

Alzheimer's disease: Efforts for discovering novel ways of therapy

Abstract

Alzheimer's disease (AD) has been under debate for many years, regarding the research that is being conducted so as to find novel ways to deal with that disease and alleviate its symptoms, making the lives of patients easier and less challenging. In this essay, there has been an approach from a slightly different perspective,, mainly focusing on certain molecular pathways, which recently have been found to be in the center of attention, concerning their involvement in the development of AD. The participation of certain cytokines was also investigated primarily of **Tumor Necrosis Factor Alpha (TNF- α)**, and other molecules like **Vascular Endothelial Growth Factor (VEGF)**, which has also been recently shown to contribute in AD pathology.

Introduction

AD is one of the most prevalent degenerative disorders in modern society. For that reason, many scientists have conducted extensive research in order to find a potential therapeutic route in order to counter that devastating disease. AD is characterized by various pathological hallmarks, including extracellular plaques composed primarily of amyloid β ($A\beta$), intracellular neurofibrillary tangles (NFTs), which are formed from hyperphosphorylated tau protein, synaptic loss and neurodegeneration in brain regions considered critical for memory and learning processes (Arora et al., 2015).

Clinically, AD is characterized by dementia that starts with a difficulty in conceiving and memorizing things and slowly progresses to become extremely severe, resulting in effecting the person's daily life. Other commonly observed clinical images include confusion, poor judgment, language disturbance, agitation and hallucinations. The usual clinical duration of the disease is eight to ten years, with a range from one to 25 years. Approximately 25% of all AD is familial (i.e. ≥ 2 persons in a family have AD) of which approximately 95% is late onset (age >60 -65 years) and 5% is early onset (age <65 years) (Bird T. D., 1998).

During the progression of the disease, there are several cytokines (TNF- α , IL-1, IL-6) that participate and various molecular pathways that intervene, all having a critical role in the further expression of AD. Investigating these components could assist us in our endeavor to find a novel therapeutic approach.

As mentioned, in AD, $A\beta$ plaques are formed in brain regions critical for memory and learning function (temporal, frontal and parietal lobe). The production of $A\beta$ mainly depends on the proteolytic processing of **amyloid precursor protein (APP)**. $A\beta$ is produced in the amyloidogenic pathway by the consecutive cleavage of β - and γ -secretase. On the other hand, its production is prevented in the nonamyloidogenic pathway by action of α -secretase, which cleaves in the center of the amyloid domain (Haass et al., 2012).

The autosomal genes responsible for the increased A β production are the ones coding for APP, presenilin 1 (PS1) and presenilin 2 (PS2). These genes demonstrate dominant transmission and are located to chromosomes 21, 14 and 1, respectively. Mutations in those genes affect the production levels of A β , specifically its highly amyloidogenic 42 amino acid variant, A β 42 (Steiner et al., 1999).

One gene that was not mentioned above but is extremely important for the age of manifestation and also the progression of the disease is the gene coding for apolipoprotein E (APOE). There are 3 different alleles that appear in the population, ϵ 2, ϵ 3 and ϵ 4. Studies have linked the appearance of the allele ϵ 4, especially in its homologous form, not only as a major risk factor for late-onset AD, but also as a contributor for lower age outbreak of AD (Perry et al., 2001). APOE is found extracellularly as both free and bound protein, as it usually bounds with tau protein, leading to NFTs formation (Strittmatter and Roses, 1995). However, of course, there are even more genes involved in that multi-dimensional disorder, for which there is going to be a brief discussion in other sections of the essay.

Another characteristic hallmark of AD, as already mentioned, is the formation of NFTs. NFTs are the result of the dysfunction of tau protein. Tau is a highly soluble microtubule-binding protein that is abundant in neurons of the CNS and, in physiological circumstances, its main function is to stabilize microtubules. There are two ways with which tau proteins control microtubules: isoforms and phosphorylation. Namely, tau phosphorylation at different sites has a different impact on its biological function and on its pathogenic role (Gong C.X. et al., 2008). Specifically, *in vitro* kinetic studies of the binding between hyperphosphorylated tau and normal tau suggest that Ser199/Ser202/Thr205, Thr212, Thr231/Ser235, Ser262/Ser356, and Ser422 are among the critical phosphorylation sites that render tau an inhibitory molecule that divides normal microtubule-associated proteins from microtubules. Further phosphorylation at Thr231, Ser396, and Ser422 promotes self-aggregation of tau into filaments (Gong C.X. et al., 2008, Alonso et al., 2004). Therefore, tau hyperphosphorylation leads to aggregation and ultimately, formation of NFTs.

In this essay, there was an effort to investigate the participation of certain molecular pathways in the manifestation of the above mentioned characteristic of AD. Moreover, the role of specific molecules, like TNF- α , IL-1 β , was also investigated, as well as their potential interactions with the studied molecular pathways. In addition, a VEGF has been recently shown to be involved in AD progression, its role was also investigated here. The ultimate goal was to eventually propose a novel therapeutic route to counter AD.

Findings and Results

Cytokine involvement in AD

Before we start elaborating on the certain role of certain cytokines during the development of AD, we are first going to give a comprehensive description of what exactly takes places in the regions of the brain which are affected by AD:

Many efforts have been made to examine the role of cytokines in the development of AD. Recently, research was held so as to identify the cytokines which were upregulated in brain regions afflicted with AD. Specifically, *Wood et al.* developed a hypothesis where cytokines are directly connected with neuronal death in AD. To test that hypothesis, they tried to make a profile of AD involved cytokines by performing a high-throughput screening of cytokine concentrations in brain regions that are highly impacted by AD, like entorhinal cortices (ECs). Interestingly, they were able to get a clear view of the cytokines that are considered most important in AD onset and progression. It was observed that TNF- α , IL-1 and IFN- γ were the most highly concentrated cytokines in these brain regions, suggesting that they play a critical role in AD, whether being neuroprotective or leading to neuronal loss.

The increased production of A β , caused by gene regulation, triggers the production of astrocytes and microglia. These cells constitute the immune system of the brain and act themselves by releasing a variety of proinflammatory cytokines, such as TNF- α , IL-1 β and IL-6. Moreover, microglia cells also produce cytotoxic and neurotoxic free oxygen radicals (ROS). As a result, A β is oxidized due to the presence of ROS and aggregates to form neurotoxic AD neuritic plaques (Perry et al., 2001). ROS also induce apoptosis signal-regulating kinase 1 (ASK1) pathway activation, leading to apoptosis and neuronal cell death (Kadowaki et al., 2005). Finally, A β also increases the production of nitric oxide (NO) in the presence of cytokines, which can also lead to neuronal death (Perry et al., 2001).

The apolipoprotein E is synthesized and released also by microglia and astrocytes in the CNS. It comprises of NF- κ B consensus sequences, thus creating a whole interacting positive feedback loop, where activation of NF- κ B pathway by A β , cytokines as TNF- α , or neuronal injury would increase the expression and release of APOE (Perry et al., 2001).

IL-1 is another cytokine expressed in proinflammatory processes. Chronic IL-1 β overexpression can become a serious obstacle in coping with tissue damage and can also lead to degenerative disease. IL-1 β has been shown to trigger a lot of toxic actions in the CNS, either acting as an autocrine or a paracrine stimulator, causing eventually, neurodegeneration (Griffin et al., 1998). Recently, studies clearly demonstrated that IL-1 β overexpression exacerbated tau phosphorylation via the activation of the p38 MAPK and the glycogen synthase kinase 3 (GSK3) pathways (Wang et al., 2015).

On the other hand, some studies have shown contradictory results, as they present a different and protective role of IL-1 β in AD. More specifically, these findings have demonstrated a protective effect of IL-1 β either by regulating microglia-A β plaque degradation and phagocyte recruitment (Shaftel et al., 2008), or by promoting APP different cleavage both in vivo and in vitro (Wang et al., 2015).

Therefore, the clear role of IL-1 β is rather intricate and remains to be further investigated.

Tumor Necrosis Factor Alpha

TNF- α is one of the main proinflammatory cytokines that plays a central role in initiating and regulating the cytokine cascade during an inflammatory response. TNF- α is produced as a type II transmembrane protein of 26kDa. Normally, it forms a stable transmembrane homo-trimeric protein, called tmTNF- α . However, when processed by TNF- α converting enzyme (TACE/ADAM17), which catalyzes a proteolytic cleavage, it forms a 17kDa monomeric protein, which is active as a soluble homo-trimeric molecule of 51kDa, called solTNF- α (Dong et al., 2015).

TNF- α is secreted by macrophages and other cells of the immune system (T cells, B cells and fibroblasts), in response of various stimuli, like lipopolysaccharide (LPS), mitogens and viruses (Perry et al., 2001). As soon as it is produced by activated macrophages, it triggers the production of other cytokines and chemokines (IL-1, IL-6, IL-12,) acting as a paracrine stimulator, along with IFN- γ .

TNF- α is expressing its diverse biological functions via its interaction with two different transmembrane receptors, 55kDa TNF Receptor 1 (TNFR1) and 75kDa TNF Receptor 2 (TNFR2). Looming evidence suggests a completely different response of TNF- α when interacting with each receptor. Its involvement in AD will be further investigated later on.

Except for its main role, TNF- α also limits and terminates inflammation, promotes angiogenesis and enhances damage repair (Perry et al., 2001). It is suggested that the amount of TNF- α is proportional to the severity of the immune response, but whether the former triggers the latter or vice versa, is yet unclear.

TNF- α and AD

TNF- α is upregulated in the brains of AD patients (Perry et al., 2001). This upregulation leads to the previous mentioned cascade of events, namely stimulating the production of other cytokines and chemokines, and activating intrinsic molecular pathways. That interaction leads to diverging responses via various molecular pathways, thus rendering the role of TNF- α in AD still under investigation.

For instance, TNF has been suggested to promote cell death locally (Djik et al., 2014), whereas also interacting with molecular pathways and eventually leading to neuronal loss and apoptosis (ASK/JNK pathway) (Margevicius et al., 2015), while it also interacts with cell-surviving pathways, like nuclear factor-Kappa b pathway (NF- κ B), promoting A β degradation (Djik et al., 2014).

TNF gene is highly polymorphic when compared to other cytokine genes (Perry et al., 2001). The two main polymorphisms which are correlated with AD via different ways are located in positions 238, 308. Specifically, gene constructs consisting of the A allele of the -308 SNPs appear to have higher transcriptional activity than with the -308 G

allele. On the other hand, G allele of the TNF -238 polymorphism was associated with higher TNF production. However, both these results are still highly controversial (Perry et al., 2001). Lastly, there is a microsatellite polymorphism TNFa, located approximately seven kilobases upstream of TNF, ((Perry et al, 2001). Summing up, these 3 separate polymorphisms were incorporated into a TNF haplotype 2(A)-1(G)-2(99bp), which was associated with AD, implying a significant role regarding the transition to the the chronic inflammatory state of the disease (Perry et al., 2001).

Recently, there has been a clear connection between the interaction of one of TNF- α , and an AD-related neuronal cell cycle event cascade (CCEs) (Bhaskar et al., 2014). Pro-inflammatory cytokine TNF- α is increased in serum and CSF of AD patients (Culpan et al., 2011). Neuronal CCEs may contribute as a coping mechanism against cellular stress, as they are observed to many neurodegenerative diseases, including AD. So, *Bhaskar et al.* suggested that during AD, A β -activated-microglia-derived TNF- α induced a neuronal CCEs that was mediated through the activation of neuronal JNK pathway, member of the MAPK, essential for neuronal cell proliferation and survival, but also contributes to AD pathologies (Margevicius et al., 2015). Furthermore, in vitro experiments have shown that exposure to TNF- α led to significant decrease of protein levels of ECE-2, a A β degrading enzyme (Culpan et al., 2011).

TNF receptors

TNF can interact with two distinct transmembrane receptors, TNF-Receptor 1 (TNFR1) and TNF-Receptor 2 (TNFR2). TNFR1 can be found in all types of cells, while TNFR2 has been found only in some types, like immune and endothelial cells (Dong et al., 2014). Concentration levels of TNFR proteins, though, can be regulated by cytokines, mainly interferons (Sedger and McDermott, 2014). They both contain four N-terminal cysteine-rich repeats in their extracellular domains (CRD) (Wajant et al., 2003), whereas TNFR1 differs from TNFR2 by the presence of intracellular death domains (DDs) (Dong et al., 2014). Binding to each one of these receptors mediates different cellular responses. Specifically, TNFR1 signaling leads to the production of pro-inflammatory factors (cytokines and chemokines), and creates a pro-apoptotic environment in neuronal tissue, whereas TNFR2 signaling is correlated with cell-proliferation and survival responses (Naude et al., 2014), after co-operation with TNFR1 (Dong et al., 2014). These responses constitute a result of complex molecule interactions which will be discussed thoroughly.

TNFR1 Signaling

After a plethora of *in vitro* studies, TNF- α has been shown to induce cell death mainly via binding with TNFR1, mainly in its soluble form, through a complex network of interactions via its cytoplasmic death domain (Wajant et al., 2003, Sedger and McDermott, 2014). There are two different complexes, comprising, however, of similar molecules, that can be assembled in the TNF-TNFR binding process, complex I and II (Dong et al., 2014). The formation of either of one of these complexes firstly requires TNF binding to TNFR1 after undergoing conformational shift which takes place after interacting with pre-ligand assembly (PLAD), which is located inside the CRD (Sedger and McDermott, 2014). This PLAD-dependent process is considered essential for the

trimer TNF- α /TNFR formation and ligand-induced signaling (Sedger and McDermott, 2014, Chan et al., 2000). After binding, PLAD permits the release of silencer of death domain (SODD), a TNFR inhibitor (Sedger and McDermott, 2014) along with the recruitment of ‘‘death signaling inducing signaling complex (DISC) proteins, including TNFR-associated death domain (TRADD), Fas associated protein with death domain (FADD) and the TNFR-associated factor (TRAF)-1 (Sedger and McDermott, 2014). That complex rapidly recruits receptor-interacting protein 1 (RIP1) which is subjected to modification by non-degradative poly-ubiquitin chains (Dong et al., 2014). TNFR associated factor -2 (TRAF2), is also recruited by TRADD, along with inhibitor of apoptosis proteins one and 2 (cIAP1 and 2). All these molecules comprise the complex I (Dong et al., 2014). Along with RIP1 ubiquitinated, that complex mediates NF- κ B activation, leading to cell survival, by simultaneous activation of other interacting antiapoptotic pathways .Specifically, catalytic I κ B kinase complex is activated, and subsequently, acting as a regulatory unit of the IKK γ /NEMO complex, which in its turn phosphorylates the inhibitor of the kappa-B (I κ B). Further on, its decomposed by the ubiquitin-proteasome, leading, ultimately, to NF- κ B activation, which acts as transcription factor of antiapoptotic genes, along with cellular FLICE like inhibitory protein (cFLIP) activation, which proceeds to inhibit caspase 8 release from complex II (Dong et al., 2014).

On the contrary, in cases where complex I fails to activate NF- κ B, complex II leads to prompt apoptotic processes (Dong et al., 2014). Specifically, after failure of activation of NF- κ B, FADD and pre-caspase 8 are recruited to form complex II. Pre-caspase 8 activation is subsequently triggered by FADD, producing caspase 8, which suppresses NF- κ B activation and initiates apoptosis (McAlpine and Tansey 2008).

TNFR2 signaling

Unlike TNFR1 signaling, it is not yet well understood how TNFR2 signaling works. Briefly, after binding primarily with tmTNF, TNFR2 can regulate NF- κ B activation either via TRAF2 which acts as a key mediator, recruiting cIAP1 and 2 and TRAF1, inducing NF- κ B activation, or via the phosphoinositide 3-kinases (PI3K)-protein kinase B/serine-threonine kinase (PKB/Akt) signaling pathway (Dong et al., 2014). On the other hand, TNFR2 has also been connected with apoptotic processes, but only when co-operating with TNFR1 signaling (McAlpine and Tansey, 2008).

Findings have demonstrated that TNFR2 interacts, via TRAF2, with the MAPK family pathways, specifically p38 MAPK and JNK, producing different cell responses, depending on whether the exposure is acute, thus promoting cell survival, or chronic, leading to apoptosis (Dong et al., 2014).

TNF receptors and AD

Very recent data has shown that in AD, TNF is demonstrating a higher affinity to TNFR1 compared to TNFR2 (Dong et al., 2015). Furthermore, TNFR1 protein levels were increased in post-mortem AD brains, whereas TNFR2 protein levels were significantly low (Dong et al., 2015). More data comes to support TNFR2 beneficial

role, as in an *in vitro* study, using the SH-SY5Y neuroblastoma cell line, they silenced TNFR2 gene and observed increased neurotoxic effect of A β (Shen et al., 1997, Dong et al., 2015). Despite the fact that neuroblastoma is not considered a completely valid system for AD research, such findings remain scientifically interesting and advocate the hypothesis that TNFR2-regulated responses can contribute to AD confrontation. On the contrary, silencing TNFR1 gene led to alleviation of learning and memory deficits in mice carrying APP23 mutation, which causes increased A β plaque formation, (He et al., 2007), illustrating TNF connection with APP processing (McAlpine and Tansey 2008). A more profound investigation into the equilibrium of TNF binding to its two receptors might probably be the key to grasp its precise role in neurodegenerative disorders, like AD.

Summarizing the amassed information, we can suggest that TNF plays a critical role in the onset and progression of Alzheimer's. This occurs via binding to TNFR1 and initiating a combination of intricate and interacting pathways, like the already mentioned JNK pathway. On the other hand, it seems that TNF can also play a neuroprotective role, via binding to TNFR2 and promoting cell survival through NF- κ B activation. Further on, there is going to be an extensive talk on the participation of other molecular pathways, to which also TNF demonstrates essential involvement, so as to try to better understand the mechanics of that disorder.

MAPK family

MAPK (Mitogen Activated Protein Kinases) are Ser/Thr protein kinases, enzymatically inactive in their physiological form, which are activated by a variety of stimuli, like mitogens, oxidative and osmotic stress, heat shock and pro-inflammatory cytokines. Their activation is induced by double phosphorylation inside its activation loop, which consists, amongst others, of the compulsory sequence Thr-X-Tyr (X can be glutamic acid, glycine or proline), as the last step of the sequential activation of 2 other kinases MEKKs and MEKs. MEKKs themselves are activated either via phosphorylation by another kinase, or by interaction with monomeric G proteins (Ras, Rho). MEKs, on the other hand, are Thr/Tyr kinases that recognize MAPKs and phosphorylate them in their activation loop (Brunet and Pouyssegur, 1997).

This super-family is subdivided into 3 groups: ERKs (extracellular signal-regulated kinases), p38 MAPK, and JNKs (c-Jun NH₂-terminal kinases). The fact that this super-family participates into numerous different cell responses raises the question of how the substrate specificity and selectivity is ensured, so that the correct response is regulated every time. Firstly, this is ensured by the interaction between MAPKs and MEKs. Specifically, the area of MEKs that is related with substrate specificity is located near the N-terminal and does not interact with the activation loop. Regarding MAPKs, their N-terminal is responsible for stimuli-recognition, while their C-terminal appears to regulate the message transduction to the substrate.

Therefore, all these interactions guarantee the correct cell response according to the initial stimuli (Brunet and Pouyssegur, 1997). Upon activation, these cascades execute various functions, such as promoting cell proliferation, cell differentiation, and cell

survival. On the other hand, prolonged activation of these pathways can also lead to apoptosis. .

MAPK and AD

In the recent years extensive research has been conducted around MAPK pathways and their connection with AD. In fact, all MAPK pathways appear to have been activated in neurons with increased A β production, thus suggesting their involvement in AD pathogenesis (Arora et al., 2015).

One of the MAPK super-family members that has been most thoroughly examined so far is the p38 MAPK family. P38 MAPK activity in human brains was investigated long ago (Munoz and Ammit 2009, Hensley et al., 1999). Post-mortem brains of AD patients lead to correlation of increased p38 MAPK phosphorylation with neuritic A β plaque formation as well as NFT formation. Furthermore, other findings of post-mortem AD patients showed increased concentration of MKK6, which is a p38 MAPK upstream activator, maintaining strongly that p38 MAPK contributes to AD pathogenesis (Munoz and Ammit 2009). Hence the reason why there have already been many efforts to target the p38 MAPK pathway as a pharmaceutical route to counter AD (discussed below).

In vitro experiments using hybrid neuroblastoma cells expressing α 4 β 2-nAChRs, sensitive to small A β concentrations, have shown that prolonged exposure of A β induced activation of both the ERK and the JNK pathway, and at the same time, increased expression of PHF-tau and increased levels of ROS. Specifically, ERK activation preceded JNK activation, maintaining their involvement in late phase neurotoxicity (Arora et al., 2015).

AD pathology has always been characterized by extracellular accumulation of A β peptides, derived from proteolytic processing of APP. However, the proteolytic processing of APP not only produces the extracellular A β , but also generates an A β intracellular domain (AICD), further advocating the complexity of AD. Elevated levels of AICD are highly cytotoxic and, in addition, tend to alter signaling pathways, by binding to various cell signaling proteins (Margevicius et al., 2015). One of them is JNK interacting protein 1 (JIP1), member of the JNK family. In a recent study, *Margevicius et al.* demonstrated the involvement of JNK in AD pathogenesis. Specifically, using transgenic mice overexpressing AICD, they conducted a series of tests to finally arrive to the conclusion that AICD interacts with JIP1, thus activating the JNK pathway and leading to synaptic loss in an age-dependent manner, via increasing tau phosphorylation. JIP1 directly interacts with tau (Ittner et al., 2009), promoting the hypothesis of facilitating further tau hyperphosphorylation via JNK/tau interaction (Margevicius et al., 2015).

These findings strongly advocate the fact that AD is a multidimensional disease, as it does not only depend on the extracellular A β plaques and the increased tau phosphorylation, but also on the AICD, which in its part affects signaling pathways, thus promoting AD neurotoxicity by loss of synapses in the brain. Therefore, an effective therapy should have multiple targets rather than signaling targeting of the degradation of A β plaques.

In the past years, there has been research examining AD from different perspectives who, finally, converge, in common hallmarks of AD pathology, as elevated A β levels and increased tau phosphorylation. A recently conducted study investigated the correlation between glucose deprivation and AD (Lauretti and Pratico, 2015).

Glucose is the main source of energy for the human body and of course for the CNS. Under physiological conditions neuronal cells are entirely dependent on a continuous supply of glucose for their correct functioning. CNS is extremely sensitive to lack of glucose, which has been observed in many degenerative disorders (Lauretti and Pratico, 2015).

So, in that study they observed that glucose deprivation lead to p38 MAPK–mediated tau phosphorylation.

This finding demonstrates that tau phosphorylation leading to NFTs formation, major diagnostic of AD pathology, is dependent on many different pathways, making AD confrontation very complicated.

This study went on and investigated the involvement of ASK1 to glucose deprivation-induced tau phosphorylation. ASK1 is part of the MAPK family and research has already shown its involvement in AD pathogenesis (Kadowaki et al., 2005), and the possibility for ASK1 to be a new therapeutic target (Song et al., 2014). ASK1 is activated upon several of internal or external stress and then initiates various cellular responses, like cell differentiation, cell survival and apoptosis, by activating in its turn, other signaling pathways.

In this case, under glucose deficit, ASK1 was activated and later on activated p38 MAPK pathway, which mediated increased tau phosphorylation. By downregulating ASK1 mRNA, resulting in decreased protein levels, it was observed that neuronal cells did not demonstrate any change in pP38 levels and also, the intracellular levels of tau phosphorylated were also intact compared to controls (Lauretti and Pratico, 2015).

This amount of information help to strongly advocate the participation of ASK1 in AD pathogenesis, either by ROS mediated A β -induced ASK1 activation, where ASK1 activation led to JNK activation and neuronal cell death (Kadowaki et al, 2005), or by glucose deprivation, where upon activation, ASK1 upregulated p38-MAPK pathway, which mediated increased tau phosphorylation.

VEGF and AD

VEGF displays broad biological functions including modulation of angiogenesis, vasculogenesis, vascular permeability, vascular remodeling, vascular survival, neurotrophic activity and inflammatory response (Religa et al., 2013). Many studies have shown VEGF's alleviating effects to degenerative diseases like AD, as VEGF has been proved to be a strong contributor to memory repair by improving vascular survival (Religa et al, 2013), and has been suggested as a potent therapeutic agent for neurodegeneration (Storkebaum and Carmeliet, 2004).

Recently though, there has been speculation that VEGF might contribute to AD manifestation, as AD might be an angiogenesis-dependent disorder, and abnormal endothelium activation might lead to amyloid deposition and neuronal death (Chiappelli et al. 2006).

When VEGF's effect on AD brain was tested in presence of physiological A β concentration for the first time, something contradictory to existing evidence was observed. Specifically, VEGF was shown to cooperate with A β and ultimately led to neuronal death, via downregulation of pro-survival pathways like p38 MAPK, JNK, ERK1/2 etc., and upregulation of cascades of the NF- κ B family (data not shown) (Wood et al., 2015).

Moreover, treatment of neurons with VEGFR1/2 inhibitor, Axitinib, led to rescue from VEGF's pro-death activity in presence of A β (Wood et al., 2015).

Discussion

AD is a devastating disease and has been tormenting millions of people worldwide, hindering them from doing everyday activities. We have seen so far that there have been several approaches to override that disorder, all of them eventually converging to countering AD's pathological hallmarks, namely A β plaques, NFTs, loss of neuron synapses and, of course, neuronal death.

TNF- α and AD

The role of cytokine involvement in AD has been thoroughly investigated. The past years great leaps have been made as regards the identification and the role of cytokines crucial for AD progression.

TNF, along with its member TNF- α , is arguably the most important cytokine involved in both early onset of AD via regulation on diverse molecular pathways. Different polymorphisms of the TNF gene has been connected with higher concentration of TNF, leading to elevated levels of A β in AD brains (Perry et al, 2001). TNF- α has been shown to regulate A β -induced CCEs, ultimately leading to neuronal death (Bhaskar et al., 2014).

However, TNF has also shown neurotrophic effects to neuronal tissue, interacting with cell survival pathways, like NF- κ B. Moreover, it is known that TNF is also essential in the inflammatory response process. TNF- α deficient mice have shown inability concerning formation of primary B cell follicles and follicular dendritic cell networks (Pasparakis et al., 1996). Furthermore, TNF knockout mice, when exposed to heat-killed *Corynebacterium parvum*, they demonstrated a delayed or no initial response, eventually leading to abnormal inflammatory response and cell death (Marino et al., 1997).

So far, many drugs targeting TNF have been developed (Infliximab, Etanercept, Adalimumab etc.) mainly having anti-TNF properties and working as TNF- α inhibitors. These drugs were primarily meant as treatments for other diseases such as rheumatoid arthritis, psoriatic arthritis, Crohn's disease, and so on. (Cheng et al., 2014).

TNF receptors

TNF receptors mediate different cellular responses in AD. So far, evidence suggests that TNFR2 receptor orchestrates cell-beneficial responses, while TNFR1 in most cases has an opposite effect. Therefore, such different behaviors that derive from TNF's ligation to its 2 receptors explain its pleiotropic effect in degenerative disorders, as AD, and, also promote novel research efforts.

Drugs that target in inhibiting TNFR1 signaling processes would probably demonstrate promising results regarding countering AD, as it is probable that such an approach would probably promote TNFR2 signaling, thus up-regulating NF- κ B pathway and subsequently p38 MAPK and JNK pathways, leading to cell survival.

From another perspective, further investigation focusing on TNF gene polymorphisms and their correlation with TNF overexpression and chronic exposure, would possibly assist in finding the designated concentration of TNF that is needed for physiological inflammatory responses and, at the same time, not inflicting neurodegeneration via increased A β plaque deposition.

Furthermore, better understanding the exact role of TNFR2 in AD, might provide new insight to the therapeutic strategies that could be generated, as recent data suggests that TNFR2 mediated pathways are the ones that render TNF a neuroprotective agent in neurodegenerative disorders.

MAPK pathways

MAPK pathways have been recently highly incorporated in the efforts of understating AD progression, as they take part in manifestation of AD pathologies, like NFT formation

Studies have demonstrated that all the members of the MAPK superfamily participate with diverse ways in AD development. For instance, JNK and ERK pathways activation due to prolonged A β exposure, have been linked with enhanced tau phosphorylation, elevated levels of ROS and reduced MAPK phosphatases (MKPs) thus promoting NFT formation and neurotoxicity (Arora et al., 2015). In addition, JNK member JIP1 has been shown to have a significant impact in AD progression, interacting with tau (Ittner et al., 2009) and promoting tau hyperphosphorylation (Margevicius et al., 2015).

On the other hand, p38 MAPK has also been shown to be upregulated in the AD brain, under glucose deprivation and to facilitate further tau phosphorylation (Lauretti and Pratico, 2015) and it has been certified that contributes to AD pathology (Munoz and Ammit 2009).

Finally, ASK1, also member of the MAPK family, has also been connected with AD, as under certain conditions, it has shown to be upregulated and subsequently activating

other MAPK pathways, like JNK (Kadowaki et al, 2005), or p38 MAPK (Lauretti and Pratico, 2015), promoting apoptosis.

Many efforts have already been made to counter AD through altering the regulation of the mentioned pathways. Shutting down each pathway at a time led using designated inhibitors has already shown some promise (Kadowaki et al., 2005, Araki et al., 2010, Arora et al., 2015 Lauretti and Pratico, 2015) via reducing tau phosphorylation or A β deposition. The involvement of these molecular pathways, as examined in this essay, is summarized in **Figure 5**.

It is known that the upregulation of these pathways by extracellular stimuli is important for immune response and cell survival. However, we observed that chronic exposure to these activated kinases leads to neurotoxicity and neuronal death in AD. Therefore, it might be interesting for further research to try to downregulate all the pathways of the MAPK family, using multiple upstream inhibitors (e.g. MEK or MEKK inhibitors). The high specificity and selectivity of substrates that derives from the variety of stimuli that activate these pathways and was discussed previously, should also be taken into account. This might aid to better understand the total impact of the MAPK cascade to AD progression and, at the same time, provide an insight regarding the implications that might rise considering the ability of proper immune response.

VEGF

VEGF is a substance that has never been associated with AD pathology, up till recently. In presence of A β in the AD brain, VEGF demonstrated neurotoxic behavior, working along with A β to decrease neuronal viability (Wood et al., 2015).

These evidence support the fact that VEGF has contradictory functions in the AD brain, depending on the presence or absence of A β (**Figure 6**). Therefore, using VEGF as a therapeutic agent might be proved to be rather problematic, as it has demonstrated both alleviating and deleterious functions in the manifestation of AD. Nevertheless, efforts have already been made so as to find a VEGF independent way in order to access the VEGF-downregulated pro-survival pathways. For instance, a growth neurotrophic factor called BDNF has been thoroughly tested in rodent models and has already shown to have substantially protective effects against neuronal damage in AD (Nagahara et al., 2009). Treatment of neurons with BDNF led to stimulation of pro-survival pathways, independently of VEGFR (Wood et al., 2015).

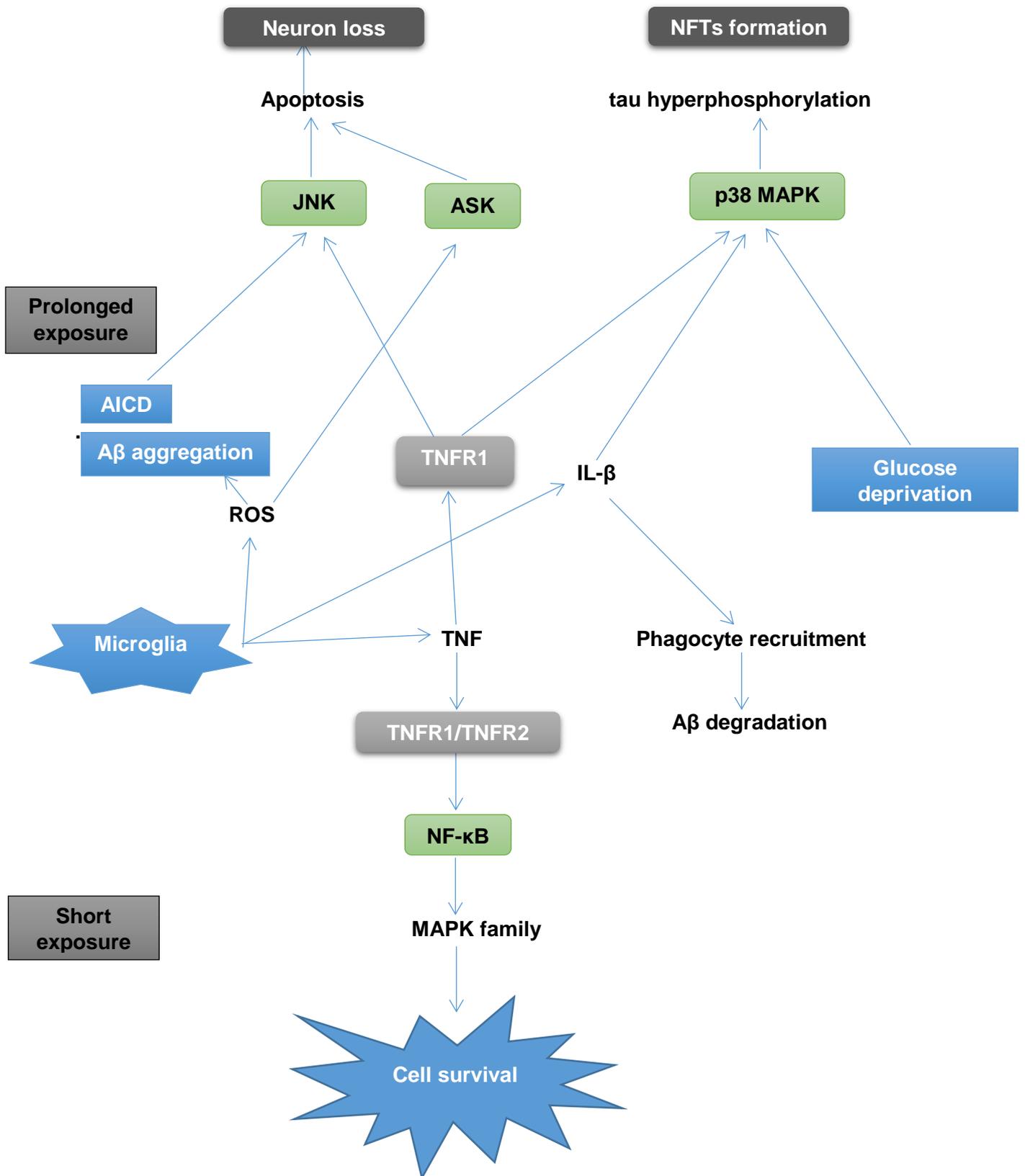


Figure 5. Summarization of the involvement of the MAPK and the NF-κB pathways to Alzheimer's disease, as investigated in this essay.

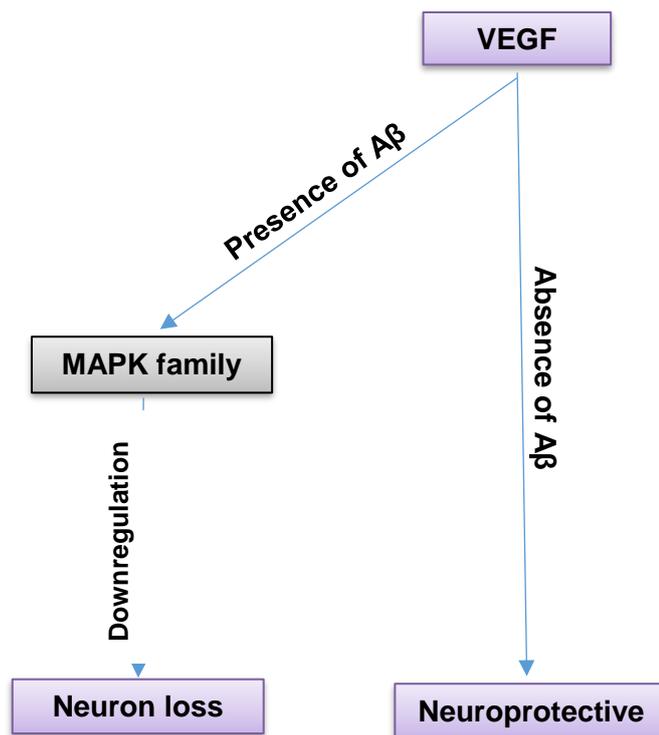


Figure 6. Involvement of VEGF in AD. It is illustrated that the presence or absence of physiological concentrations of A β modulates the function of VEGF, rendering it neurotoxic or neuroprotective, respectively.

Conclusion

Thus far, concrete evidence exist to support that AD is a multi-dimensional disorder, comprising of multiple interacting complex molecular networks, each of them containing many different components. Therefore, a multi-dimensional disease requires a multi-dimensional therapeutic strategy. Combining the above mentioned propositions for each different aspect that was examined in this essay, namely TNF targeting via its receptors, as well as MAPK and VEGF pathways, a novel therapeutic route is created, so as to discover new aspects of AD pathology that could help scientists in their perpetual endeavor to eradicate that disease.

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