Implications of Neuroimmunity in Alzheimer’s Disease: A Review

Katriel Lee

1Transylvania University, Lexington, Kentucky 40508

AD is characterized cognitively by memory, problem-solving, and language difficulties. It is estimated that 5.4 million Americans currently have Alzheimer’s disease (AD). The cognitive difficulties in AD are reflected in the brain through the accumulation of amyloid-β (Aβ) in cerebral amyloid angiopathy (CAA), neurofibrillary tau tangles, neuronal tissue atrophy, and neuroinflammation, but the exact cause of AD is still in question. However, evidence suggests that differences in neuroimmune function—the central nervous system’s ability to resist disease—may play a role in the development and progression of AD. This paper largely relates neuroimmune changes to the amyloid hypothesis of AD. This approach relates AD to Aβ production and clearance, and therefore, targets Aβ for treatment uses. A thorough literature search revealed evidence that the blood-brain barrier (BBB), glial cell mediation and effects on neuroinflammation, and cerebrospinal fluid (CSF) and interstitial fluid (ISF) drainage systems are changed in AD. These changes seem to be detrimental for the AD brain and Aβ accumulation. Future research should be conducted regarding characterization of the lymphatic system in the human dura, the balance of helpful and harmful effects of activated microglia, the driving forces of paravascular CSF-ISF exchange, the effects of sleep on neuroimmunity, and genetic risk factors for neuroimmune dysfunction.

Abbreviations: Aβ – amyloid-β; AD – Alzheimer’s Disease; BBB – blood-brain barrier; CAA – cerebral amyloid angiopathy; CSF – cerebrospinal fluid; ISF – interstitial fluid; RAGE – receptor for advanced glycation end products; SAS – subarachnoid space

Keywords: Alzheimer’s Disease; Neuroimmunity

Introduction

A 67-year-old man begins to exhibit cognitive decline. He cannot seem to reason as logically as he could before and has difficulty remembering small events (Braak and Braak, 1997). Eventually, he goes to a physician who orders an MRI that reveals some classic changes in brain structure associated with Alzheimer’s Disease (AD). This information, along with his cognitive deficits, contributes to his saddening diagnosis of AD (McEvoy 2011; Wahlund et al., 2005). AD is typically cognitively characterized by memory, problem-solving and language difficulties (Keene et al., 2017), and it is estimated that 5.4 million Americans of all ages had Alzheimer’s disease in 2016 (“Alzheimer's disease facts”, 2016). If this is not striking enough, estimates predict that if the rates of AD continue, by 2050, the cost to our healthcare system will be over one trillion dollars and will increase government spending on Medicare and Medicaid by about five-fold (“Changing the Trajectory of Alzheimer’s Disease”, 2015). Not only is this a national health crisis, this disease has saddening impacts on families affected. Caretakers often find it challenging to devote time to AD patients, and the symptoms of AD can be heartbreaking. Ultimately, AD can even indirectly cause death via complications from severe dementia such as pneumonia or malnutrition (“Alzheimer's disease facts”, 2016).
These negative consequences (memory difficulties, cognitive deficits, death, etc.) of AD may be the result of an inappropriate neuroimmune response in the brain (Abbott et al., 2010; Bakker et al., 2016; Kimelberg and Nedergaard, 2010; Schafer and Stevens, 2015; Weller et al., 2008; Zlokovic, 2008). Neuroimmunity can be characterized as the central nervous system’s (or in particular, the brain’s) ability to resist disease. The neuroimmune mechanisms utilized to safeguard the brain are vast and include the blood-brain barrier (BBB) (Abbott et al., 2010; Zlokovic, 2008), glial cell mediation and effects on neuroinflammation (Kimelberg and Nedergaard, 2010; Schafer and Stevens, 2015), and cerebrospinal fluid (CSF) and interstitial fluid (ISF) drainage systems (Bakker, et al., 2016; Weller et al., 2008).

Neuroimmune systems are not perfect and can sometimes become compromised. It was hypothesized that the above components of neuroimmunity play a detrimental role in AD. Research thus far has shown that multiple aspects of immune regulation in the brain are changed in AD (Abbott et al., 2010; Bakker et al., 2016; Kimelberg and Nedergaard, 2010; Schafer and Stevens, 2015; Weller et al., 2008; Zlokovic, 2008). Components of neuroimmunity that were stated earlier—the BBB, glial cell mediation and neuroinflammation, and CSF and ISF drainage systems—will be discussed with respect to the changes caused by AD.

Additionally, research about neuroimmunity and AD is constantly updating, particularly recently in light of the discovery of lymphatic vessels in the mouse brain (Aspelund et al., 2015; Louveau et al., 2015). This paper will also briefly discuss the significance of this recent discovery.

**Alzheimer’s Disease Characteristics**

AD is characterized by progressive cognitive decline and neurovascular dysfunction (Iadecola, 2004; Zlokovic, 2005). Physically, amyloid-β (Aβ) peptide plaques accumulate on the blood vessels and brain parenchyma (Deane and Zlokovic, 2007; Hardy, 2006; Rovelet-Lecrux et al., 2006). Amyloid deposits on blood vessels of the CNS are also known as cerebral amyloid angiopathy (CAA) (Bakker et al., 2016; Silva et al., 2008). Although typically Aβ may modulate synaptic activity and neuronal survival (Pearson and Peers, 2006), in excess it is considered to be neurotoxic and can induce oxidative stress in endothelial cells (Deane et al., 2003). Neurofibrillary tangles (composed of hyperphosphorylated tau protein) are also a common indicator of AD and are correlated with cognitive deficits and neuronal loss (Lee et al., 1991; Santacruz et al., 2005; Tanzi, 2005). Neuronal atrophy is also a significant aspect of the pathology of AD (“The Changing Brain”, 2008). Figure 1 shows a visual image of the cortical changes of the AD brain.

![Figure 1: Changes of the AD brain include cortical loss and enlarged ventricles. Used with permission from the National Institute on Aging (“The Changing Brain”, 2008).](image-url)
In summary, anatomically, the accumulation of Aβ in CAA, neurofibrillary tangles (Lee et al. 1991; Santacruz et al., 2005; Tanzi, 2005), neuronal tissue atrophy, and neuroinflammation are hallmarks of AD, but the exact cause of AD is still in question. This paper largely relates neuroimmune changes to the amyloid hypothesis of AD. This is the approach that considers the main hallmark of AD to be Aβ accumulation (Selkoe and Hardy, 2016). In this view, amyloid production and clearance needs to be targeted for treatment. Therefore, this study relates how changes in the BBB, glial cell mediation and neuroinflammation, and CSF and ISF drainage systems are related to Aβ accumulation.

The Blood-Brain Barrier

Researchers began to suspect the existence of a blood-brain barrier (BBB) in the late 19th century when basic dyes injected into the bloodstream did not appear to stain the brain (Davson and Churchill, 1967). On the most basic level of understanding, the BBB functions to separate the blood from the fluids of the brain. It also controls exchange of solutes between the blood and fluids of the brain (Banks and Erickson, 2010). The BBB is composed of endothelial cells that line capillaries in the brain and spinal cord of mammals, and it works to protect the brain in a variety of ways. It acts as a physical barrier (with tight junctions), a transport barrier (with transport mechanisms to control solute levels), and a metabolic barrier (with enzymes to break down certain molecules in transit). Using these methods, the BBB regulates ions, controls certain neurotransmitters, prevents many macromolecules and neurotoxins from entering the brain, and moderates brain nutrition (Abbott et al., 2010). Below, some of these mechanisms are briefly explained.

A specific ionic composition is optimal for synaptic signaling in the brain, so it is important for the BBB to help control ion regulation. For example, too much or too little potassium in the brain could cause decreased or increased excitation of neurons and can potentially be detrimental. Ideally, the concentration of potassium is maintained at around 2.5-2.9 mM in the CSF and ISF, while the blood plasma maintains a potassium concentration around 4.5 mM. In addition to K⁺ concentration, the BBB actively regulates Ca²⁺ and Mg²⁺ concentration and pH (Abbott et al., 2010).

The blood plasma also carries high levels of glutamate, an excitatory amino acid neurotransmitter (NT). If too much glutamate enters the brain, neural tissue can be damaged (Abbott et al., 2010). Excessive activation of NMDA (N-Methyl-D-aspartic acid) receptors by this neurotransmitter in the brain is also implicated in AD. Much of the neuronal cell loss in AD is restricted to glutaminergic neurons, so NMDA receptors are targeted in some current treatments (Hynd et al., 2004). A fully functional BBB is crucial to prevent excess influx of glutamate, and it also helps keep the central and peripheral nervous system NTs separate (Abbott et al., 2010).

It is also crucial to prevent certain macromolecules from entering the brain. Proteins carried in the plasma such as albumin, pro-thrombin, and plasminogen can be damaging to nervous tissue because they have the ability to induce apoptosis (cell death) through cellular activation. Clearly, preventing these large molecular weight proteins from passage into the brain is an important task that the healthy BBB completes (Abbott et al., 2010).

Additionally, neurotoxic substances in the blood are kept out of the brain due partially to this protective barrier. If an excess of neurotoxins was present in the brain, the natural rate of cell death could be accelerated to debilitating levels (Abbott et al., 2010). For example, it has been shown that lead poisoning can cause nerve damage and can potentially lead to neurodegenerative disorders (Sanders et al., 2009).

In addition to preventing entry of certain levels of ions, macromolecules, and neurotoxins into the brain, the BBB must also allow entry of certain helpful substances into the brain. The BBB has low passive permeability to some water-soluble nutrients and metabolites. There are also transport systems expressed in the BBB.
in order for these important substances to reach the brain as needed. For example, transporters in the BBB supply the CNS with glucose and amino acids necessary for metabolism (Abbott et al., 2010). In sum, the BBB provides the necessary balance of keeping out “bad” and letting in “good”.

Unfortunately, when the BBB properties cannot effectively meet the changing needs of the CNS, disease conditions may arise (Banks and Erickson, 2010). As mentioned earlier, the accumulation of protein Aβ is associated with AD (Deane and Zlokovic, 2007; Hardy, 2006; Rovelet-Lecrux et al., 2006). With regards to the BBB, this means something might not be functioning properly with macromolecule regulation.

Relevant to this discussion is the receptor for advanced glycation end products (RAGE). The RAGE influx transporter transports Aβ across the BBB into neurons and the brain parenchyma, and generally RAGE expression increases in affected cerebral blood vessels, microglia, and neurons in AD models (Deane et al., 2003). Compared to RAGE expressed in low levels in most areas of the BBB in the healthy, young brain, the aging and AD brain exhibits increased RAGE expression. This can have many effects. The effects associated with AD onset and progression include transcytosis of Aβ into the brain parenchyma, a cascade resulting in secretion of proinflammatory cytokines, and suppression of cerebral blood flow (CBF) (Zlokovic, 2008).

Deane et al. (2003) explored some of these effects in transgenic mice that expressed mutant precursor proteins for Aβ. In the mice that had the precursor, there was an increase in RAGE-dependent brain capillary uptake. When antibodies were introduced specifically for RAGE, there was inhibited brain capillary uptake and transport of Aβ in a dose-dependent manner. To be more certain that RAGE was mediating these effects, controls were done with fucoidan (inhibitor of Aβ binding to type A macrophage scavenger receptor), antibodies to β1 integrin, the RHDS sequence of Aβ, and nonimmune IgG. Aβ BBB transport was shown to be unaffected by these (Deane et al., 2003). This experiment aligns with the idea that increased RAGE activity is associated with increased Aβ in the brain, which can contribute to the detrimental Aβ plaques in AD.

Additionally, this paper discusses the effects of Aβ-RAGE on CBF. Blocking RAGE using RAGE-specific antibodies was effective in preventing Aβ-induced CBF decrease. They also demonstrated that RAGE-null mice with introduced Aβ only had minor changes in CBF compared to age- and strain-matched controls. Also related to CBF is the vasoconstrictor endothelin-1 (ET-1). Deane et al. (2003) also showed an increase in ET-1 in cerebral vasculature after infusion of Aβ. Overall, they demonstrated that RAGE-dependent transport of Aβ results in production of ET-1 along with expression of proinflammatory cytokines that together cause decreased CBF (Deane et al., 2003).

As mentioned earlier, inflammation is also associated with the progression of AD. Deane et al. (2012) explored the effects of a high affinity Aβ/RAGE inhibitor they derived from tertiary amides called FPS-ZM1. In mice, they showed that FPS-ZM1 suppressed mRNA and protein levels of proinflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin (IL) 1β (IL-1β), IL-6, and monocyte chemotactic protein-1 (MCP-1) in the cortex and hippocampus. Overall, this demonstrated that inhibiting RAGE led to decreased neuroinflammatory response that is usually associated with AD (Deane et al., 2012).

RAGE transports Aβ into the brain, but equally important is low-density lipoprotein receptor related protein 1 (LRP1). LRP1 is the efflux Aβ transporter in the BBB (Zlokovic, 2008), and having compromised LRP1 may cause the elevated Aβ levels seen in AD. Aβ is removed via a three-step process through this transporter. First, LRP1 of the brain endothelium binds Aβ, which initiates the process. Then, soluble LRP1 (sLRP1) binds the plasma Aβ. Subsequently, cellular clearance is mediated by the LRP1 receptor (Zlokovic, 2011). When Aβ levels are excessive in the brain, LRP1 begins to lose its normal protein conformation, which may be one contributor to the vicious cycle of elevated Aβ levels in the brain of AD patients (Zlokovic, 2008).

The function of LRP1 as related to AD is explored by Sagare et al. (2007). In their
experiment, they utilized a LRP recombinant cluster IV (LRP-IV) in mice. Some mice had a mutation of the gene that encodes the Aβ precursor protein- the Swedish mutation of the amyloid precursor protein (sw-APP). Mice with this mutation were treated with low doses of LRP-IV. The result was increased CBF, improved operant learning, and improved spatial and recognition memory. These results were not found for wild type mice with LRP-IV or vehicle treatment. Additionally, Aβ40 levels in the mutant LRP-IV treated mice versus vehicle-treated mutant mice were reduced by 72% in the hippocampus and >80% in the cortex. This demonstrates the importance of LRP-IV to remove Aβ from the brain, and the authors note this importance as well in relation to Alzheimer’s disease treatment (Sagare et al., 2007).

Another study by Uden et al. (2002) demonstrated the neuroprotective benefits of LRP1 on the BBB in mice. Transgenic (tg) mice overexpressing human APP (hAPP) and receptor-associated protein (RAP)-deficient (RAP-/) mice were crossed and yielded 4 groups of mice: non-tg that expressed endogenous mRAP (RAP+/+), non-tg that did not express endogenous mRAP (RAP-/-), hAPP tg expressing both copies of endogenous mRAP, and hAPP tg lacking endogenous mRAP (hAPP tg/RAP-/-). The hAPP tg/RAP-/- and non-tg RAP-/- mice had reduced LRP immunoreactivity by about 80%. hAPP tg and hAPP tg/RAP-/- mice developed AD-like plaques around 10 months. Additionally, hAPP tg/RAP-/- mice had a higher plaque load (Uden et al., 2002). This appears to support the idea that LRP is crucial in preventing these plaques.

### Glial Cells and Inflammation

Glial cells are ubiquitous in the brain. In fact, it is estimated that there are 3 times more glia in the brain than neurons. A variety of different types of glial cells carry out functions to assist in neural signal propagation, modulate synaptic signaling, rehabilitate the brain after damage, and facilitate neuronal development (“Neuroglial Cells”, 2001). Research suggests that microglial cells, a type of glial cell, affect the progression of AD (Takeuchi, 2010). Microglial cells are mainly derived from hematopoietic stem cells, and are sometimes compared to macrophages of tissue because they scavenge cellular debris from sites of injury. After brain damage, the number of microglia at the site of injury increases in order to promote inflammation (“Neuroglial Cells”, 2001), and a similar accumulation of microglia is seen in the AD brain (Barger and Basile, 2001).

Mittelbronn, Dietz, Schluessener, and Meyermann (2001) explored the effects of microglia on neuroinflammation and immunity. This study used 20 human subjects with no clinical neurological symptoms or diseases and no neuropathological alterations. Using immunolabeling, they found that cell surface markers CD68, MHC-II and AIF-1 (important regulators of immune function) are constitutively expressed on microglia in the brain (Mittelbronn et al., 2001). These are important regulators of immune function, especially MHC-II. MHC-II molecules are important because they bind portions of pathogenic cells and display them for T-cells. Subsequently, the pathogen can get eliminated by other immune cells (Janeway et al., 2001).

Table 1 provides a concise list of the receptors found to be expressed on microglia, and Table 2 lists secretory products of microglia that have interesting consequences with immunity and inflammation. Usually, all of these receptors and products of microglia work to help the brain defend itself. For example, cytokines usually help to regulate inflammation and modulate cell growth, survival and differentiation (Ramesh et al., 2013). Even the free radical nitric oxide can have a positive role in the non-AD brain by increasing neuronal excitability (Balez and Ooi, 2016). Each of the secretory products can be very beneficial, but in the case of AD, there may be something wrong.
It is important to note that the accumulation of activated microglia is a prominent finding in AD (Barger and Basile, 2001). As seen in the two tables, microglia have a variety of receptors and secretions, so it is easy to see how these individual components can work together in many different ways. In the case of AD, one cascade involves microglia as antigen-presenting cells (APC) producing neurotoxic molecules. Also, microglia activation allows the toll-like receptors (TLRs) to trigger a proinflammatory cascade by secreting cytokines like the ones listed in Table 2, chemokines, glutamate, etc. (Cabezas et al., 2014). Excessive release of these substances can be problematic. For example, large amounts of glutamate can lead to neuronal damage through excessive excitation (Abbott et al., 2010) and is especially detrimental in the AD brain (Hynd et al., 2004).

Barger and Basile (2001) studied the effects of glutamate using rats. Secreted APP (sAPP) is a form of the Aβ precursor protein known to increase microglia activation. So, in order to study effects of activated microglia, they utilized rats with sAPP treatment. Because Ca\(^{2+}\) is heavily involved with neuronal survival, this is the initial marker they used when looking at primary hippocampal neurons. They found that a gradual \([\text{Ca}^{2+}]\) increase was observed after the control medium was added, but after adding the medium from sAPP-activated microglia, there was a larger, more rapid elevation of \([\text{Ca}^{2+}]\). These results were reversed when an ionotropic glutamate receptor inhibitor, 2-amino-5-phosphonovaleric acid (APV), was introduced. This suggests that a glutamatergic agonist is released by microglia (Barger and Basile, 2001).

Additionally, their paper explored the effects of the role of cysteine-glutamate antiporters as a mechanism of glutamate transport across the cell. This transporter seems to play an important role in the problematic aspects of glutamate because at high extracellular glutamate concentrations, cysteine is depleted from the cytosol. This cysteine is necessary for glutathione synthesis, which plays a role in moderating cell proliferation and antioxidant defense. Therefore, with cysteine deprivation, oxidative stress occurs. To test the role of this transporter in the sAPP triggered glutamate release, microglial cultures were exposed to either cysteine free or cysteine containing medium. The cysteine free medium

---

**Table 1: Microglial cell membrane receptors**

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Most Relevant to the Current Discussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-II</td>
<td>TLR</td>
</tr>
</tbody>
</table>

Receptors reported in the literature, whose generation may be influenced by the state of activation as well as by the anatomical location, age, and animal species from which the microglia are derived.

**Table 2: Secretory products of microglia**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Chemokines</th>
<th>Nitric Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-16, IL-23, TNF-α</td>
<td>CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES</td>
<td>Perivascular cells, CNS, microglia</td>
</tr>
</tbody>
</table>

Secretory products reported in the literature whose generation may be influenced by the state of activation as well as by the anatomical location, age, and animal species from which the microglia are derived.
group showed increased release of glutamate into the medium, but the absolute concentrations of glutamate released were diminished. Then, microglia were treated with a-aminoadipate (AAA), an inhibitor of the cysteine-glutamate antiporter. With AAA, sAPP did not stimulate glutamate release. This is evidence of the idea that the cysteine-glutamate antiporter plays an important role in the potentially harmful effects of release of glutamate (Barger and Basile, 2001). Overall, this study demonstrates the mechanisms and magnitude of glutamatergic damage that microglia can cause through over-activation and contributes to the overall picture of microglia harming the AD brain.

There are more examples of these cases in which, although microglia try to remove Aβ from the brain, activation of the microglia itself can be harmful (Takeuchi, 2010). Figure 2 shows products that can be counterproductive in red and helpful products in blue. For example, the proinflammatory cytokines TNF-α, IL-1β, IL-6, and MCP-1 were already mentioned in the BBB section. Release of these substances can be neurotoxic, resulting in cell death (Block et al., 2007). However, neurotrophic factors such as brain-derived neurotrophic factor (BDNF) can be released and is neuroprotective and promotes neuronal growth (Takeuchi, 2010).

Microglia can also have effects on neurogenesis. A study by Ekdahl et al. (2003) highlights the consequences of large numbers of microglia in the brain. Rats had stimulating/recording electrodes implanted in their right ventral hippocampus. In some of these rats, a brain infusion cannula was also inserted. Additionally, some rats only had the cannula. The rats, excluding controls, were electrically induced to have status epilepticus (SE), which is a form of brain damage (repeated seizures) linked to inflammation. Lipopolysaccharide (LPS) (or a control vehicle) was introduced to the rats as a means to induce inflammation. Additionally, minocycline, a microglia inhibitor, was introduced to some rats while others got the control vehicle. Through tissue analysis with 5-bromo-2'-deoxyuridine (BrdU) (used for in-vivo cell labelling) as a marker of neurogenesis, they found that there was a significant negative correlation between the number of new neurons and the number of activated microglia. They also found that minocycline was able to restore the previously inhibited neurogenesis. This demonstrates the impact that too many activated microglia can have on the brain and also shows that preventing them from activating can be beneficial for neurogenesis (Ekdahl et al., 2003).

CSF and ISF Drainage

As described before, the BBB allows passage of ISF and nutrients through the brain and also allows passage of certain molecules out of the brain. Then drainage systems must do their job to “clean up” soluble metabolites and waste, and one such waste product is Aβ (Silva et al., 2008). The brain can accomplish this and fluid volume regulation by draining cerebrospinal fluid (CSF) and ISF. CSF is extracellular fluid in the ventricles of the brain and the subarachnoid space (SAS) and is largely produced by choroid plexus. ISF is extracellular fluid formed from the active transport of solutes across the BBB (Bakker et al., 2016).

Appropriate drainage of these fluids is crucial. The importance of CSF drainage is highlighted by the fact that the total volume of CSF is replaced every 5-7 hours. To accomplish this CSF exchange, CSF uses “pulsatile” motion.
to create some net flow from the ventricles to the SAS. The flow of CSF is considered to be mostly paravascular because it occurs between the pia mater and glia limitans, enclosing the vascular wall of blood vessels (Bakker et al., 2016). As for ISF, movement of water and other solutes across the capillary endothelium efflux route contribute to its flow. Eventually, in the healthy brain, ISF drains by bulk flow along the basement membranes of cerebral arteries, which can be considered perivascular drainage (Bakker et al., 2016). Figure 3 is a visual representation of the described process of perivascular drainage (Silva et al., 2008).

The mathematical model by Schley et al. (2006) suggests that there are “pulse cycles” in these artery walls. These pulse cycles include periods when fluid and solutes are driven in the opposite direction of blood flow. The functioning of this system is dependent partially upon the flexibility of the artery walls. Usually, the artery wall will change in accordance with the vessel expanding and recoiling for the pulse wave (Schley et al., 2006).

CAA is a hallmark of AD (Deane and Zlokovic, 2007; Rovelet-Lecrux et al., 2006) and can result from decreased draining of Aβ (Schley et al., 2006). According to the mathematical theory, the efficiency of draining fluid and solutes from the brain is dependent upon the artery wall expanding and contracting as needed (Schley et al., 2006). This is problematic because stiffening of arteries can accompany age and can cause the brain to drain with reduced force. Therefore, the brain would have less protection from deposition of toxic levels of insoluble Aβ. Figure 4 is a visual representation of these detrimental changes and shows the relation between a less proficient drainage system and CAA implicated in AD (Silva et al., 2008).

This claim was corroborated by Iliff et al. (2013). They used in vivo two-photon microscopy in mice in order to observe pulsatile motion. They were able to observe this pulsatile motion once the CSF tracers were injected. Additionally, they attempted to reduce this pulsatility by performing a unilateral ligation of the internal carotid artery. Although there were no changes in systemic blood pressure, this resulted in significantly reduced vascular pulsatility along penetrating cortical arteries. They also wanted to determine if reducing the pulsatility resulted in a slower paravascular CSF-ISF exchange. Results revealed that the ligation significantly slowed movement of the CSF tracer into and throughout the cortex. Going even further, they wanted to test the reverse effects and determine if increasing pulsatility could increase the rate of CSF-ISF exchange. So, by administering a β1 adrenergic agonist (dobutamine), they successfully elevated pulsatility, which also resulted in accelerated CSF-ISF exchange. They mentioned that despite these findings, more research needs to be done.
on the mechanical source driving this paravascular CSF-ISF exchange (Iliff et al., 2013). Overall, this demonstrates more evidence that reduced pulsatility could be a factor in AD neurodegeneration due to lower Aβ clearance.

Silva et al. (2008) also presents atherosclerosis as a cause of stiffening arteries that can contribute to diminished Aβ clearance. Beach et al. (2007) explored this link between atherosclerosis and AD through autopsies of patients diagnosed with neuropathological vascular dementia (VaD) and non-AD dementias. They found that with increasing atherosclerotic grade, the odds ratio for the diagnosis of AD and VaD increased. It also increased the odds ratio for neuritic plaque density and higher neurofibrillary tangle stage (Beach et al., 2007) which is also implicated in AD. This provides support for the idea that atherosclerosis is involved in AD.

In summary, it appears that much of the literature supports the idea that decreased function of CSF and ISF drainage can contribute to increased Aβ deposition and the consequences that follow, like dementia.

Another interesting aspect of this system is the connection it has with sleep. Xie et al. (2013) assessed diffusion of tetramethylammonium using two-photon imaging in mice. They found that states of anesthesia or sleep are associated with a 60% increase in the interstitial space and increases the CSF-ISF exchange. This is relevant because a state of sleep would be more effective in clearing Aβ. In fact, the paper mentions that this 60% increase contributes to more efficient convective clearance of Aβ and other compounds (Xie et al., 2013). Perhaps this is an area that needs more focus in research in the future. This is especially important considering that AD is associated with abnormal sleep—this could be a vicious cycle for patients. Sleep disruptions are common for normal aging as well (Vitiello and Borson, 2001), so finding a distinction in the pathophysiology of sleep changes in the normal versus the pathological brain may be helpful.

Central Nervous System Lymphatics

The lymphatic system is crucial for blood volume regulation and immunity in the human body. It accomplishes blood volume regulation by returning interstitial fluid to the blood, and it assists in immunity by releasing lymphocytes and removing foreign matter (Srebnik, 2002). Many of the studies discussed throughout this review were written under the impression that lymphatic vessels in the central nervous system did not exist, or, at best, the idea was speculated but not confirmed. In fact, the previous prevailing concept was that T-cells and other lymphocytes exited the brain through venous drainage (Dissing-Olesen et al., 2015). However, a recent discovery of lymphatics in the brain may be crucial to our understanding of immunity in the brain and how it relates to diseases like AD (Aspelund et al., 2015; Louveau et al., 2015).

Louveau et al. (2015) discovered functional lymphatic vessels in the dural sinuses while looking for T-cell gateways into and out of meninges of mice. They did so by preparing a dissected mouse brain meninges and using immunohistochemistry to stain endothelial cells, T-cells, and MHC-II-expressing cells. They found a high concentration of stained cells near the dural sinuses. The presence of these cells indicates the possibility that this is a site where immune cells are recirculated (Louveau et al., 2015).

To analyze more accurately the exact location of these cells, coronal sections of the dura mater were stained for T cells and endothelial cells. The location was confirmed, and they noticed a portion of T cells in a linear alignment. These unexpected findings led the researchers to test the vessels for markers that are associated with lymphatic endothelial cells (LEC). First, they established that the vessels do not belong to the cardiovasculature. They also detected expression of the main LEC transcription factor. The presence of LEC in the meninges was also confirmed by flow cytometry. This, among other indicators, demonstrates that the CNS has lymphatic vessels. Although these findings were in mice,
they found a similar structure in the human dura, but more studies need to corroborate the findings to fully characterize this system (Louveau et al., 2015).

Around the same time as these findings, Aspelund et al. (2015) also were exploring the possibility of lymphatic vessels in the brain. They used immunofluorescence against six lymphatic markers: LYVE1, PROX1, PDPN, CCL21, VEGFR3, and PECAM1. They also reported an extensive network of lymphatic vessels in the meninges underlying the skull bones. Additionally, they found that the dural lymphatic vessels are similar to conventional lymphatic vessels in that they have low levels of PECAM1 but high levels of LYVE1, PROX1, PDPN, CCL21, and VEGFR3. These researchers made similar observations to Louveau et al. (2015), concluding that there is strong evidence of lymphatic vessels in the dura mater (Aspelund et al., 2015).

The review by Dissing-Olesen et al. (2015) describes the combination of these two studies to characterize the vessels. The lymphatic network seems to contain more lymphatic valves at the base of the brain and exits the skull in foramina next to arteries, veins, and cranial nerves. However, the two papers (Louveau et al., 2015; Aspelund et al., 2015) do not perfectly align concerning the exact path of drainage of these vessels, so more research is needed (Dissing-Olesen et al., 2015).

Overall, these findings open the door to much more research concerning AD. Perhaps this lymphatic system plays a role in clearing Aβ, and it may allow the immune system access to the brain, which has many interesting implications. If so, how would sleep, memory, or stress affect this system? Additionally, if this system is implicated in Aβ clearance, the lymphatics could be targeted for AD treatment. The discovery of these vessels is met with excitement but also uncertainty.

**Discussion**

From this review and synthesis of some old and some current research, it is clear that neuroimmunity as implicated in AD is a multifaceted issue. What is known is that the BBB (Abbott et al., 2010; Zlokovic, 2008), glial cell mediation and effects on neuroinflammation (Kimelberg and Nedergaard, 2010; Schafer and Stevens, 2015), and cerebrospinal fluid (CSF) and interstitial fluid (ISF) drainage systems (Bakker et al., 2016; Weller et al., 2008) are changed in AD. The altered BBB in AD models exhibits high levels of RAGE influx transporters and compromised LRP1 efflux transporters, which contribute to increased neuroinflammatory response and decreased CBF in mice models (Deane et al., 2003; Deane et al., 2012; Sagare et al., 2007; Uden et al., 2002; Zlokovic, 2008). Increased levels of microglia due to Aβ in the AD brain can trigger proinflammatory cascades, which can lead to neuronal damage and decreased levels of neurogenesis (Barger and Basile, 2001; Cabezas, et al., 2014; Ek Dahl et al., 2003; Hynd et al., 2004). Lower pulsatility of arteries can diminish the CSF-ISF exchange rate and therefore can contribute to accumulation of Aβ plaques (Iliff et al., 2013; Schley et al., 2006; Silva et al., 2008).

Though the influence of Aβ is undeniable, other factors may work in tandem with this phenomenon to produce the neurological effects seen in AD patients. Exploring the implications of the malfunction of each of these systems can bring scientists closer to deciphering the puzzle of AD, but there is still so much to learn. For example, new research gave a novel look into the possibility of lymphatic vessels in the human brain (Aspelund et al., 2015; Louveau et al., 2015). We can speculate on what the discovery of lymphatics in the brain means for AD; however, this recent finding needs much more thorough exploration in its basic characterization before it can be studied in relation to AD treatments or etiology (Dissing-Olesen et al., 2015). Other areas of exploration are also necessary. For example, the effects of microglia in AD are complex because microglia may help remove Aβ plaques at the cost of worsening brain damage (Sardi et al., 2011). More research may be able to help clarify the question of whether or not they are more helpful by inducing neuroprotective inflammation or harmful in excess numbers as seen in AD. If more research can clarify the
ideal levels of microglia in the AD brain, treatments could specifically target microglia and/or their resulting cascades. Additionally, it would be helpful to more thoroughly understand the driving forces of paravascular CSF-ISF exchange and the effects of sleep on this system. This research would have practical importance for both AD patients and the general public, especially considering the sleep difficulties that many Americans experience in such a career-oriented, fast-paced society (“Insufficient Sleep”, 2015). Furthermore, although the causal relationship of amyloid-beta in AD has support in the literature (Deane and Zlokovic, 2007; Hardy, 2006; Rovelet-Lecrux et al., 2006), neurofibrillary tau tangles also play a role (Lee et al., 1991; Santacruz et al., 2005; Tanzi, 2005). It would be useful to explore both of these hallmarks hand in hand to see the causal role they may play in AD. There is also some information about the links and interactions between these two features, but the literature is incomplete (Hernández et al., 2010). Exploring these links and the causal role of these plaques and tangles could bring us closer to finding more effective treatments.

Although neuroimmunity appears to play a large role in AD (Barger and Basile, 2001; Cabezas et al., 2014; Deane et al., 2003; Deane et al., 2012; Ekdahl et al., 2003; Hynd et al., 2004; Iliff et al., 2013; Sagare et al., 2007; Schley et al., 2006; Silva et al., 2008; Uden et al., 2002; Zlokovic, 2008), this is only a small piece to the puzzle. Genetic factors have also been identified, including apolipoprotein E-e4 (APOE-e4), which could account for up to a fourth of AD cases (“Alzheimer's disease facts”, 2016). When proceeding with future research, it would be useful to also keep genetic factors in mind as it may be helpful in characterizing the disease and potentially facilitating more individualized treatment for patients with varying genetic predispositions.

Conclusion

Overall, the full picture of AD is still incomplete, but research advances continue each day. Perhaps one day the knowledge of this
disease will be complete enough to not only slow its progression, but to halt it all together. With our current knowledge, the BBB, glial cell mediation, and CSF and ISF drainage systems provide promising therapeutic targets in order to prevent detrimental Aβ accumulation or side effects of the brain “overreacting” (excessive microglia activation) in an attempt to remove Aβ. Within the BBB, downregulating RAGE or upregulating LRP1 could minimize influx of Aβ into the brain or maximize efflux of Aβ out of the brain, respectively. Additionally, partially suppressing microglial response could reduce release of potentially harmful proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and MCP-1. Within the CSF and ISF drainage systems, there could be many biomedical interventions possible. However, behavioral intervention and guidance may also be worthwhile given the link between more sleep and increased CSF-ISF exchange. Finally, as we continue to learn more about the brain’s lymphatic system, we may begin to develop additional means of treating this complex disease.

Acknowledgements

I would like to thank Dr. Margaret Upchurch and Dr. Rebecca Fox at Transylvania University for guiding me through the process of writing this review.

Corresponding Author

Katriel Lee
Transylvania University
Katriellee30@gmail.com

References


Implications of Neuroimmunity in Alzheimer’s Disease: A Review


