ells from AD patients [99]. GSK3β plays a crucial role in the regulation of cell cycle events through its ability to phosphorylate β-catenin. Phosphorylated β-catenin is recognized by ubiquitin and targeted for proteasomal degradation [100]. Consequently, signals that modify GSK3β activity are expected to alter β-catenin levels. In the nucleus, β-catenin interacts with transcription factors TCF (T-cell factor)/LEF (lymphoid enhancing factor) [101] to activate genes that allow cell cycle progression as cyclin D1 and c-myc [102].

On the other hand, GSK3β has been identified as an important regulator of inflammation [103] promoting the production of several pro-inflammatory cytokines such as IL-6, IL-1β, and TNFα, as well as decreasing the levels of the anti-inflammatory cytokine IL-10. Data from Avila's group demonstrated that GSK3β overexpression in neurons leads to the appearance of a unique pattern of cytokines in the brain in vivo that is detrimental for appropriate neuron maturation [80; 104].

GSK3β is a key transducer of the canonical Wnt signaling, the components of which are involved in AD [105]. In the canonical pathway, members of the Wnt family interact with Frizzled, a seven-transmembrane receptor, and with an additional receptor that corresponds to either low-density lipoprotein receptor-related protein 5 (LRP-5) or LRP-6. Activation of this pathway leads to the inhibition of GSK3β through a cascade of intracellular reactions that involve protein kinases and adaptor proteins [102]. Inestrosa and coworkers have provided evidences that Wnt pathway is modulated by Aβ in AD [106]. Moreover it has been suggested that Wnt pathway might play a role in the early induction of the cell cycle in neurons [107]. As far as we know, the role of Wnt signaling pathway on peripheral cells from AD patients has not been investigated.

Impaired apoptosis in AD lymphocytes

The molecular cascades leading to the loss of neuronal populations in AD have not been fully delineated. However it appears that apoptosis is the final fate of vulnerable neurons. Careful examination of brains after autopsy for signs of apoptosis has provided, compelling evidence that apoptosis is increased in AD brains compared with brains from non-demented [108]. Several markers of apoptosis, such as nuclear DNA fragmentation, and activation of caspases were reported to be elevated in AD brains [109; 110].

It is now widely accepted that an aberrant re-initiation of the cell cycle activation in vulnerable neurons is one important inducer of neuronal apoptosis [31; 111; 112]. There is a significant body of evidence pointing to a role for neuronal cell cycle proteins in the modulation of stress-induced apoptosis, including the neuroprotective effects shown by certain CDK inhibitors [113]. Neuronal apoptosis, however, differ of classical apoptosis in the long time taken for susceptible neurons to die. It appears that they are highly protected against rapid apoptotic cell death. Even if the apoptotic cascade is initiated,

the lack of the downstream caspases [114] leads to a long agony, instead of rapid apoptosis [115]. As a consequence, neurons survive for long periods of time in the G_2 phase of the cell cycle becoming more vulnerable to stress according to the "two-hit hypothesis" [116; 117]. The need of two hit for effective neuronal death provides an explanation for the low amounts of neuronal apoptosis in AD (less than one in 10,000 at any given time show signs of apoptosis) [118]. This apoptosis avoidance, together with differences in the signal transduction pathways activated by growth factors in the brain, may represent a defense program for susceptible neurons in AD, in analogy with neoplasia. Indeed some authors have considered AD pathology as an abortive neoplastic disorder [29]. Interestingly, lymphoblasts from sporadic AD patients show tumor-like features. Work from our laboratory demonstrated that compared with lymphoblasts from non-demented subjects, AD cells have a higher proliferative activity and increased resistance to apoptosis induced by serum deprivation [50; 119]. Selective impairment of mechanisms involved in cell death has been also reported in fibroblasts from AD patients. The protective mechanism of AD fibroblasts against H_2O_2 was related to an impairment of cell cycle arrest and a diminished induction of apoptosis [120].

Increased resistance of AD lymphoblasts to serum deprivation also depends on Ca^{2+}/CaM signaling [50; 119]. Therefore, CaM seems to play a pivotal role in transmitting proliferative/survival signals from the plasma membrane to the nucleus. Whether CaM contributes to cell proliferation or apoptosis may depend on cellular CaM levels and/or activity, as well as the presence of growth-stimulatory signals. The lower vulnerability to death of AD cells was associated with decreased NF-κB DNA-binding activity [50] and higher levels of the CDK inhibitor p21 [48; 121]. The involvement of NF-κB activation in inducing apoptosis was previously reported in HEK 293 cells [122] and conversely inhibition of NF-κB was shown to prevent cell death induced by the

oncogenic protein, latent membrane protein 1 (LMP1) in Rat-1 cells [123]. Further work aimed at studying the molecular mechanism involved in the distinct Ca^{2+}/CaM-mediated regulation of survival of AD lymphoblasts revealed a CaM/CaMKII-dependent downregulation of the ERK1/2 signaling pathway [119]. It is well known that the kinetics and duration of ERK1/2 activation are important factors in determining the cellular response [124; 125]. In lymphoblasts derived from AD patients we observed an enhanced and transient activation of ERK1/2 in AD lymphoblasts associated with increased proliferation, whereas serum starvation induced a sustained activation of ERKs, although lower than in control cells. These results are in consonance with previous work in which a persistent activation of ERK1/2 was associated with cell cycle arrest and apoptosis in different cell types [126-128].

As already mentioned, the higher resistance of AD lymphoblasts to death induced by trophic factor deprivation was accompanied by increased levels of p21 [48]. Several reports pointed out that in addition to being an inhibitor of cell proliferation, p21 might protect cells from apoptosis [129]. For example, it has been reported that upregulation of p21 blocked the oxidative stress-induced death of human myeloma U266 cells [130] and rendered resistance to chemotherapy drugs in other types of cancer cells [131]. Thus the increase in p21 cellular content in AD may confer these cells a survival advantage. The transcription of p21 was found to be upregulated in AD cells by the forkhead box O3a factor (FOXO3a). The reduced ERK1/2 activation of ERK1/2 prevents the phosphorylation of FOX3a and subsequent translocation and degradation via a murine double minute (MDM2)-mediated ubiquitin proteasome pathway, thereby allowing the accumulation of this transcription factor in the nucleus [48]. In addition to the increased transcriptional activation of *p21* gene observed in AD lymphoblasts, we also found an increase in the cytosolic content of p21 protein in AD cells. The

cytoplasmic p21 is thought to be a positive modulator of cell survival [43; 132]. Upregulation of p21 has also been associated with blockade of oxidative stress induced apoptosis in fibroblasts from AD patients [133].

Impairment of mechanisms involved in cell death was previously reported in peripheral cells from AD patients [120; 133-135] although there are conflicting results as to whether cells from AD patients are less or more vulnerable to situations that promote cell death. Most likely these discrepancies result from the different cell types and stress-inducing conditions used. For example, in contrast with lower sensitivity to serum deprivation-induced death in AD lymphocytes, these cells appears to be more susceptible to oxidative stress-induced apoptosis [136-138]. The impaired apoptosis mediated by oxidative stress was even observed in MCI patients [137], although enhanced susceptibility to H_2O_2-induced death in peripheral lymphocytes correlates with dementia severity and enhanced death in AD patients is attributable to a Poly (ADP-ribosyl) polymerase-1 (PARP)-dependent increase in the apoptosis/necrosis ratio [138].

The other two important branches besides ERK1/2 of the Mitogen-activated protein kinases (MAPK) signaling, p38 and the c-Jun-N-terminal kinase (JNK), have also been associated to neuronal apoptosis in AD brain [130]. Impaired regulation of expression and activity of these two kinases have been documented in brain and cell models of AD [139; 140], and it has been reported that pharmacological modulation of p38/JNK activity attenuated neuronal cell death [141]. Recent work has reported that both phosphorylated p38 and phosphorylated JNK levels were significantly increased in lymphocytes from AD patients compared with healthy controls, in agreement with the increased expression of phosphorylated p38 and phosphorylated JNK in AD brains [142]. These authors found that phosphorylated p38 and JNK levels in AD lymphocytes

were positively correlated with disease duration and severity, suggesting the peripheral alteration of these two kinases is more a consequence of a CNS disease mechanism rather than being linked to casual mechanisms. It was suggested that determination of peripheral changes in the expression of the p38 and JNK could provide useful biomarkers of disease progression.

Systemic alterations in mitochondrial dysfunction and in the proteasome activity

Mitochondrial dysfunction and impairment of the ubiquitin-proteasome system (UPS) are important features of AD shared by the CNS and peripheral cells [143; 144]. Mitochondria are the main source of energy and the primary source of reactive oxygen species (ROS). Therefore, impairment of mitochondrial function leads to decreased energetic and increased production of free radicals that can in turn have an impact on cellular functions with consequent neuronal loss. On the other hand, dysfunction of the UPS has been associated with the deposition of ubiquitinated protein aggregates [143] and widespread disruption of the proteostasis network. The UPS provides 80% to 90% of the proteolysis of the short-life proteins and ensures, as chaperon-molecules, the right conformation and hence the correct function of the proteins [145].

Mitochondrial dysfunction has been documented in peripheral cells from AD patients. For example, it has been reported alterations in the electron transport chain activity in lymphocytes from AD patients [146]. Another study showed decreased mitochondrial membrane potential in AD lymphocytes, together with a reduced rate of respiration and a significant impairment of total oxidative phosphorylation (OXPHOS) capacity, suggesting mitochondrial uncoupling that led to a failure in the maintenance of cellular energetics [147].

Increased oxidative stress has been also described in lymphocytes from AD patients. The leakage of ROS from mitochondria can be detected by different parameters such as

the direct measurement of ROS levels, the antioxidant status or DNA oxidation. Many authors have found elevated levels of oxidative DNA damage and increased ROS levels in peripheral lymphocytes [59; 135; 148-151] that in some cases were also present in MCI patients, suggesting that oxidative stress may represent an early event in the pathogenesis of AD. One of these studies also pointed out the correlation of the higher levels of oxidative DNA damage in peripheral lymphocytes with lower plasma levels of antioxidants [150]. Lower plasma antioxidants have been also described by other groups [152-154], together with lower levels of glutathione in lymphocytes of patients with familial AD [155].

Increased lipid and protein oxidative damage have also been found in mitochondria isolated from patients with AD [156]. These results were in agreement with the increased lipid peroxidation products found in lymphocytes from patients with the familial form of AD, carrying mutations in *amyloid protein precursor* (*APP*) or *presinilin 1* (*PS1*) genes [157] and in the PS1M146L transgenic mice model of AD [158]. In addition, a recent study pointed out the increased susceptibility of AD lymphoblasts to cell death induced by H_2O_2 compared to healthy controls [136].

Several studies in AD lymphocytes have suggested the involvement of p53 signaling in the pathogenesis of AD, due to its role in the response to DNA damage and in cell cycle control [40; 52; 159; 160]. Buizza and coworkers found that oxidative imbalance altered the conformation of p53 protein in lymphocytes from AD patients. They suggested that the oxidation of the p53 could make the protein dysfunctional and represent an early marker of oxidative alterations [161].

It is worth to highlight that lymphocytes from AD patients represent a useful tool to test the effect of mitochondrial-related therapeutic approaches for AD. The cholinesterase inhibitor rivastigmine, already in use for AD, has been shown to enhance the

mitochondrial electron transport chain in lymphocytes from AD patients [162], and galantamine or melatonin treatments appear to protect AD lymphocytes from cell oxidative damage [163; 164]. In addition, peripheral measures of mitochondrial dysfunction might be helpful in developing biomarkers to combat AD [165; 166].

Alterations in the UPS activity in the CNS and peripheral cells from AD patients might also have detrimental consequences for cell survival [167; 168]. In the AD brain changes in the UPS have been linked to the accumulation of neurofibrillary tangles [169], and it has been reported that ubiquitin mediates Aβ neurotoxicity-inducing apoptosis [170].

Changes in the proteasome activity have been observed in peripheral tissues including increased levels of ubiquitin in cerebrospinal fluid (CSF) of AD patients [171] and a reduction in proteasome activity in CD45 T-lymphocytes from the elderly [172]. Work in our laboratory demonstrated selective impaired degradation in the UPS of key proteins involved in the Ca^{2+}/CaM-dependent regulation of survival/death of lymphoblasts from AD patients. First, we found that CaM itself is degraded at a lower rate in AD lymphoblasts than in control cells, allowing the accumulation of the protein in AD cells [59]. The impaired CaM degradation in AD lymphoblasts was associated with higher basal ROS levels when compared with control cells. Treatment of lymphoblasts with antioxidants like glutathione (GSH) or trolox reduced ROS levels and restored CaM degradation rate [59]. By contrast, the UPS-dependent degradation of the CDK inhibitors p27 and p21 is enhanced in AD cells [53; 173]. The altered rate of degradation of these proteins does not appear to be the result of nonspecific alteration of general protein degradation in lymphoblasts from AD cells in consonance with reports from other groups [174; 175]. Nevertheless, the ubiquitin-protein ligase system seems to be affected in PBMCs from AD patients, although they don’t show significant

reduction in global proteasome activity [175]. It was proposed that the determination of two of these ubiquitin-ligases, E1 and E2, in combination with other blood-cell markers may be useful as biomarkers for AD diagnosis [175].

The activation of the NF-κB transcription factor depends on the degradation in the UPS of the associated inhibitory molecule of the IκB family [176]. Besides its widely known role in inflammation and immune responses, NF-κB is involved in the control of cell division and apoptosis [177; 178]. Postmortem brain tissues from AD patients have revealed altered NF-κB activity in cells in the vicinity of amyloid plaques [179]. Moreover, it has been shown that conditioned medium from Aβ-stimulated glial cells triggers neuronal cell division [180], suggesting that the inflammatory process may be one of the mitotic pressures in AD. Deregulated NF-κB activity has been reported in PBMCs and lymphoblasts from AD patients associated with changes in cell survival [50; 51]. On the other hand, enhanced UPS-dependent degradation of IκB appears to underlie the beneficial effects of anti-inflammatory molecules in AD [181].

Role of altered lymphocytes response in AD pathogenesis

Apart from the molecular alterations observed in lymphocytes derived from AD patients in relation to the mechanisms involved in the control of cell survival/death described above, a large body of evidence indicates the existence of other alterations in systemic immune responses in AD. Changes in lymphocyte and macrophage distribution together with the presence of autoantibodies, inflammatory factors and cytokine production have been described in AD patients [8; 9; 182-189]. Moreover increased expression of Toll-like receptor 2 and 4 (TLR2, TLR4) have been found on PBMCs from AD patients [190]. It is believed that both TLRs are involved in neuroinflammation, due to their ability to bind amyloid-β [191]. In addition, global approaches such as microarray analysis of PBMCs and lymphocytes from AD patients show a prominent gene

deregulation when compared to age-matched controls [16; 17]. Changes in patterns of immune mediators in the periphery have been linked to AD and were used to predict disease progression [192].

However, despite the evidence for the existence of a communication between the CNS and the immune system, the involvement of immune alteration in the instigation and/or progression of AD is far from being completely understood.

Functional integrity of the immune system has been linked to the functional integrity of the brain [193]. It was shown for example that immunocompromised mice display impaired hippocampal functions [194], and the cognitive alterations associated with the immune deficiencies could be rescued by immunomodulation [195; 196]. On the other hand, it was reported that, the triple-transgenic mouse (3xTg-AD), show premature immunosenescence [197]. 3xTg-AD mouse, which harbors APPSwe and tauP301L transgenes on a mutant Long-chain ceramide is elevated in presenilin 1 (*PS1M146V*) knock-in background, develops both Aβ plaques and neurofibrillary tangles with a temporal- and regional-specific profile that closely mimics their development in the human AD brain [198]. Taken together, these studies suggest that circulating immune cells play an essential role in brain function and support the involvement of systemic immunity and inflammation in behavioral and cognitive deficits, such as those in AD. In other words, the immune cells present in the CNS may have neuroprotective effects. However, accumulation of immune cells in brain areas or uncontrolled cell response could eventually increase the oxidative and inflammatory processes and contribute to the instigation or progression of neurodegeneration [199]. Apparently acute activation of the circulating immune cells within the CNS has different consequences for the young and aged/or diseased brain. In the latter conditions, systemic inflammation

impacts on resident microglia to adopt a more aggressive phenotype with the enhanced synthesis of pro-inflammatory mediators.

On the other hand, abnormalities found in circulating immune cells could be the downstream result of AD processes leading to a deficient control of peripheral processes. The brain regulates key biological processes throughout the organism by releasing molecules into the blood and CSF. Proteins, such as amyloid-β, or inflammatory mediators from the CNS may cause systemic immune reaction. Therefore, communication between the CNS and the immune system in AD could thus influence both the lymphocyte distribution in the blood and the production of immune mediators [15; 200]. PBMCs from AD patients show increased production of several cytokines, chemokines, as well as increased expression of growth factors and chemokine receptors, after in vitro stimulation by Aβ [188]. Amyloid-β was reported to stimulate the proliferative response of lymphocytes from AD patients [201]. Cytokines, chemokines, and growth factors regulate diverse cellular processes, including proliferation and survival, and they are important for the development and function of the hematopoietic and nervous systems. In Fig. 1 we have summarized schematically the bidirectional communication between the CNS and the immune system, highlighting the reported changes in lymphocytes signaling.

The possibility should also be considered that both changes in the CNS and systemic alterations share common underlying etiological processes. AD has been proposed to be a multifactorial disease that affects both CNS and systemic processes [202]. Most likely, there is no a single trigger that could explain AD etiology, but several interconnected processes, that together with genetic risk factors, determine the clinical manifestations of dementia.

Concluding Remarks

The studies summarized here suggest that AD is accompanied by changes in peripheral cells, particularly those in the immune system. Changes that occur in lymphocytes from AD subjects include cell cycle deregulation, alterations in cell viability, proliferation, apoptosis, oxidative metabolism, proteasome activity, calcium homeostasis, and cellular signal transduction systems. At present it is not possible to conclude whether immune changes play a significant role in the development of cognitive decline, or by contrast they are downstream consequence of brain pathology. Nonetheless, evaluation of systemic alterations may be useful to further characterize immune dysfunction during disease progression, and their influence on AD pathology. On the other hand, because of their easy accessibility, peripheral cells may be of great importance for identification of biological markers of AD, and a suitable platform for monitoring the efficiency of novel therapies.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest

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Legend to Figure 1

Figure 1

Communication between the CNS and the immune system: Changes in signaling pathways in AD lymphocytes.

As the consequence of chronic neuroinflammation and neurodegeneration, Aβ peptides and pro-inflammatory mediators can reach the blood through altered BBB. The CNS-derived molecules could then induce changes in key signaling pathways that control the survival/death lymphocyte's fate. Conversely, increased production of cytokines and chemokines by activated lymphocytes may impact the CNS contributing to exacerbate the neuroimflammation. Robust arrows denote reported activation in lymphocytes derived from AD patients.

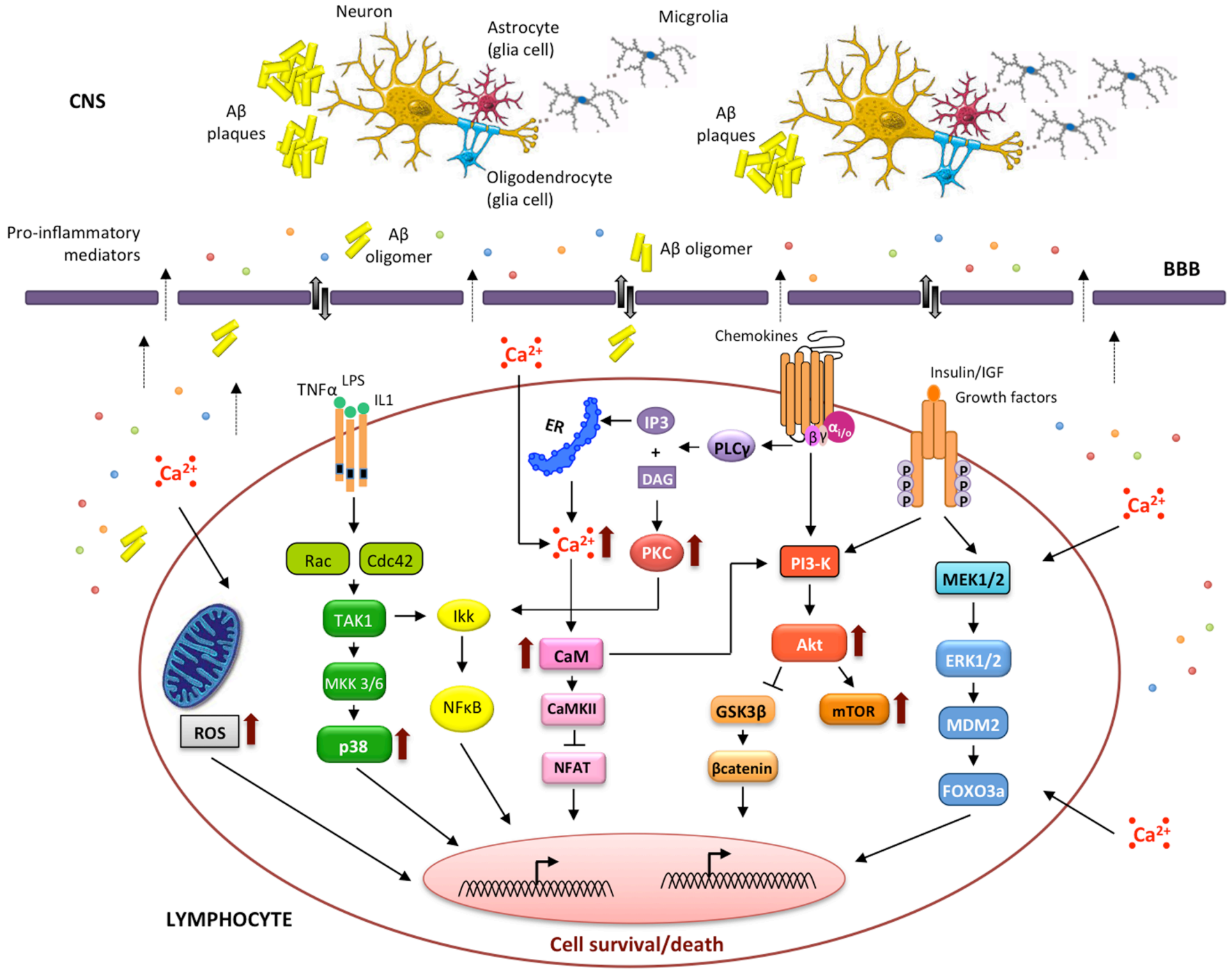
CNS
Neuron
Astrocyte
(glia cell)
Micgrolia
Aβ
plaques
Oligodendrocyte
(glia cell)
Aβ
plaques
Pro-inflammatory
mediators
Aβ
oligomer
Aβ oligomer
BBB
Chemokines
Insulin/IGF
Growth factors
TNFα
LPS
IL1
ER
IP3
+
DAG
PLCγ
βγ
αi/o
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Rac
Cdc42
TAK1
Ikk
MKK 3/6
NFκB
p38
ROS
PKC
CaM
CaMKII
NFAT
PI3-K
Akt
GSK3β
βcatenin
mTOR
MEK1/2
ERK1/2
MDM2
FOXO3a
LYMPHOCYTE
Cell survival/death

Figure 1