# Blood-Brain Barrier Integrity and Clearance of Amyloid-β from the BBB

Irsalan Cockerill, Joy-Anne Oliver, Huaxi Xu, Bingmei M. Fu, and Donghui Zhu

**Abstract**

Alzheimer's disease, a type of dementia, affects memory, behavior, and cognitive processes in affected individuals. It is one of the prominent diseases, accounting for 60–80% of dementia cases and affecting a significant population of persons over the age of 65 years. While rare, Alzheimer's disease (AD) may affect the younger population as well. With such a widespread number of persons affected with AD, scientists have undertaken the initiative to develop a cure for this devastating disease; however, it has been deemed quite challenging. A dysfunctional blood-brain barrier, with impaired ability to clear amyloid-β from the brain, has been directly linked to the development of Alzheimer's disease. The blood-brain barrier restricts the flow of many substances into and out of the brain and serves as a selective and protective barrier to the brain. A proper functioning blood-brain barrier contributes to the maintenance and integrity of the brain. In turn, different systems and mechanisms within the blood-brain barrier are set in place to facilitate mediated passage of materials and substances between the brain and the bloodstream. In relation to AD, the mediation of amyloid-β clearance is of great importance in maintaining the blood-brain barrier's integrity.

## 1 Introduction

Performing ordinary activities such as eating, drinking, or exercising can affect the blood composition in innumerable ways, including shifts in salt levels to increases or decreases in the lipid or amino acid content in the blood. Blood travels throughout the body via the circulatory system, and through this system the blood transports oxygen, carbon dioxide, and nutrients into and out of the various tissues of the body. Metabolites that are toxic to the brain can even be present in the bloodstream; however these "toxic" metabolites

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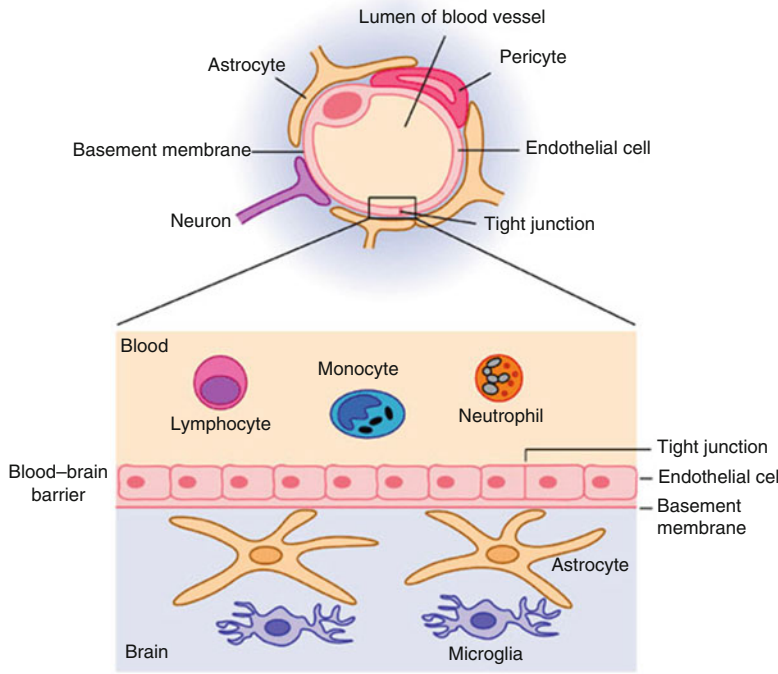
may be beneficial to the body. The blood-brain barrier (BBB) blocks the streaming of harmful materials present in the bloodstream from entering the central nervous system (CNS) and maintains brain homeostasis by regulating the movement of compounds across the endothelium of cerebral capillaries (Kreutzer et al. 2011). It acts as a defense mechanism, consisting of the endothelial cells lining the blood vessels in the brain that are "glued" together very tightly by structures known as tight and adherens junctions and other structures that combine to facilitate the functioning of the BBB. For decades, scientists have focused on ways to penetrate the BBB in designing many drug delivery methods and techniques in the prevention and treatment of neuro diseases such as Alzheimer's and dementia.

The function of the brain and the study of the BBB serve as a vital integral to the investigation of brain diseases such as Alzheimer's disease and dementia. Scientists spend a great deal of time focusing on the vital aspects that make up the BBB structure and how these characteristics contribute to its specific functions. They have also developed a central concept to understanding the factors that contribute to the degradation of brain functioning and the cause of Alzheimer's disease. Additionally, they have used these concepts in designing appropriate drug delivery techniques in combating this devastating disease.

## 2 Blood-Brain Barrier Structure and Function

Neurons present in the CNS are between 8 and 20 μm from the capillaries and require a constant supply of oxygen and nutrients (Tajes et al. 2014). Nutrients and oxygen leave the blood to enter the brain through the endothelial layer that lines the blood vessels of the blood-brain barrier. The endothelial cells of the BBB consist of numerous transport systems that enable necessary materials required for proper brain functioning but do not easily cross the barrier (Fig. 1). Studies have shown that small molecules of sizes less than ~800 Da can cross the BBB easily because they are small enough to pass through tight junctions. However, molecules larger than ~800 Da




The blood–brain barrier (BBB)

**Fig. 1** Structure of the blood-brain barrier including its components. The BBB is not a rigid structure but a dynamic interface with a range of interrelated functions, resulting from the presence of extremely effective tight junctions, trans endothelial transport systems, enzymes, and regulation of leukocyte permeation, which thereby generate the physical, transport, enzymatic, and immune regulatory functions of the BBB (Joan Abbott and Friedman 2013). There are three cellular elements of the brain microvasculature that compose the BBB—endothelial cells, astrocyte endfeet, and pericytes (Aarts and Arundine 2003)

have difficulty crossing the BBB (Tajes et al. 2014).

Restrictive permeability is key for the ideal functioning of the BBB. The main function of the endothelium is to block the passage of unwanted materials and toxins from entering the brain. The presence of tight and adherens junctions between the endothelial cells forming the BBB allows for the cells to be packed together in such a way that there is almost no space between them. The presence of the BBB ensures high specificity of nutrients reaching the neurons in the CNS despite the surrounding environmental influences of the harmful substances in the body.

The endothelium allows for rapid-free diffusion or gaseous exchange of small particles such as oxygen from the blood into the brain and carbon dioxide from the brain into the blood (Zhao et al. 2015a). Hydrophilic molecules such as glucose cannot pass through the endothelium as easily as oxygen and carbon dioxide do and require receptors and channels to facilitate their transport into and out of the brain (Zhao et al. 2015a).

The general components of the BBB are quite similar in morphology of the peripheral cells; however, there are notable modifications that are specific to maintaining ideal functioning and homeostasis of the BBB. For it to be maintained, the endothelial cells of the BBB consist of the following notable difference from peripheral cells:

- *The presence of intercellular tight junctions and the absence of fenestration*. The BBB is primarily composed of a considerable number of endothelial cells. The significance of the endothelial cells is to act as a barrier, preventing the passage of various materials into and out of the brain. Fenestration refers to the presence of pores in the capillaries that allow the passage of large molecular weight materials. Fenestrated capillaries are found in the intestinal mucosa, renal glomeruli, pancreas, endocrine gland, and other tissues of the body. Because the BBB focuses on preventing the free passage of materials into the brain, fenestration is not present.
- *The low level of non-specific transcytosis (pinocytosis) and paracellular diffusion of hydrophilic compounds*. Non-specific transcytosis involves the movement of various macromolecules across the interior of the cells through vesicles that are captured from outside the cell. Unlike other endothelial cells of the body, the absence of the high quantity of non-specific transcytosis benefits the BBB by facilitating the selectiveness to the materials that enter the brain tissue. Paracellular transport refers to the transfer of substances across the epithelium through the intercellular space between the cells. Tight and adherens junctions make up the intercellular spaces and cause limited space to be present between the cells of the endothelium. Because of this, the passage of hydrophilic or water-loving materials is not possible as long as the BBB is fully functioning.
- *The presence of a large number of mitochondria in endothelial cells*. Mitochondria in the endothelial cells forming the BBB provide energy in the form of ATP through respiration and regulate cellular metabolism and gene expression. They play a crucial role in maintaining the integrity of the BBB through the miR-34a-mediated mechanism. MicroRNA 34a, also referred to as miR-34a, is a microRNA that the human body encodes using the miR-34A gene. The miR-34a participates in post-transcription regulation of gene expression by affecting the stability and translation of mRNAs during gene expression. When a specific miRNA binds to its target on a messenger RNA (mRNA), it can inhibit the expression of that mRNA. MicroRNAs are referred to as master regulators of gene expression and are known to regulate up to 30% of the protein coding genes in the human genome. One single miRNA can bind to a target and regulate more than 100 different transcripts. With such ability, the presence of mitochondria in the endothelial cells of the BBB is crucial for the regulation of the integrity of the barrier through its role on gene expression.

- *The polarized expression of membrane receptors and transporters.* These are responsible for the active transport of blood-borne nutrients to the brain or the efflux of potentially toxic compounds from the cerebral to the vascular compartment. Nutrients and other materials enter and exit through the endothelial cells of the BBB via transport systems. The diffusion of substances into the brain can be divided into two processes: paracellular (substances travel between cells) and transcellular (substances travel across cells). Substances that move paracellular are limited as they are subjected to tight junctions. However, substances that move transcellular primarily travel via active transport. Apart from the paracellular diffusion (sucrose) and the transcellular diffusion (ethanol) mechanisms that occur at the BBB, other types of transcellular transport mechanisms include ion channels, e.g., $K^+$ gated, ion-symport channels (e.g., $Na^+/K^+/Cl^-$ cotransporter), ion-antiporter channels (e.g., $Na^+/H^+$ exchange), facilitated diffusion (e.g., glucose via GLUT-1), active efflux pumps (e.g., P-glycoprotein), active-antiporter transport (e.g. $Na^+/K^+$ ATPase), and receptor-mediated endocytosis (e.g., transferrin and insulin). The two main efflux transporters in the BBB are multidrug resistance gene (MDR1) and breast cancer resistance protein (BCRP). They are both expressed on the apical membrane of the endothelium, pumping substances from the cell into the bloodstream, and function by preventing xenobiotics from crossing the BBB.

## 2.1 Tight Junctions

Tight junctions are located in the apical section of the paracellular pathway between adjacent endothelial cells. Tight junctions primarily form a seal to avoid paracellular diffusion of solutes into the brain. Additionally, they regulate the lateral diffusion of nutrients and other particles between the apical and basolateral plasma membrane domains that maintain the plasma membrane and lipid polarity. Tight junction functions are enhanced by the presence of astrocytes and pericytes. Their presence in the BBB shares similar characteristics to those of epithelial tight junctions but is a specific difference that allows BBB tight junctions to fulfill their functions. These BBB tight junctions are sensitive to ambient factors (Wolburg 2000). Tight junctions consist of a number of proteins, primarily claudins, occludins, and junctional adhesion molecules JAMs that directly contribute to maintaining their viability. Other proteins that contribute to the structure and functioning of tight junctions include PDF motif MAGUK (ZO-1, 2 and 3), non-PDF motif cingulin, 7H6 ZONAB, Rab13, PK C heterotrimeric G protein, and catenin (120 and p100) (Wolburg 2000).

## 2.2 Adherens Junctions

Adherens junction is the junction present in the BBB whose cytoplasmic face is linked to the actin cytoskeleton. They are responsible for keeping contiguous cells together and therefore assist in maintaining the structure of tight junctions. Adherens junctions are present in the endothelial cells of the BBB and are primarily comprised of transmembrane glycoproteins of the cadherin armadillo superfamily. The transmembrane glycoproteins mainly consist of vascular endothelium cadherin also known as VE-cadherins that have the ability to form homotypic adhesive complexes with adjacent cells in the presence of $Ca^{2+}$. The Armadillo superfamily acts as an anchor through cytoplasmic plaque that links the glycoproteins to the cytoskeleton.

## 2.3 Astrocytes

Astrocytes are star-shaped glial cells that connect neurons directly to the endothelial cells of the BBB. They play a very important role in the BBB by providing structural support of the endothelial cells and reinforcing the BBB function by the endothelial cells. Specific contribution of astrocytes to the BBB is not as prominent as those of tight junctions and endothelial cells (Weiss et al. 2009).

## 2.4 Pericytes

Pericytes engulf the basal membrane that surrounds the endothelial cells of the BBB. They contribute to the regulation of endothelium proliferation, angiogenesis, and inflammatory process. Pericytes also regulate specific gene expression in the endothelial cells and deter polarization of astrocyte endfeet surrounding CNS blood vessels.

## 2.5 Transport Across the BBB

Nutrients, ions, and other molecules travel across the BBB through two general mechanisms (Fig. 2):

### 2.5.1 Paracellular Transport

Movement of particles is between the endothelial cells, through the junctional complex. The paracellular pathway is described as a passive transport process involving the movement of hydrophilic molecules across the barrier, depending on their electrochemical, hydrostatic, and osmotic gradient. Tight junctions primarily mediate paracellular transport, and the movement of particles is dependent on solute concentration. The contractility and adhesive forces of the endothelial cytoskeleton control the permeability of the junction complex of the tight junctions through which paracellular transport occurs (Tajes et al. 2014). The dynamic equilibrium between both forces accounts for this type of transport through the BBB. However, although this is one of the primary transport mechanisms, it plays a minor role in developing brain-targeted drugs in the treatment of many brain-related diseases such as dementia and Alzheimer's.

### 2.5.2 Transcellular Transport

It refers to the movement of nutrients, ions, or particles across the endothelial cells of the BBB that may or may not require energy. Through transcellular pathway, particles move across the luminal and abluminal membrane of the capillary

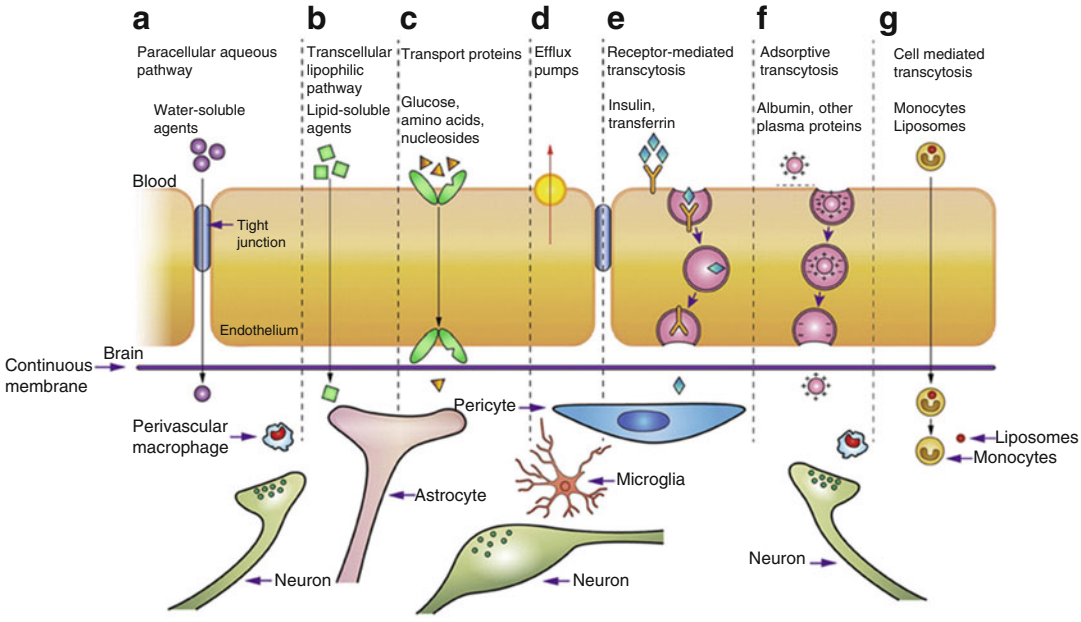



**Fig. 2** Transport pathways across the blood-brain barrier. (**a**) Paracellular pathway for water-soluble agents, (**b**) transcellular pathway for lipid-soluble molecules, (**c**) carrier-mediated pathway, (**d**) efflux transport system, (**e**) receptor-mediated transcytosis pathway, (**f**) absorptive transcytosis pathway, and (**g**) cell-mediated pathway (Liu 2012)

endothelium via mechanisms such as receptor-mediated transcytosis, efflux transport systems, endocytosis of positively charged molecules, and carrier-mediated transport (Tajes et al. 2014).

### 2.5.3 Receptor-Mediated Transcytosis

A form of active transport that is temperature dependent and is the primary transport mechanism used by the brain's endothelial cells. High-molecular-mass proteins, such as insulin, and low-density proteins and lipoproteins such as leptin, transferrin, and insulin-like growth factor (IGF) require this type of transport. Receptor-mediated transcytosis requires a two-step process: (1) the formation of an endocytic vesicle where the receptor-ligand recognition facilitates the formation of coated pits. These pits become endocytic vesicles and engulf the ligand. (2) Endosomal fusion dissociates the receptor from the ligand, and the contents are released via exocytosis. Some of the vesicles fuse with lysosomes and lose the content due to factors such as low pH and enzyme-mediated hydrolysis and, therefore, never make it across the BBB and into the CNS. This pathway is safe and effective in CNS drug delivery because of its specificity and lack of size and lipophilicity dependence (Tajes et al. 2014).

### 2.5.4 Efflux Transport System

This mechanism actively transports particles via ATP and concentration dependence and is responsible for the removal of materials out of the CNS, into the systemic circulation, therefore eliminating the buildup of compounds that would have entered through the BBB via different pathways. The prototypic efflux transporter is known as the glycoprotein P (gp-P), present on the luminal membrane of the endothelial cells, and it is present in high concentrations within the capillaries of the brain to maintain the brain's vitality. The gp-P is a member of the class of multidrug resistance receptors, well known for being an ATP-dependent remover of anticancer drugs, antibiotics, immune system suppressors, or ionic channel modulators. There are additional efflux transporter systems that do not use the same mechanism as gp-P as they do not have the ability to hydrolyze ATP, a critical ability of the ATP-binding cassette (ABC) transporter family. The other systems include monocarboxylate transporters (MCTs) and organic anion transporters/organic anion transporter polypeptides (OAT/OATPs) (Tajes et al. 2014). However, these systems are unable to transport materials against their concentration gradient and therefore participate in bidirectional transport of materials via ion or substrate gradients based on the material concentration on both sides of the barrier.

### 2.5.5 Charged Compound Interaction

This transport mechanism occurs when positively charged compounds interact with negatively charged compounds of the endothelial cell membrane to facilitate absorptive-mediated endocytosis to occur at the BBB. This pathway is mainly used by scientists to increase the delivery of conjugated drugs across the BBB during drug delivery into the CNS (Tajes et al. 2014).

### 2.5.6 Carrier-Mediated Pathway

A form of saturable transport that mediates the exchange of particles between the systemic circulation and the brain's parenchyma. This process can be ATP dependent or ATP independent. The protein carriers that participate in this pathway are located in the luminal and abluminal membrane and are usually polarized. Within the BBB, there are numerous carrier systems that promote the selectiveness of substrates and other molecules that travel through the barrier. Glucose, for instance, is supplied through the GLUT-1 transporter by concentration gradient through passive transport (Tajes et al. 2014). Passive transport is defined as the movement of molecules such as ions and nutrients across cell membranes without the need of ATP. Other compounds such as amino acid require different carriers depending on their physiochemical properties.

## 3 Alzheimer's Disease and Amyloid-β

Alzheimer's disease is a central nervous system (CNS) disease, caused by the dysfunction of the blood-brain barrier (BBB). Alzheimer's disease, being the most common type of dementia, affects over 36 million individuals worldwide and is quite prevalent in persons of age 65 years and older. Symptoms of Alzheimer's begin from memory loss and cognitive impairment and can progress in severity as time progresses. Depending on how severe the disease is, individuals have to be institutionalized and may soon die. If left untreated, the occurrence of Alzheimer's disease can become widespread where it can become a threat to the worldwide healthcare system.

Amyloid-β is a protein constituent that is considered the hallmark of Alzheimer's disease. Individuals with this disease are known to have clumps of deposited Aβ within the brain tissue that form plaques and contribute to nerve cell death. The presence of Aβ in the brain tissue causes the destruction of synapses from an early stage prior to its accumulation and further damage. Synapses are referred to as contact points where nerve cells relay signals to one another. Amyloid-β originates as a solitary molecule and favors the formation of small clusters that are soluble and able to travel freely into the brain. The accumulation of clusters leads to the plaque formation which becomes deposited throughout the brain tissue. However, these cluster forms are capable of binding strongly to a receptor on the nerve cells that set into motion a cascade of events that erodes the synapses with other nerve cells. Therefore, scientists have developed a hypothesis—*the amyloid cascade hypothesis* states that amyloid-β (Aβ) decomposition in the CNS initiates a cascade of molecular events that cause neurodegeneration, leading to Alzheimer's disease onset and progression.

The presence of synapses between nerve cells contributes to memory storage, planning, thought processes, and emotions. Once synapses degradation begins to occur, persons experience loss of these attributes—the symptoms of Alzheimer's disease. The presence of a protein receptor, paired immunoglobulin-like receptor B also known as PirB, present on the nerve cells on the brains of mice, binds strongly with Aβ. The PirB in the brain reduces plasticity in the nervous system and weakens synapses. When Aβ binds to PirB, it breaks down cofilin activity—a protein that breaks down actin, a building block protein that maintains the structure of the synapses—and revs up the synapses degradation. The main receptors for amyloid-beta peptide (Aβ) transport across the blood-brain barrier (BBB) from the brain to the blood and the blood to the brain are low-density lipoprotein receptor-related protein-1s (LRP1) and receptor for advanced glycation end products (RAGE), respectively (Deane et al. 2009). Because the BBB plays a vital role in maintaining CNS homeostasis, its dysfunction results in the onset of Alzheimer's and many other brain diseases.

## 4 Amyloid-β Clearance Mechanisms from the Brain

Insufficient amyloid-β (Aβ) clearance is a hallmark in the pathogenesis and progress of Alzheimer's disease (AD). Neurons predominantly secrete Aβ into the interstitial fluid (ISF) upon amyloid precursor protein (APP) hydrolysis. Several clearance mechanisms exist which include low-density lipoprotein receptor-related protein 1 (LRP1)-mediated transport, cerebral parenchymal cells, and choroid plexus secretion and cerebrospinal spinal (CSF) production, while heavy metals may impair clearance. Understanding the biochemical pathways at work can further advance our knowledge of the disease and lead to the development of effective therapies.

### 4.1 LRP1-Mediated Clearance of Aβ

LRP1 is a member of the low-density lipoprotein receptor family which includes LRP1B, megalin/LRP2, very-LDLR (VLDLR) apolipopro-

tein E receptor 2 (ApoER2)/LRP8, sortilin-related receptor (SorLA/LR11), LRP5, and LRP6 (Kanekiyo and Bu 2014). LRP1 has been widely studied for its implication in AD. It is a receptor for more than 40 different ligands and participates in Aβ metabolism by binding directly or indirectly to Aβ. LRP1 is ubiquitously expressed all throughout the body and highly concentrated in the liver, lung, and brain (Kanekiyo and Bu 2014). It plays an integral part in brain hemostasis. APP undergoes processing via α, β, and γ secretase; the latter two confer a more amyloidogenic pathology, whereas α-secretase produces soluble sAPPα. The transmembrane APP undergoes cleavage of its N-terminal domain by β-secretase followed by C-terminal cleavage by γ-secretase, producing Aβ which is a small protein of 39–43 amino acids long; $Aβ_{40}$ and $Aβ_{42}$ have an intrinsic tendency to self-assemble into SDS-stable oligomers which form insoluble β-pleated sheet structures termed amyloid fibrils (Bhattacharjee et al. 2014). β, followed by γ-secretase processing, results in exocytosis of Aβ in neurons, and LRP1 mediates uptake of Aβ in brain parenchyma (by microglia, neurons, and astrocytes). As a major endothelial surface receptor, LRP1 mediates clearance of Aβ across the BBB. Cell membrane-bound LRP1, concentrated on the abluminal endothelial side, assists with transcytosis. Phosphatidylinositol binding clathrin assembly (PICLAM) protein is encoded by the PICLAM gene and is involved in internalization and endocytosis of membrane receptors. It is abundantly expressed in the brain capillary endothelium and has been shown to affect Aβ metabolism and transport through the BBB (Zhao et al. 2015b). In a detailed study conducted by our group, the precise mechanism of PICLAM-mediated Aβ migration from the brain to the blood was discovered. *Picalm*$^{+/-}$ heterozygous mice were generated for the study and displayed significant retention of $Aβ_{40}$ and $Aβ_{42}$ by 38 and 36% following intracerebral administration of Aβ, respectively (Zhao et al. 2015b). Upon Aβ binding to LRP1, phosphatidylinositol binding clathrin assembly protein (PICLAM) binds to the YXXXL intracellular domain tail of LRP1, triggering a conformational structural change and driving the clearance of brain Aβ transcytosis to systemic circulation (Zhao et al. 2015b). Clathrin heavy chain (CHC) and clathrin adapter protein α-adaptin (AP-2) were also found to be essential for proper internalization of Aβ, a mechanism confirmed by siRNA knockdown of PICALM, CHC, and AP-2. *Picalm* deficiency diminished $Aβ_{40}$ and $Aβ_{42}$ efflux across BBB by 41 and 61%, respectively, a finding that was further validated by lower plasma levels of 48% Aβ40 and 65% $Aβ_{42}$ compared to *Picalm*$^{+/+}$ controls (Zhao et al. 2015b). During this process, Aβ is directed away from Rab 7, a GTPase that directs late endosomes to lysosomes resulting in ligand degradation, and ushered toward Rab 5 and Rab 11, GTPases responsible for recycling endosomes and transcytosis and exocytosis of ligands (Zhao et al. 2015b). Peak co-localization between PICALM and Rab5 was higher than that of PICALM and Rab11, suggesting transfer of endocytotic vesicles from Rab5 to Rab11 is a rate-limiting step (Zhao et al. 2015b). In the liver, soluble-LRP1 (sLRP1) is synthesized and secreted into the peripheral circulation where it sequesters recently emigrated Aβ across the BBB, leading to clearance from the kidneys. Coimmunoprecipitation of sLRP1-bound Aβ in neurologically normal humans has indicated that circulating sLRP1 can sequester 70–90% of plasma Aβ (Ramanathan et al. 2015). This precise mechanism creates an "Aβ sink" where systemic concentration of Aβ is kept low enough, thus driving Aβ transcytosis across the BBB. The precise mechanism is detailed in Fig. 3.

### 4.2 Brain Parenchymal-Mediated Clearance of Aβ

Brain parenchyma cells represent an alternate pathway for clearance of Aβ (Fig. 4). In neurons, APP undergoes processing where it is secreted into ISF. From there, neighboring neurons can uptake Aβ in a LRP1-mediated endocytosis fashion. It can be further degraded in the lysosome. Disturbances in lysosome functionality have been implicated in intraneuronal Aβ

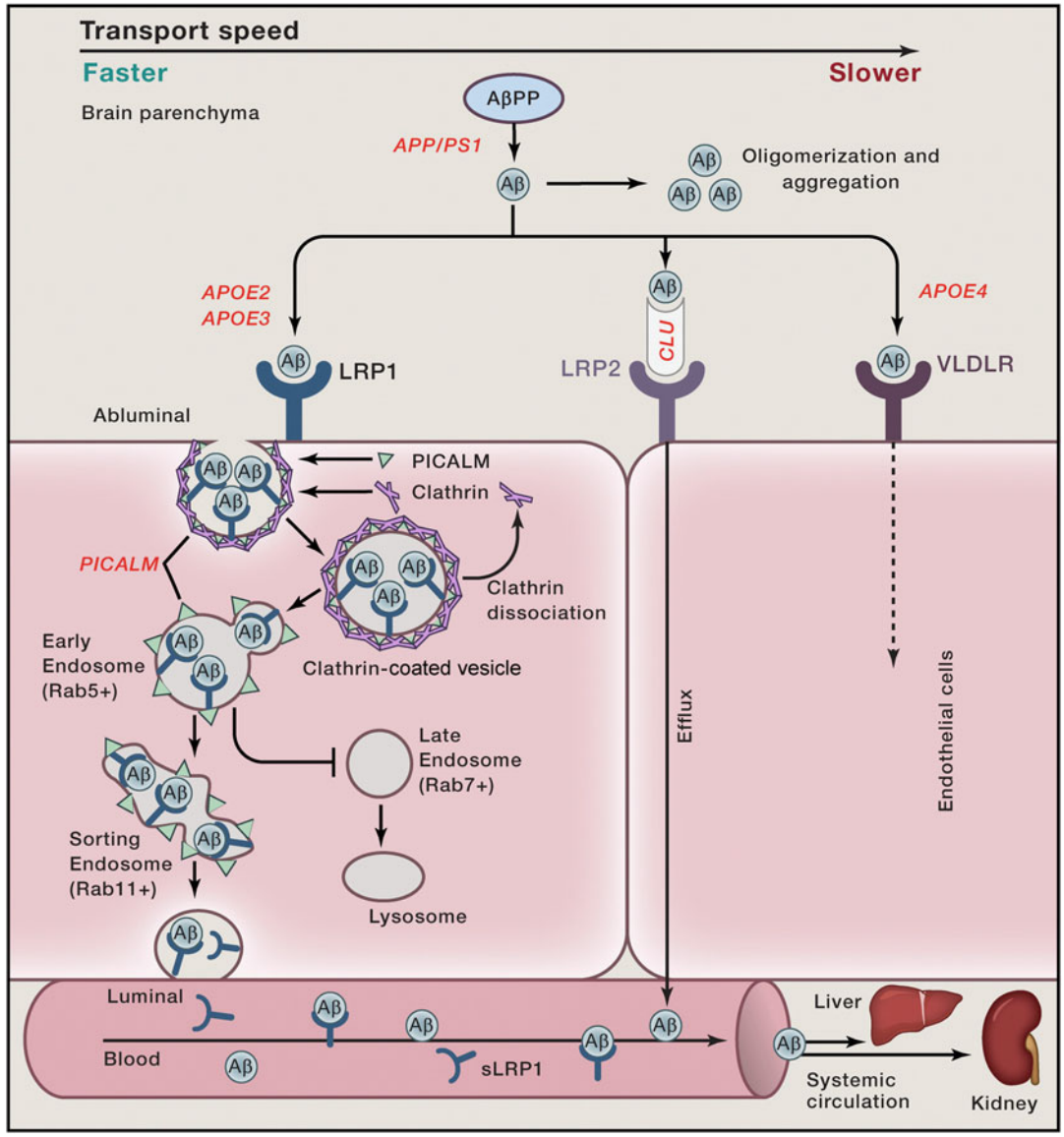



**Fig. 3** APP undergoes processing by α secretase, generating sAPPα, while β and γ secretase sequentially produce Aβ. Binding of Aβ to APOE2, APOE3, or APOE34 dictate transport speed through BBB. APOE2 and APOE3-AB result in PICALM-mediated transcytosis via Rab 5 and Rab 11 through BBB. sLRP1 is directed toward the liver and kidneys for systemic clearance. Slower Aβ clearance is controlled by APOE4 γ-secretase processing, resulting in Aβ oligomerization and limited clearance via VLDLR. Courtesy of Zhao et al. 2015a

aggregation and deposition. LRP1 knockdown mice display higher concentrations of ISF Aβ, exhibiting exacerbated AD pathology.

Microglia represent another clearance mechanism of Aβ. Soluble Aβ (sAβ) is taken up in a macropinocytosis manner and transported to lysosomes, whereas large Aβ fibrils are taken up by multicomponent cell surface receptors. Phagocytosis of apoptotic cells via LPR1 has also been demonstrated, suggesting an alternate pathway of Aβ clearance for microglia. Interestingly, inhibition of LRP1 via antagonists such as lactoferrin, α2macroglobulin (α2M), and RAP results is diminished Aβ uptake (Kanekiyo

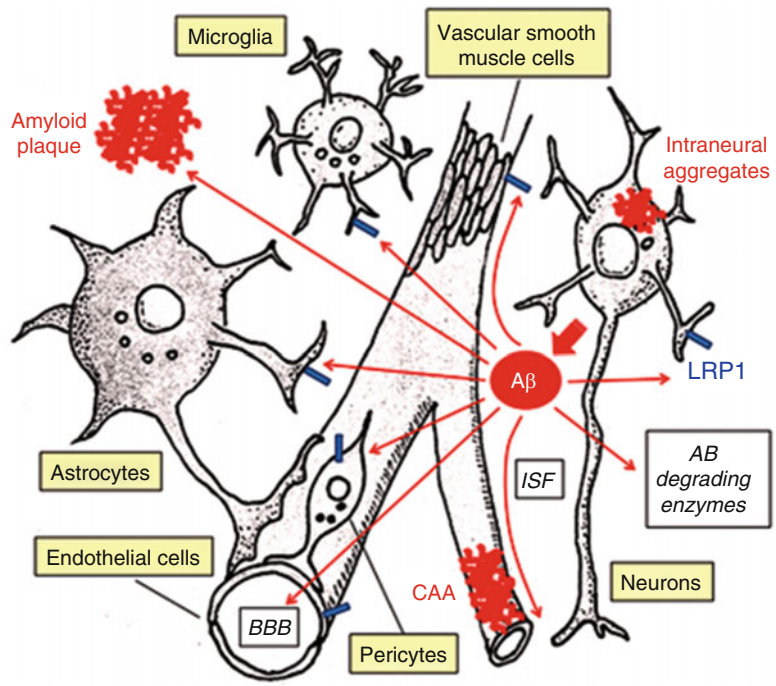



**Fig. 4** Neuronal secretion of Aβ can be cleared through several mechanisms. Aβ can be assimilated by microglia, astrocytes, pericytes, endothelial cells, or transported across BBB. Aβ may also be enzymatically processed by insulin-degrading enzyme (IDE) or neprilysin. Failure in Aβ clearance may result in amyloid plaques, synaptic dysfunction, and neuronal loss. Adopted from Kanekiyo and Bu 2014

and Bu 2014). Interestingly, in a human BBB model performed by Bernas, microglia initiate blood-derived monocyte recruitment through secretion of RANTES, otherwise known as "regulated on action," normal T cell expressed and secreted, a chemotactic cytokine (Zhagi et al. 2009). Emigrating monocytes are attracted to neuritic plaques where they intrude into neurons, harvesting Aβ. Subsequently, monocyte-derived macrophages become swollen and attempt to emigrate through the BBB. Compared to controls, AD macrophages acquire a swollen phenotype and upregulate apoptotic pathways, namely, caspases-6, 7, and 8 (Zhagi et al. 2009). Through engorgement of Aβ and apoptosis, migrating macrophages burst spilling fibrillar Aβ into the vessel wall (Zhagi et al. 2009), known as cerebral amyloid angiopathy, CAA. Apparently, decreased phagocytosis of Aβ results from downregulation of β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase (MGAT-3), an enzyme important in phagocytosis of Aβ. Increased vessel angiopathy activates inflammatory pathways, resulting in increased ROS, oxidative stress, and chemokines, further exacerbating the disease. Aβ deposition within cerebral vessels also results in loss and degeneration of pericyte and smooth muscle cells. Mice defective in pericytes express higher levels of intercellular adhesion molecule 1 (ICAM-1) in the brain endothelium, and larger numbers of $Gr1^{+}$ leukocytes infiltrate into the brain parenchyma (Zenaro et al. 2016), demonstrating the importance of pericytes in regulating the immune response.

Pericytes are located on the abluminal side of the BBB and cover approximately 25% of their circumference. Pericytes express LRP1 and adenosine triphosphate-binding cassette transporter B1 (ABCB1) which have both been shown to play roles in Aβ clearance. As part of the neurovascular unit (NVU), pericytes are critical for the development and stability of the BBB and regulate blood flow through capillaries through controlling cellular contraction/relaxation (Zenaro et al. 2016). Additionally, endothelial secreted platelet-derived growth factor BB (PDGF-BB) binds to basement membrane heparan sulfate proteoglycan, initiating pericyte proliferation, migration, and recruitment (Zhao et al. 2015b). Transgenic null mice have demonstrated PDGF-

BB that is imperative for CNS microvessels as complete loss leads to rupture, microaneurisms, and embryonic lethality. Aβ deposition has been shown to increase PDGRβ shedding from pericytes, demonstrating yet another degenerative effect of the amyloid cascade. A major biochemical pathway at work involved APOE; APOE binds to LRP1 on localized pericytes, activating and/or suppressing the proinflammatory cyclophilin A, an isozyme involved in cis-trans isomerization of proline imidic peptide bonds, nuclear factor kB (CypA-NFkB) pathway; the APOE4 variant causes activation of this pathway, while APOE3 translates into suppression (Zhao et al. 2015b). Secretion of APOE occurs at the level of the astrocyte.

Astrocytes cover up to 90% of brain microvessels and rely on bidirectional feedback from pericytes in maintaining BBB integrity. Their presence in the NVU is essential in maintaining tight junctions of the BBB. Astrocytes close to plaques express LRPs and can internalize Aβ in an APOE-dependent manner. Some studies have shown intra-astrocytic Aβ to induce dysfunction, while others have shown that Aβ can be degraded. Speculation surrounds the idea that astrocytes may traffic Aβ close to endfeet where abluminal endothelial cells and pericytes may eliminate AB via ABCB1/LRP1 (ElAli and Rivest 2013). They express relatively low levels of ABCB1 and elevated levels of ABCA1, a known factor in APOE lipidation (ElAli and Rivest 2013), resulting in reduced Aβ aggregation. As such, astrocytes contribute to Aβ clearance in making Aβ more diffusible through the BBB. Aquaporin-4 (AQP4) is abundant in astrocyte processes adjacent to cerebral microvessels, and its expression is key in uptake and degradation of Aβ (Zenaro et al. 2016). The reallocation of AQP4 from astrocyte end processes to non-endfeet has been observed in several models of AD, suggesting increased depolarization and communicational interference with pericytes (Zenaro et al. 2016). Additionally, astrocytes mediate neuroinflammation through active secretion of cytokines and chemokines, establishing a link between the NVU and immunoregulatory processes. Given this information, astrocytes mediate several aspects of Aβ clearance and may reduce and/or contribute to AD pathology.

### 4.3 Choroid Plexus Removal of Aβ

The choroid plexus (CP) is a network of capillaries and cuboidal epithelial cells lining the four ventricles of the brain. As a lining of the ventricular system, the epithelial cells produce cerebrospinal fluid (CSF) and actively filter blood and plasma. Therefore, they serve as a secondary barrier, the blood-CSF barrier, paralleling that of the BBB. Fluid is filtered into and out of the CSF. Indeed, many of the tight junction proteins, zonula occludens (ZO-1), and Aβ clearance proteins are present, RAGE, LRP1, and gp-P (Gonzalez-Marrero et al. 2015). Choroid plexus maintains proper integrity, pressure, and composition of CSF through secretion of ions, nutrients, metabolic precursors, and proteins (Lun et al. 2016). Two proteins, transthyretin (TTR) and aquaporin-1 (AQP-1), are often used as benchmarks to gauge the health of CP. AQ1 is a transmembrane protein of the CP involved in transporting water from blood to CSF, while TTR is a transport protein involved in binding thyroxine and retinol-binding protein bound to retinol. TTR has also been found to bind Aβ and thus prohibiting the formation of Aβ fibrils. As such, TTR is an important indicator in the pathogenesis of AD.

Gonzalez-Marrero et al. investigated the effects of triple transgenic (3xTg-AD)-induced mice, harboring presenilin 1 (PS1/M146 V), APPSwe, and tauP301L on CP; these animal models mimic the critical hallmarks of AD as presenilin 1 mutants mimic overproduction of $A\beta_{42}$ through interaction with γ-secretase, APPSwe accelerates Aβ production because it is a transgene coding for a 695 amino acid isoform of Aβ, and tauP301L results are tau hyperphosphorylation. 3xTg-AD mice display behavior alterations, cognitive deficits, and accelerated Aβ plaques and NFT starting from 12 months (Gonzalez-Marrero et al. 2015). Their

results indicate 3xTg-AD mice exhibit age-related pathology at 16 months similar to AD (Gonzalez-Marrero et al. 2015). Additionally, they found more than twofold increases in $A\beta_{42}$ in the stroma and altered cellular distribution patterns as compared to non-Tg mice; 3xTg-AD showed uniform $A\beta_{42}$ throughout the CP, while controls displayed apical $A\beta_{42.}$ CP can receive up to 5–10 times more blood flow than other brain regions, and thus maintaining hemostasis and vascular perfusion is pertinent in assessing proper removal of Aβ. Further analysis revealed ~twofold higher collagen IV deposition around CP and blood vessel basement membranes (Gonzalez-Marrero et al. 2015). Collagen IV deposition increased BM thickness and is suggestive of altered BBB integrity. According to Fick's diffusion theory, a major factor affecting diffusion across biological membranes is membrane thickness (Gonzalez-Marrero et al. 2015). Consequently, reduced permeability, oxygenation, plasma ultrafiltration, and CSF formation become less efficient, consistent with most models of AD pathology (Gonzalez-Marrero et al. 2015). TTR and AQP-1 staining also revealed >threefold and 31% lower expression in 3xTg-AD as compared to controls, respectively. Increases in cellular and stroma $A\beta_{42}$ indicate impaired Aβ metabolism and clearance. AD patients show elevated levels of Aβ in plasma due to compromised binding to sLRP1. Elevated systemic levels of Aβ can reenter the brain and CP via RAGE-mediated transport; indeed, this study also found elevated cytoplasmic RAGE expression in CP, suggesting increases in Aβ upregulate RAGE (Gonzalez-Marrero et al. 2015).

### 4.4 Heavy Metal Toxicity

Heavy metal accumulation and toxicity appear to be major variables in the pathogenesis of Alzheimer's disease and disrupt clearance mechanisms of Aβ. Aluminum (Al) is one of the most abundant elements in the biosphere, and its industrial production and environmental prevalence makes it one of the most neurotoxic elements we are exposed to daily. Al's contribution to AD is based on at least seven independent-derived observations:

1. Al strongly promotes Aβ aggregation and accumulation, known as amyloid cascade.
2. In vitro and in vivo analyses display marked increases in pro-inflammatory transcription factor NF-kB.
3. mRNA and miRNA expression induced by Al is strikingly similar to that of AD.
4. Dietary consumption of Al induces lipid peroxidation, oxidative stress, apoptosis, and gene expression deficits.
5. Many deficits observed in AD are recapitulates in Al-treated cellular or animal models.
6. Numerous worldwide epidemiological studies correlate Al in drinking water as hydrated Al potassium sulfate, $KAl(SO_4)_2{\cdot}12H_2O$, to AD.
7. A significant number of pharmaceutical treatments rely on antioxidant and iron/aluminum chelation therapy (Bhattacharjee et al. 2013).

In fact, the scientific literature is replete with studies showing neurotoxic effects of Al.

The only oxidation state of aluminum is $Al^{3+}$, and it has a small ionic radius (50 pm) relative to zinc ($Zn^{2+}$; 74 pm), calcium ($Ca^{2+}$; 99 pm), and sodium ($Na^{+}$; 95 pm) which makes it a very effective cross-linker of proteins. Al-induced conformational changes have been demonstrated using circular dichroism (CD) spectroscopy (Kawahara and Kato-Negishi 2011; Exley et al. 1993). Within the amino acid sequence of Aβ, arginine ($Arg^5$), tyrosine ($Tyr^{10}$), and histidine ($His^{13}$) demonstrate metal-binding properties. This makes this particular amino acid sequence particularly susceptible to cross-linking. Studies have demonstrated that polymerization of Aβ occurs in the presence of Al and treatment with deferoxamine (DFO) results in dissolving of the network (Kawahara and Kato-Negishi 2011).

Al also affects iron (Fe) homeostasis as it has comparable properties. Binding of Al to ferritin, transferrin, iron regulatory protein (IRP), and/or iron-responsive elements (IRE) has profound implications in the production of free radicals via the increase in Fe concentrations. IRP is a cytoso-

lic protein which functions as a posttranscriptional switch for Fe homeostasis, binding to 5′ and 3′ untranslated regions of mRNA containing IREs. As a negative regulator, it inhibits the production of ferritin in Fe-depleted cells by blocking ferritin mRNA from translation within the ribosome. Conversely, the lack of IRP binding to mRNA untranslated regions results in endonuclease cleavage of the construct. Al has the ability to disrupt all pathways in iron homeostasis because, as mentioned previously, it can bind all relevant proteins in this pathway. Importantly, APP and ferritin contain an IRE within their mRNA, and Al has been shown to increase APP production in experimental animal models (Kawahara and Kato-Negishi 2011).

Several studies have exhibited increased Al deposition in the brain of AD patients, especially in areas of hippocampal formation and occipital lobes. Al >2.00 μg/g dry wt is considered pathologically concerning, while >3.00 μg/g dry wt is considered pathologically significant and thus will contribute to exacerbation of AD pathology, earlier onset, and more aggressive etiology (Mirza et al. 2017). In a recent study of 12 humans, 7 females and 5 males, autopsy-confirmed familial AD diagnosed patients, postmortem tissue samples were obtained from occipital, frontal, parietal, and temporal lobes. The samples were analyzesd for Al content using validated lumogallion and fluorescence microscopy. Eleven of 12 individuals had at least one tissue sample with a pathologically significant (>3.00 μg/g dry wt) content of Al (Mirza et al. 2017). The brains of nine individuals displayed Al content >5.00 μg/g dry wt, while five of these had at least one sample upward of >10.00 μg/g dry wt (Mirza et al. 2017). Furthermore, Al appears to show a differential deposition in arteries that supply the brain and CNS (Bhattacharjee et al. 2013). A postmortem, case-controlled study of AD patients unveiled a concentration gradient of Al while dissecting the celiac, femoral, aorta, vertebral, common carotid, internal carotid, basilar, middle cerebral, and posterior cerebral arteries (PCA). The PCA had a ninefold increase in Al, amounting to 54.2 μg/g dry wt (Bhattacharjee et al. 2013). The PCA provides major blood supply to the hippocampus, suggesting a possible correlation between increased Al affinity for the PCA and hippocampal dysfunction. The concentration gradient of Al may be related to its increased affinity for phosphates covalently linked to lipids lining the cerebral vasculature.

## 5 BBB Dysfunction and Impaired BBB Clearance

The BBB provides a physical separation of blood from components of the brain. However, dysfunction of the barrier is highly implicated in numerous neurodegenerative diseases, including autism, Parkinson's disease, dementia, multiple sclerosis, HIV-1 encephalitis, severe hypertension, AD, etc. (Haorah et al. 2007). Increasingly elevated levels of reactive oxygen species (ROS), impaired insulin resistance, and elevated cholesterol esters play important roles in BBB dysfunction.

### 5.1 ROS Exacerbate Alzheimer's Disease

ROS are natural byproducts of cellular metabolism. They are generated in response to mitochondria's production of adenosine triphosphate (ATP). During this process, hydrogen ions are pumped across the inner mitochondrial membrane. Lastly, oxygen is reduced to form water. This process inevitably produces small amounts of superoxide ($\cdot O_2^-$) which can react with water to form hydroperoxyl ($HO_2$) and hydroxide (OH). Subsequently, ROS can lead to lipid peroxidation, damage to local tissues and DNA, activation of apoptosis, and recruitment of immune cells to the site of inflammation. Therefore, they play a leading role in nearly all aspects of neurodegenerative diseases. They may act alone or in response to other factors and represent complex interactions of cellular metabolism in conjunction with their environment. Matrix metalloproteinases (MMPs) have been shown to be upregulated in response

to ROS. Specifically, ROS-mediated activation of MMP2 and MMP9 has demonstrated degradation of basement membrane proteins and subsequent loss of BBB integrity. Additionally, ROS-induced phosphorylation of tight junction proteins (claudin-5, occludin, ZO-1) via upregulation of protein tyrosine kinase (PTK) (with diminished protein tyrosine phosphatase) triggers leaky BBB. Furthermore, loss of BBB stability leads to infiltration of leukocytes and exacerbated brain inflammation. Fibrinogen, albumin, thrombin, hemoglobin, and immunoglobulins have been discovered in postmortem studies of AD and amyotrophic lateral sclerosis (ALS), exemplifying the pathological consequences of BBB disruption (Fig. 5).

## 5.2 Insulin Resistance and Its Role in Vascular Hemostasis

Vascular pathologies are believed to contribute significantly to the spectrum of neurodegenerative diseases, including dementia, mild cognitive impairment, AD, etc. Until recognition of the significance of neuritic plaques and neurofibrillary tangles (NFTs), vascular dysfunction was the prevailing view surrounding cognitive deficits in AD (Mullins et al. 2017). Insulin signaling has profound consequences on vascular hemostasis, BBB functionality, and AD exacerbation. Insulin can be produced de novo in the brain, but the general consensus is that insulin's actions are primarily pancreas-derived. Its vasodilation properties couple PI3K signaling to increased generation of nitric oxide (NO) in endothelial cells, a potent vasodilator. NO diffuses freely into nearby smooth muscle cells resulting in production of cyclic guanosine monophosphate (cGMP) and thus regulating vasoconstriction. Furthermore, NO inhibits platelet aggregation, monocyte adhesion, and thrombosis, all of which damage the vessel wall (Mullins et al. 2017). Microvascular disruption leads to production of ROS and potentiates overexpression of receptor for advanced glycation end products (RAGE), the endothelial receptor responsible for plasma Aβ transcytosis from the blood to the brain (Mullins et al. 2017).

Binding of insulin to brain endothelium elicits autophosphorylation of insulin receptor substrate-1 (IRS-1) and activation of several pathways, including PI3K/Akt, leading to membrane localization of glucose transporters (GLUTs), primarily GLUT 1 and GLUT 3 in the brain, and mediation of glycogen synthase kinase-3β (GSK-3β)-regulated hyperphosphorylation of tau proteins, a trademark of neurofibrillary tangles in AD (Mullins et al. 2017). A detailed analysis by Talbot et al. uncovered significant downregulation of insulin pathway IR → IRS-1 → PI3K and greatly reduced signaling in response to IGF-1 in the IGF-1R → IRS-2 → PI3K pathway of AD patients without diabetes relative to normal controls (2012). They found that IRS-1 and IRS-2 activation is primarily separate around physiologically relevant concentrations (~1 nM) and responds to insulin and IGF-1, respectively, but this response can crosstalk under conditions of hyperinsulinemia (~10 nM) or excessive IGF-1 (~10 nM) (Talbot et al. 2012). Additionally, modest downregulation occurred at the level of IR, while major reductions occurred downstream: 90% for IRS-1 phosphotyrosine, pY, (IRS-1 pY), 89% for Akt1 pS, 76% for GSK-3β $pY^{216}$, 83% for GSK-3β $pS^{9}$, 74% for mTOR $pS^{2448}$, and 90% for ERK2 $pT^{185}/pY^{187}$ (Talbot et al. 2012). Their analysis indicated this extensive downregulation must occur, not from IR inhibition but from bottom-up effects (Talbot et al. 2012). Consistently, they found excessive phosphorylation of IRS-1 at critical serine sites, S312, S616, and/or S636, resulting in suppression of IRS-1. Phosphorylation at these sites was also significantly correlated with basal activation of GSK-3, inhibitor of kappa B kinase (IKK), JNK, mTOR, and PKCζ/λ. IR-sensitizing drugs appear to ameliorate this inhibition and improve Aβ pathology (Talbot et al. 2012). In contrast to Chia et al., Talbot found overall suppression of GSK-3, refuting the hypothesis of GSK-3-mediated hyperphosphorylation of Tau protein; these conflicting results warrant more research. In conclusion, excessive phosphorylation of IRS-1 $pS^{616}$ had the largest and most negative effects

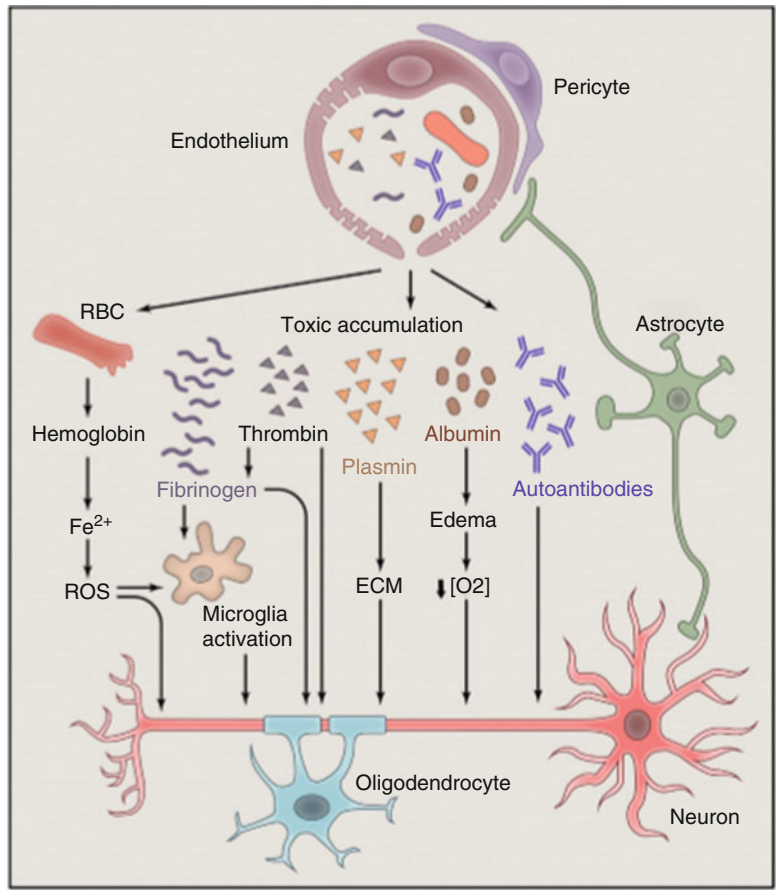



**Fig. 5** Effects of disrupted BBB and leakage of toxic blood components into the cerebrum Dysfunctional pericyte-endothelial and astrocyte-endothelial signaling results in localized breakdown of the BBB. As BBB becomes leaky, proteins from the blood escape into local tissues, triggering inflammation. $Fe^{2+}$ derived from red blood cells acts as a pro-oxidant and producer of ROS. Fibrinogen triggers activation of microglial cells, promoting neuroinflammation and demyelination. Additionally, fibrinogen, thrombin, and plasmin activate cleavage of extracellular matrix proteins and detachment of neurons, leading to cell death. Albumin results in hypoperfusion, hypoxia, and localized edema. Production of autoantibodies against neuron components such as myelin is possible via loss of immune privilege. Courtesy of Zhao et al. 2015b

on episodic memory score and appears to be a major factor in cognitive decline associated with AD (Talbot et al. 2012).

## 5.3 Cholesterol Effects on AD

Hypercholesterolemia and defective lipid metabolism have a strong link to AD. Grimm's work established a pertinent link between high cholesterol, activation of β and γ-secretase, and pathogenesis of AD (2008). They found inhibition of cholesterol through administering lovastatin, an inhibitor of HGM-CoA reductase, and extraction of cholesterol by methyl-β-cyclodextrin treatment reduces β and γ-secretase activities (Grimm et al. 2008), thereby reducing the intracellular concentrations of $Aβ_{40}$ and $Aβ_{42}$. Production of cholesterol begins with the addition of two molecules of acetyl-CoA to form acetoacetyl-CoA. Another reaction of acetoacetyl-CoA with acetyl-CoA produces 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), whereby HMG-CoA reductase

acts to form mevalonate. In fact, statins exert their cholesterol-lowering effects through inhibition of this enzyme, which is the rate-limiting step in the process. Through more than 20 enzymatic steps, cholesterol is produced. Cholesterol is used by every cell in the body where it is concentrated in lipid rafts within the cell membrane. It increases membrane packing by interacting with membrane phospholipids and, in doing so, maintains cell fluidity. Cholesterol also serves as a precursor for vitamin D, bile acids, and the biosynthesis of steroid hormones, including testosterone and estrogen. The brain houses approximately 25% total cholesterol and is predominantly found in the non-esterified form (Gamba et al. 2015). Because cholesterol and lipoproteins are unable to cross the BBB, neurons rely on astrocyte de novo synthesis and secretion of APOE-cholesterol complexes. Of note, 24-hydroxycholesterol (24-OH), an oxidation product of cholesterol, is secreted by the neuron and combines with nuclear liver X receptor (LXR), and they are secreted to neighboring astrocytes, where they facilitate transcription and production of APOE; APOE is the brain's principal cholesterol transport system (Gamba et al. 2015). In essence, neurons secrete 24-OH-LXR to astrocytes where it promotes production of APOE, and then astrocytes secrete the newly produced APOE-cholesterol complexes back to the neuron (Gamba et al. 2015).

APOE is an apolipoprotein in that its primary function is to transport lipids, fat, and cholesterol throughout lymphatic and circulatory systems. It is found on the surface of chylomicrons and intermediate density lipoproteins (IDLs). APOE is synthesized peripherally in the liver and by macrophages but also by astrocytes within the central nervous system.

Implications of APOE polymorphisms in AD are believed to be related to cholesterol transport. APOE has been shown to colocalize with cholesterol and Aβ fibrils in senile plaques. Of interest, the APOE4, but not APOE2 and APOE3 variant, may confer increased susceptibility to late-onset AD, synergizes with Aβ toxicity, and is more susceptible to cleavage than APOE3 (Gamba et al. 2015). In part, it is because of indirect activation of the proinflammatory CypA-NFkB matrix metalloproteinase 9 (MMP-9) pathway, which leads to breakdown of BBB through destruction of basement membrane and tight junctions (Gamba et al. 2015). As mentioned previously (Fig. 5), destruction of the BBB tight junctions can lead compromised blood flow and hypoxic conditions, accumulation of blood proteins and leukocytes, and further potentiate the inflammatory condition of the brain. According to the vascular two-hit hypothesis, an initial vascular insult to the brain (*hit 1*) elicited by hypoxia, hypoperfusion, or a disrupted BBB proceeds observed amyloid pathology in AD (Gamba et al. 2015). Accumulated Aβ (*hit 2*), predominantly an effect of faulty Aβ clearance in late-onset AD, now triggers a pathological cascade of neuronal injury, cognitive decline, and AD dementia (Gamba et al. 2015).

Several oxysterols have been implicated in Alzheimer's disease. Because oxysterols can cross the BBB, more than 20 different metabolites have been associated with AD, but two cholesterol oxidation products are highly implicated. 24-OH is produced almost exclusively in the brain by the enzyme cholesterol 24-hydroxylase (CYP46A1), and 27-hydroxy cholesterol (27-OH), to a lesser extent, is produced by the enzyme cholesterol 27-hydroxylase (CYP27A1). 27-OH is subsequently converted into 7α-hydroxy-3-oxo-4-cholestenoic acid (7-OH-4-C) by the enzyme CYP7B where it exits the brain via the BBB and gets excreted following processing by the liver. However, 27-OH can flow from systemic circulation to the brain, serving as a potential link between intracerebral and extracerebral pools of cholesterol, and may contribute to hypercholesterolemia in the brain (Gamba et al. 2015). 24-OH is the primary cholesterol metabolite of the brain and has been shown to promote brain protection through its efflux and inhibition of Aβ production. During early stages of the disease, higher levels of 24-OH have been found in the CSF and peripheral circulation as compared to healthy controls (Gamba et al. 2015). However, late stages of AD exemplify lower plasma levels of 24-OH,

suggesting decreased oxidation of cholesterol by the enzyme CYP46A1, loss of neurons and ongoing demyelinization, and/or reduced transport through the BBB (Gamba et al. 2015).

## 6 Conclusion

After more than 100 years since the first description, AD now affects about 30 million people worldwide. Age is a significant biological risk factor. While we are gaining knowledge of underlying genetic predispositions, molecular mechanisms, and potential etiologies of this complex disease, there is still much we do not know. Genetic predispositions contribute to approximately 1–10% of familial AD cases, while environmental factors appear to have the biggest impact. The two common hallmarks of AD, Aβ plaques and Tau NFTs, may be the result of upstream events, or they may be at the center of AD pathogenesis. With that being said, Aβ appears to have detrimental effects on many aspects of the disease, including loss of synaptic plasticity, neuronal death, compromised BBB integrity, increased expression of RAGE and implicated clearance by LRP1, increased leukocyte extravasation across inflamed endothelium, and elevated levels of oxidative stress leading to ROS and continuous neuroinflammation. Several clearance mechanisms exist involving enhanced metabolism and proteolytic cleavage of Aβ or clearance from the cortex to systemic circulation. As complications of BBB integrity arise from activation of MMP-2 and MMP-9, ROS, cholesterol metabolism, and impaired insulin signaling, amplified Aβ deposits in and around microvessels, known as cerebral amyloid angiopathy (CAA), become evident. As Aβ fibrils accumulate, neural and ISF accumulation results in a cascade of significant brain degeneration and atrophy of hippocampal and cortical structures.

As late-onset AD accounts for ~90% of cases, environmental effects and epigenetics are at the core of AD. As heavy metals, specifically Al, are known as neurotoxins implicated in neurodegenerative diseases, this calls into question their increasing presence in our environment. Al is present everywhere in the biosphere and yet has no biological role. Several studies have found increased deposition of Al in occipital, frontal, temporal, and parietal lobes of early- and late-onset AD. In fact, one study suggested it is the deposition of Al which leads to earlier onset and more aggressive etiology of AD (Mirza et al. 2017). Interestingly and importantly, Al deposition has been found in cerebral blood vessels supplying critical structures of the brain, including a ninefold increase in the PCA which immediately supplies blood to the hippocampus, a major area of the temporal lobe affected in AD. Alterations in endothelium have profound consequences on downstream blood flow, typically resulting in hypoxic conditions to the tissue. This may explain the substantial damages inflicted on the hippocampus.

Another confounding factor in AD is the increasing prevalence of insulin resistance. This may explain why diabetics are at much higher risk of developing AD. The rate of insulin transport across the BBB is slowed by obesity and aging. Additionally, high circulating levels of free fatty acids in the bloodstream promotes IR. As seen here, hyperphosphorylation of IRS-1 leads to diminished insulin signaling. This effect can be seen as downregulated GLUT receptors and aberrant endothelial NO responses. A disruption in endothelial-mediated NO signaling has grave consequences for cerebral blood flow. The parenchyma relies on adequate blood flow for oxygenation, enzymatic and cellular function, and retrieval of important nutrients, and without it, cells can undergo apoptosis or take on an inflammatory state. Adequate blood flow is also imperative for clearance of Aβ. Impaired perfusion combined with the neurotoxic effects of Al, Al deposition in the PCA, and augmented collagen thickening of the basement membrane create an environment for BBB dysfunction and pose significant challenges in clearance in Aβ. For this reason, AD is largely believed to be a vascular disease.

Atherosclerotic plaques have been observed in a significant number of clinical studies which led to initial hypotheses of AD being a vascular disease. As cholesterol is a primary component of

vascular plaques, it should come as no surprise that cholesterol is heavily implicated in AD. In fact, statins have shown some benefit in experimental studies. In senile plaques, APOE binds and colocalizes with cholesterol. The APOE ϵ4 variant is considered a substantial risk factor in AD. Given the presence of oxysterol metabolism within the brain, their transport to and from the brain, and implications in AD, further studies are warranted to decipher the exact role they play in the disease.

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