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REVIEW

Neuroinflammation, immune system and Alzheimer disease: searching for the missing link

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Abstract Due to an increasingly aging population, Alzheimer disease (AD) represents a crucial issue for the healthcare system because of its widespread prevalence and the burden of its care needs. Several hypotheses on AD pathogenesis have been proposed and current therapeutical strategies have shown limited effectiveness. In the last decade, more evidence has supported a role for neuroinflammation and immune system dysregulation in AD. It remains unclear whether astrocytes, microglia and immune cells influence disease onset, progression or both. Amyloid-β peptides that aggregate extracellularly in the typical neuritic plaques generate a constant inflammatory environment. This causes a prolonged activation of microglial and astroglial cells that potentiate neuronal damage and provoke the alteration of the blood brain barrier (BBB), damaging the permeability of blood vessels. Recent data support the role of the BBB as a link between neuroinflammation, the immune system and AD. Hence, a thorough investigation of the neuroinflammatory and immune system pathways that impact neurodegeneration and novel exciting findings such as microglia-derived microvesicles, inflammasomes and signalosomes will ultimately enhance our understanding of the pathological process. Eventually, we should proceed with caution in defining a causal or consequential role of neuroinflammation in AD, but rather focus on identifying its exact pathological contribution.

Keywords Alzheimer disease · Neuroinflammation · Blood brain barrier · Inflammasome · Signalosome · Derived-microglia microvesicles

Introduction

Dementia is a clinical syndrome characterized by the development of multiple cognitive deficits that are severe enough to interfere with daily functioning, including social and professional capacity. Cognitive deficits include memory impairment and at least one of other cognitive domains, such as aphasia, apraxia, agnosia or disturbances in executive functioning [1].

Alzheimer disease (AD) is the most common cause of dementia in the elderly, accounting for 60–70 % of all cases [2]. The global trend in the phenomenon of population aging has a dramatic impact on public health and healthcare costs throughout the world. Due to the aging of the population, dementia has become a major challenge to elderly care and public health [3].

AD is strictly a neuropathological diagnosis determined by the presence of neurofibrillary tangles (NFT) and senile plaques in the brain of patients with dementia. The concept of linking AD to neuroinflammation emerged in the early 90s when it became evident that an altered immune response observed in AD is hard to overlook [4]. Afterward, abundant evidence was found for a causal role for cerebral immunological processes in the pathology of AD. Neuroinflammation is primarily driven by microglia, perivascular myeloid cells and astrocytes, acting as triggers

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for AD pathogenesis either independently or in combination with amyloid [5].

In AD, glial cell activation leads to impaired regulation, resulting in increased cytotoxicity and decreased microglial defensive functions. Impaired activation results in oxidative stress, neuroinflammation and neuronal dysfunction, all of which induce production and aggregation of amyloid-β (Aβ) and additional neuronal dysfunction [6]. Inflammatory activation and glial cell dysregulation could be secondary to several stimuli or injuries throughout life, as well as natural aging [7]. In the microglia of aged mice, an increased expression of cytokines and an exacerbated inflammatory response to pathological changes can occur [8]. Furthermore, during aging, accumulation of oxidative damage to DNA, telomere shortening and decreased telomerase activity have been observed in the mitochondria of microglia [9]. An increased reactive oxygen species (ROS) production has been observed in aged rodents [10] Mitochondria-derived ROS determines increased production of the inflammatory cytokine IL-1β by microglial cells [11].

As a matter of fact, in both aging and neurodegenerative diseases, neuroinflammation and chronic dysregulated microglial activation appear to lead to deleterious effects inducing malfunction and damage to the brain.

Altered immune response and neuroinflammation in Alzheimer disease

Neuroinflammation is an inflammatory response that takes place within the central nervous system (CNS) during a neurodegenerative process or following a neuronal injury. The main effectors of neuroinflammation, which are astrocytes, microglia, innate and adaptive immune cells converge in a context- and time-dependent manner either with neuroprotective or with neurotoxic effects. It has now become evident that neuroinflammation is a prominent pathological hallmark of several neurodegenerative diseases including AD, Parkinson's disease and Amyotrophic Lateral Sclerosis [12]. Under normal and healthy conditions, astrocytes, which are the most abundant cell type within the CNS, are typically found in a resting state. Activation of astrocytes follows an acute or chronic injury, where the cells adopt a different morphology, become proliferative, express the intermediate filament and glial fibrillary acidic proteins releasing pro-inflammatory cytokines, growth factors as well as producing nitric oxide (NO) [13].

Neuropathologically, AD brains are characterized by massive accumulation of NFTs. NFTs are composed by paired helical filaments (PHFs), of which the main constituent is the tau protein. Other hallmark lesions of AD are neuritic plaques (NPs), constituted by extracellular amyloid peptide aggregates, which are also associated with dystrophic neurites [14]. Amyloid precursor protein (APP) processing occurs via two pathways: (a) the amyloidogenic and (b) the non-amyloidogenic pathway. In the amyloidogenic pathway, the APP is proteolyzed by β-secretase. This truncation occurs at the N-terminus of APP. This cleavage produces a soluble N-terminal fragment of APP (sAPPβ) and a C-terminal transmembrane fragment (β-CTF). β-CTF is inserted into the membrane by γ-secretase and generates the Aβ peptide and the APP intracellular domain. Depending on the site of γ-secretase cleavage, two main species of Aβ are generated: Aβ 40 and Aβ 42 aminoacids. Aβ 42 is more hydrophobic and more prone to aggregate compared to the Aβ 40 peptide. In the non-amyloidogenic pathway, APP is cleaved by the α-secretase. This cleavage occurs in the middle region of Aβ and produces a N-terminal fragment of soluble APPα (sAPPα) and a C-terminal transmembrane (α-CTF) fragment. The sAPPα hinders both neurotrophic and neuroprotective functions. Similarly, to β-CTF, α-CTF generates a 23–25 aminoacid peptide designated as p3 after being cleaved by γ-secretase. Aβ aggregates promote an inflammatory response mediated by activated microglia and astrocytes that may arouse altered signaling pathways, leading to neurodegeneration. Long-term activation of the innate immune system is able to trigger an inflammatory cascade that converges on alterations of the cytoskeleton (including aggregation of tau protein and formation of PHFs and microtubular disassembling), thus promoting neuronal degeneration [15] (Fig. 1).

The Aβ itself causes activation of microglia and astrocytes through Toll-like Receptors 2, 4, 9 (TLRs); activated microglia produce neurotoxic molecules and are conveniently located to the vicinity of the NPs. The proinflammatory cascade generated by microglial activation results in the release of cytotoxic molecules such as cytokines, chemokines, matrix metalloproteinases and complement factors. These cytotoxic molecules may enhance neuronal neurodegeneration by increasing sensitivity to free radicals. The neurotoxicity mediated by microglial cells depends on ROS and cytokines.

Dysfunction of the blood brain barrier (BBB) has been proposed to represent a possible link between the immune system and AD. The brain is an immune-privileged organ isolated from self-immune reaction by BBB, whose function is to prevent macromolecules from reaching brain tissue. The integrity and the permeability of BBB could be altered by multiple microtraumas, microvascular pathologies and inflammation, thus leading to the abolition of the immunological CNS privilege [16, 17].

In addition to the mechanisms involved in neuroinflammation, we will discuss how the accumulation of Aβ in blood

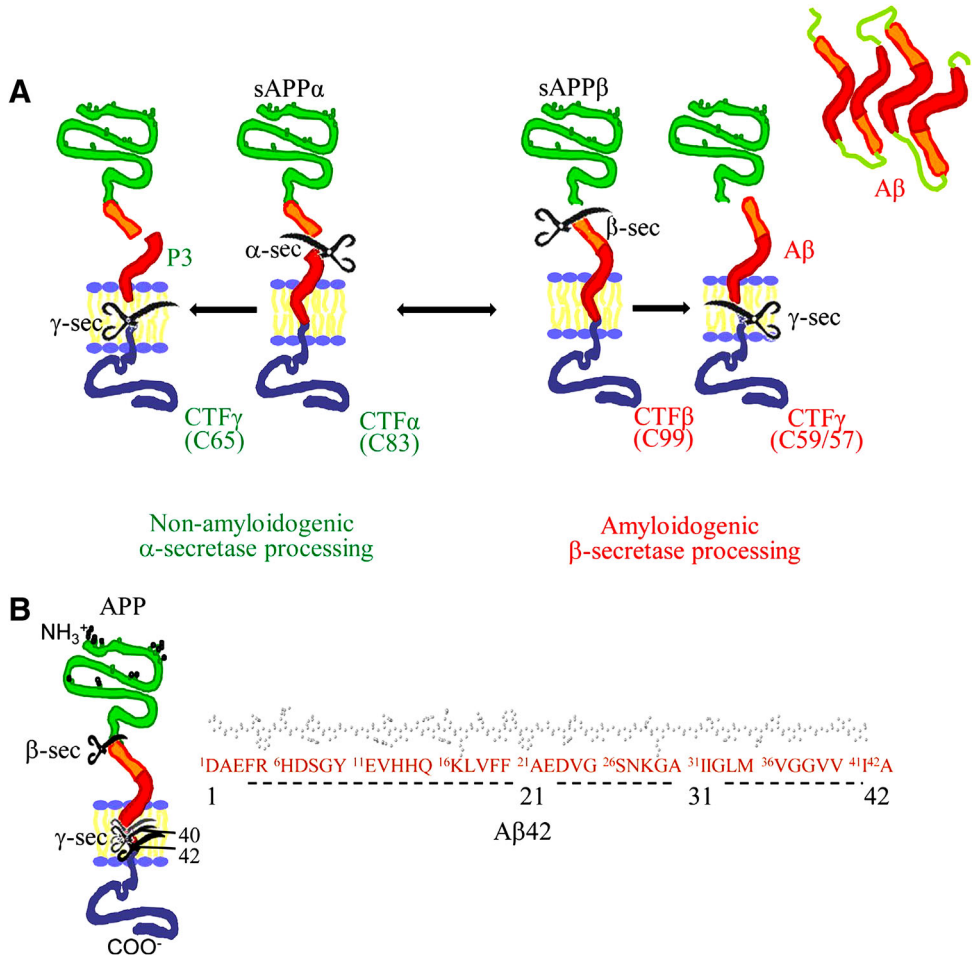

Fig. 1 **a** Aβ peptide is the cleavage product of a type I membrane glycoprotein; β-amyloid precursor protein (β-APP). APP can be cleaved alternatively by two secretases leading to a different pathway each. Cleavage by α-secretase results in the production of soluble APP and CTFα (C83). CTFα is then cleaved by γ-secretase enzyme to produce P3 and CTFγ (C65) or AICD in a pathway called non-amyloidogenic processing of APP as it precludes the generation of amyloidogenic Aβ-peptide. Alternatively APP cleavage by β-secretase to produce CTFβstub (C99), which is then acted upon by γ-secretase to produce CTFγ (C59/57) or AICD and amyloidogenic Aβ is called amyloidogenic APP processing. According to several hypotheses that put major impetus on Aβ as the sole trigger of AD pathogenesis, once starts accumulating then Aβ leads to the full disease cascade. **b** Depending on membrane bilayer thickness and spatial placing of γ-secretases, APP can be cleaved either into less amyloidogenic Aβ40 or more amyloidogenic Aβ42

vessels and neuroinflammation affect the BBB tight junctions of endothelial cells, modifying their permeability and concomitant implications in AD pathology. Finally, we will discuss about the most recent developments in the field, such as the role of microglia-derived microvesicles, signalosomes and inflammasomes toward AD pathogenesis.

The blood brain barrier and T lymphocytes: a possible link between neuroinflammation and Alzheimer disease

The barriers of the CNS are cellular interfaces shielding the vulnerable homeostasis of the brain or spinal cord parenchyma from the dynamic environment in the trunk and peripheries. The largest brain barrier surface area is formed by endothelial cells of the parenchymal CNS capillaries referred to as the BBB. Further endothelial CNS barriers are formed by the pial vessels of the meninges and the inner retinal vasculature. The endothelial BBB establishes a physical barrier by inhibiting a paracellular diffusion of water-soluble molecules via complex tight junctions interconnecting brain microvascular endothelial cells and by limiting transcellular diffusion due to a low pinocytotic activity of the brain microvascular endothelium. The CNS strictly controls immune cell entry across its barriers and localizes its immunosurveillance under physiological conditions in perivascular and subarachnoidal spaces, leaving the CNS parenchyma untouched [18].

Blood born T lymphocytes migrate into non-inflamed tissues to perform immune surveillance and into inflamed tissues to deliver effector functions. In both cases,

lymphocyte migration is controlled at two critical steps: (1) the arrest and firm adhesion of circulating cells on endothelial surfaces; (2) the migration through the endothelium into the interstitium and, from there, to tissue microenvironments [19].

The BBB had been considered as a strong barrier to immune cells until seminal studies demonstrated T cell extravasation across the BBB. In vivo imaging experiments performed on laboratory mice and rats established that initial contact formation between T cells and the healthy BBB of the spinal chord or the non-inflamed blood retina occur in the eye. In contrast to the peripheral tissues, where reaching the endothelial layer suffices to reach the tissue parenchyma, in the CNS the T cells reaching the CSF-drained perivascular or leptomeningeal spaces are tightly sealed off from the CNS parenchyma by the glia limitans. T cell entry into the CNS parenchyma involves, therefore, two differently regulated steps: (1) the multistep transmigration of T cells across the BBB endothelial wall into the perivascular or leptomeningeal spaces; (2) the progression across the glia limitans into the CNS parenchyma. To extravasate from the blood stream into CNS parenchyma, T cells must enter into intimate contact with the BBB. Adhesive interactions between the T cells and the luminal surface of the blood vessels must be strong enough to resist shear forces exerted by the blood flow [20].

Three distinct protein families of adhesion molecules fulfill primary roles in the process of T cell extravasation across an endothelial monolayer, namely selectins, integrins and the cell adhesion molecules of the Ig superfamily (VCAMs) [21].

Molecular mechanisms of initial contact formation of T cells with the inflamed BBB are different from those at the non-inflamed BBB since, in addition to T cell capture, T cell rolling is specifically observed under inflammatory conditions of the CNS. T cell capture to the BBB in the absence of neuroinflammation is mediated by alpha4-integrin/VCAM-1 interactions, whereas during inflammation alpha4-integrins are no longer required for the initial contact of T cells mediated by T cell rolling and capture with the inflammed BBB. In addition to alpha4 integrin/VCAM-1 interaction, LFA-1 interaction with endothelial ICAM-1 contributes to the shear resistant arrest of encephalitogenic T cells to the inflammed BBB [22].

Diapedesis of T cells across the BBB under physiological flow in vitro can still be observed in the absence of ICAM-1 and ICAM-2, albeit at a very low frequency, which suggests additional molecules are involved in this process. This is also supported by the observation that ICAM-1-null mice succumb to experimental autoimmune encephalomyelitis [23].

Once the T cell has performed an initial contact with the endothelial surface, its interaction strength with the endothelial surface must be increased for sustained blood flow shear resistance and subsequent extravasation. This is achieved via a very fast process (in the range of milliseconds) by changing the binding affinity of T cell expressed integrins to their respective endothelial ICAM ligands from a low-affinity to a high affinity conformation. This rapid signaling is induced by the binding of chemokines, displayed on the luminal surface of the endothelial cells, to chemokine receptors present on the T cell. The luminal surface of the endothelial cells is protected by the endothelial glycocalyx, a network of negatively charged membrane-bound proteoglycans and glycoproteins. Proper functioning of chemokines in mediating leukocyte arrest has been shown to be dependent on chemokine immobilization on heparan sulfate glycosaminoglycans of the endothelial glycocalyx. Receptors for chemokines belong to the protein family of G-protein-coupled receptors. Once a chemokine engages its respective chemokine receptor in the T cell this induces a G-protein-dependent signaling cascade in the T cell affecting cytoskeletal dynamics and integrin conformation [24].

Immediately upon arrest, T cells undergo changes to their shape and spreading of their cell body toward a polarized morphology displaying a broad lamellipodium at the front and a projected uropod at the rear. Spreading and polarization of the encephalitogenic T cells is a rapid process that is completed within only 3 min. The crawling of T cells is directed against blood flow (a phenomenon never observed on non-BBB endothelial cells in vitro or in the peripheral vasculature in vivo). Taken together, the T cell adhesion steps of multi-step T cell extravasation cascade combine three sequential events, namely (1) shear resistant arrest of T cells, (2) T cell spreading/polarization and (3) T cell crawling against shear [25]. These distinct steps might be individually regulated by different adhesion and signaling molecules. ICAM-1 and VCAM-1 have redundant roles in mediating shear resistant arrest of encephalitogenic T cells to the BBB endothelial cells. In fact, only in the functional absence of both ICAM-1 and V-CAM 1 does complete abrogation of T cell arrest on the BBB occur. Interestingly, spreading, polarization and subsequent crawling of encephalitogenic effector/memory T cells on stimulated brain microvascular endothelial cells requires endothelial ICAM-1 or ICAM-2. In the absence of ICAM-1 and ICAM-2 T cells completely lose their ability to crawl against the flow, demonstrating at the same time that endothelial VCAM-1 is not able to support T cell crawling against shear on the BBB [26].

Whereas mechanosensing of flow occurs at the level of the T cells, the remarkably long distances of up to several hundred micrometers which T cells have to crawl on the BBB before finding an appropriate spot for diapedesis is a unique characteristic defined by the highly specialized

BBB endothelium. It seems that molecular cues for T cell diapedesis are sparse in the BBB leading to T cell polarization and subsequent crawling against the flow in search of sites permissive for migrating across the brain endothelium, as shown by the extremely long crawling distances demonstrated in the in vivo imaging study. Diapedesis is the last step of the multistep extravasation cascade of T cells across the endothelial layer of the microvessel. Two alternative pathways across the endothelium exist: the paracellular route through the endothelial cell–cell junctions and the transcellular route directly through the endothelial cell body. At the BBB the high complexity of endothelial tight junctions would require a highly coordinated opening and resealing of the complex BBB tight for diapedesis of T cell. Since such a process seems difficult to achieve in the paracellular pathway, the transcellular pathway of T cell diapedesis across the BBB might be the preferred route [27].

Transcellular diapedesis of T cells across an endothelial monolayer is a direct consequence of the dynamic formation and retraction of T cell protrusions—so-called invadosome-like protrusions (ILP)—during crawling. IL-2 stimulates human T cells, which cross the endothelium via the transcellular route using an endothelial pore with a diameter of 4–5 microns. The essential requirements of ILPs for transcellular diapedesis are demonstrated by biochemical inhibition of the formation of ILPs by the crawling T cells through an Src-kinase inhibitor, which results in selective inhibition of transcellular diapedesis [28].

The inflamed endothelial cells actively contribute to pore formation by attracting ILP or IL-2 activated effector/memory T cells through intracellular chemokine vesicles and membrane delivery and fusion mechanisms required for pore formation. Finally, transcellular diapedesis for T cells across the endothelium might still involve junctional molecules. For example, blocking the endothelial cell–cell adhesion molecule PECAM-1 interferes not only with paracellular diapedesis, but also with transcellular diapedesis. Thus, diapedesis of effector/memory T cells across BBB seems to require molecular mechanisms that are distinct from those involved in neutrophil diapedesis across the endothelium elsewhere [29].

The BBB has the unique ability to control a vast amount of specific and directed vesicle fusion and transport mechanism involved in the receptor mediated or adsorptive-mediated transcytosis of nutrients. Moreover, during inflammatory conditions endothelial cells of the BBB have an increased number of intracellular vesicles, which are indeed able to form elongated transendothelial channel-like structures. Thus, BBB endothelium does harbor the molecular players required for the transcellular diapedesis of T cells. The signals that might favor T cell diapedesis across the BBB via transendothelial pores over the paracellular pathway through the complex BBB tight junctions require further study.

Blood brain barrier permeability, T cell trafficking and Toll-like receptors in Alzheimer disease

Macrophages and microglia are the innate immune cells responsible for clearance of pathogens and waste products. It has been shown that peripheral blood mononuclear cells (PBMCs) and macrophages of AD patients cross the BBB, but are defective in the clearance of Aβ in neuritic plaques [30]. On the other hand, it is known that regulatory T lymphocytes (Treg) play a fundamental role in modulating the relative balance between inflammation and immune tolerance, and alterations of these cells are observed in inflammatory diseases. To better characterize the neuroinflammatory processes suggested to be associated with AD and to clarify the possible role of Treg cells in this process, Saresella and colleagues extensively analyzed these cells (CD4+ CD25highFoxp3+) in patients with either severe AD ($n = 25$) or mild cognitive impairment (MCI) ($n = 25$), comparing the results with those of two groups of healthy controls (HC) ($n = 55$) [31]. The working hypothesis is that if inflammation is a negative factor for AD, and if Treg cells play a beneficial anti-inflammatory role in the attempt to control such inflammation, it should be expected that such cells would be quantitatively and qualitatively impaired in AD compared to mild cognitive impaired patients. Results showed that the development of AD is associated with lower quantities of circulating Treg lymphocytes and, in particular, with reduced percentages of PD1neg Treg cells. These quantitative alterations are associated with qualitative changes, summarized as an increased Aβ-specific proliferation and a reduced ability of Treg to suppress such proliferation. These results, together with the preliminary observation that the lowest percentages of all subpopulations of Treg cells are seen in patients with severe AD, lend support to the inflammatory origin of AD and suggest that alterations in Treg lymphocytes may play a pivotal role in the inflammation associated with AD. In addition, it has been found that frequency, phenotypic characteristics and number/function of Tregs are significantly altered with aging [32]. The impaired condition of CD4+ T cells, so-called immunosenescence, renders transplant recipients less responsive to an allogeneic kidney grafts and all these changes contribute to the aging-related decline of immune responses and subsequently the higher risk of immune-mediated diseases, cancers and infections in aged individuals. These dysfunctions arise from alterations in each component of the immune system, but the most consistent and significant alterations are seen

in the T lymphocyte compartments, particularly within CD4+ CD25+ regulatory T cells. Further studies in this field evaluate the T cell subpopulation through the Aβ-specific cell infusate technique [33]. They point out dramatic alterations in naive and memory subsets of CD4+ cells in patients with mild AD, with greatly decreased percentages of naive cells, elevated memory cells and increased proportions of CD4+ but not CD8+ cells lacking the important co-stimulatory receptor CD28. Together, these data provide stronger evidence than hitherto presented for more highly differentiated CD4+ as well as CD8+ T cells in AD patients, consistent with an adaptive immune system undergoing persistent antigenic challenge and possibly manifesting dysregulation as a result.

Another relevant field of research could be directed toward Toll-like receptors (TLRs), which could represent an attractive therapeutic target for numerous CNS disorders as well as infectious diseases. TLRs are a family of PRRs characterized by an extracellular leucine-rich repeat domain and an intracellular Toll/IL-1 receptor domain. In mammals there are at least 10 TLRs; despite a high degree of structural similarity and each receptor has a distinct function in innate immune recognition. They are crucial for macrophage function and play an important role in the detection of non-self by the innate immune system [34]. Thus, a defective TLR system is anticipated to have far reaching consequences for abnormal Aβ phagocytosis as well as other functions in the field of innate immunity. Activation of TLRs results in many functional outcomes, including enhancement of apoptosis, secretion of inflammatory cytokines and direct antimicrobial activity [35]. Expression of TLR genes in response to Aβ stimulation has been examined using PBMCs of AD patients and control subjects. A striking difference is observed in TLR mRNA levels between PBMCs from control individuals and AD patients stimulated with Aβ. Whereas control PBMCs exposed to Aβ up-regulate TLRs, transcription of TLR1, TLR2, TLR3, TLR5, TLR8 and TLR10 are significantly down-regulated in similarly treated AD cells. It is possible that the lower expression levels of TLR genes in AD macrophages may be indicative of a more global innate immune defect beyond Aβ phagocytosis. The imbalance between Aβ production and clearance greatly influences AD pathogenesis and since neural loss is a prominent feature in AD pathology further investigations should be required in this field of research.

Neuroinflammation and peripheral inflammatory factors

Peripheral inflammation has been recognized as capable of clinically exacerbating neurological disorders [36]. In this regard, experimental studies have shown that peripheral inflammatory stimuli, such as lipopolysaccharides (LPS), cause a profound immunological response in the brain resulting in microglial activation. Interestingly, in animal models treated with systemic infusion of Gram-negative bacterial endotoxins, LPS-induced peripheral inflammation determines an inflammatory response in the hippocampus similar to that generated in the periphery. This is due to activation of microglia by increasing of systemic pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α [37]. Notably, injection of a COX-2 inhibitor significantly diminishes neuroinflammation in the hippocampus suggesting that peripheral inflammation triggers a COX-2-dependent mechanism to induce neuroinflammation in the CNS [38].

Several mechanisms have been proposed to play a role in this process. Microglia may be activated through communication by the vagal afferents [39], by active BBB transport of pro-inflammatory chemokines and cytokines, by passive transport of interleukins via the circumventricular organs [40], or by potential disruption of BBB [41].

Of note, microglial activation is associated with up-regulation of TLRs. In fact, when microglial activation is not observed, TLR expression is similar to the control group [42]. Microglia activation does not occur in TLR receptor knocked-out mice [43]. As a matter of fact, TLRs seem to play a role in this process.

Remarkably, systemic LPS challenge causes a microglial hyperactivity in the brain of aged mice, associated with higher induction of inflammatory IL-1β [44]. Moreover, microglial activation in middle-aged rodents has been associated with neurocognitive deficits [45].

These data suggest that aging occurs in the brain as microglial senescence, rendering microglia to function abnormally and eventually contributing to neurodegeneration.

Role of microglia-derived micro-vesicles in brain inflammation

Microvesicles (MVs) are small vesicles, which arise directly from the plasma membrane and are released into the extracellular environment upon cell activation. They are emerging as a novel means of cell-to-cell communication and MVs shed from the cell surface of microglia seem to play a key role in inflammatory and degenerative brain pathologies [46], in particular in AD where activated microglia constantly surround amyloid deposits [47]. The primary function of microglia is to maintain brain tissue homeostasis, to provide the first line of defense during infection or brain injury and to promote tissue repair. In response to several signals (i.e., cytokines, apoptosis, exogenous viral factors and bacteria) microglia undergo

several levels of activation and migrate to the specific sites to eliminate pathogens or to phagocytose dead cells and protein aggregates. MVs are shed by microglia upon ATP activation [48] and originate from lipid rafts, where the ATP-receptor $P2X_7$ is localized [49]. Microglia-derived MVs contain various bioactive molecules, which modulate neuron functionality and the activity of surrounding non-neuronal cells. MVs contain pro-inflammatory signals (i.e., IL-1β, proteases and MHCII) acting as amplifiers of inflammatory signals between glial cells and stimulate excitatory neurotransmission [50]. Consistent with a pro-inflammatory role of MVs shed upon $P2X_7$-receptor activation, microglia-derived MVs determine, in recipients, microglia expression of inflammatory genes in a dose-dependent manner upon MV exposure [51].

MVs of microglial origin have been detected in the cerebrospinal fluids of mice and their concentration increases in the course [52] of experimental autoimmune encephalomyelitis (EAE). Moreover, it has been observed that injection of MVs into the brain of rodents with subclinical EAE recruits inflammatory cells at the site of delivery, while genetically modified mice with impaired MVs production are largely protected from EAE [51].

Remarkably, the production of MVs is extremely high in patients with AD and that microglial MVs, either shed in vitro or isolated from cerebrospinal fluid of AD patients, promote generation of soluble neurotoxic amyloid-β species, thereby acting as potent drivers of neuronal damage. The production of MVs is very high in patients with prodromal dementia and AD, reflecting microgliosis, which typically characterizes both diseases [51].

All together these data clearly identify a correlation between microglia-derived MVs and brain damage, suggesting that microglia-derived MVs act as amplifying agents of inflammation.

Inflammasomes and signalosomes roles in Alzheimer disease

Inflammasomes

In recent years, our understanding of the innate immune system has made great progress and one of the most important achievements is certainly the molecular characterization of the inflammasome. The term inflammasome is used to denote an intracellular protein complex which plays a key role in the regulation of programmed cell death, differentiation and cell proliferation through the activation of the inflammatory caspases-1 and -11 [53, 54].

Therefore, the inflammasome plays a function similar to the apoptosome, but while the apoptosome leads to the activation of apoptotic cascades, the inflammasome activates inflammatory cascades. From a structural point of view the inflammasome is a platform of proteins that, assembled together, leads to the activation of a cascade of defensive reactions: once the inflammasome is activated, this activates other defense proteins such as interleukins inducing an inflammatory process. Inflammasomes have been characterized in a numerous variety of cells, although the focus has been mainly on epithelial cells in mucosal surfaces and immune cells of the myeloid lineage [55].

Inflammasome activation contributes significantly to host and inflammatory responses but the association of gain-of-function mutations in inflammasome genes and autoinflammatory disorders illustrates that excessive inflammasome activity can be harmful. Polymorphisms in inflammasome genes have been shown to be linked to common autoimmune diseases. The study of inflammasome signaling at the molecular level recently allowed the recognition of different inflammasome subtypes and a better understanding of their functions and of the events that regulate their activity. This includes modulation by microbial agents and their connection with human autoinflammatory, autoimmune and infectious diseases. In particular, studies on IL-1β and inflammatory caspases suggest the role of these inflammatory mediators in pathways and pathologies that may involve inflammasome activation, including neurodegenerative disorders, cancer and fertility-associated conditions [56].

A specific inflammasome named NLRP3 may play a significant role in AD pathogenesis. NLRP3 can be activated by numerous stimuli, which lead to its oligomerization. For example, a low intracellular potassium concentration is shown to induce or increase NLRP3 activity [57]. Furthermore, various pathogens are shown to induce NLRP3 such as influenza A and Neisseria gonorrhoeae [58]. Interestingly, various endogenous molecules such as cholesterol crystals and monosodium urate crystals also increase NLRP3 activity and consequently IL-1β production [59].

The phagocytosis of the fibrillar peptide Aβ, which has a key role in the pathogenesis of AD, has been found to activate the NLRP3 inflammasome, and thus the release of IL-1β, which in turn is demonstrated to be able to promote the recruitment of microglia toward exogenous Aβ in the brain, with the lysosomal damage and the release of cathepsin B leading to inflammation and neurodegeneration [60].

A relationship between the NLRP3 inflammasome and AD onset has been shown in a recent work by Heneka in APP/PS1 mice (transgenic mice developing chronic brain overload of amyloid-β) with NLRP3 and caspase-1 deficiency. These mice have reduced AD-related pathogenesis, as reflected by reduced chronic Aβ deposition and neuronal inflammation. NLRP3 inflammasome deficiency alter

microglial cells to a M2 phenotype—characterized by overexpression of arginase-1 and IL-4)—lowering the brain deposition of amyloid-β and enhancing tissue remodellind in AD mice [61].

These data suggest a key role of IL-1β and NALP3 inflammasome activation in the developing of AD and, even if there is still no evidence at this time, blocking this mechanism could be an interesting future therapeutic target.

Signalosomes

Signalosomes can be considered as signaling platforms constituted by complex lipids and proteins that may interact producing various physiological responses when activated by different extracellular stimuli. Neuronal signal transduction begins at the plasma membrane by the interactions of proteins and lipids essential for the normal cellular communication and activities. Defects in these events may determine cognitive impairments. Age-related cognitive impairment, as well as the pathological cognitive decline, is strictly related to modifications in the normal intracellular signaling cascades, which lead to defects in cellular communication and alteration of the normal neuronal activity. Indeed, lipid rafts may be considered as dynamic entities in which numerous molecules can move and interact with different kinetics in response to a variety of ligands that may ultimately favor specific protein interactions and signaling cascade activation [62].

One of the most interesting functions of signalosome is certainly the role that they could play in neuroprotection. In particular, a specific signalosome, formed by the interaction of the estrogen receptor (ER) with scaffolding caveolin-1 and a voltage-dependent anion channel (VDAC) would seem to be involved in neuroprotection against AD modulating a porin involved in Aβ induced toxicity. The importance of estrogen in modulating brain development and preservation is well known [63] and the ER may play a key role in neuroprotection through the activation of the MAPK signaling pathway and thereby reduce amyloid-beta induced neurotoxicity [64]. The interaction of ER with neuronal lipid rafts and VDAC is important to stabilize this complex and to ensure its neuroprotective function [65].

In AD the function of this signalosome seems to be compromised, due to alteration of the lipid rafts or proteins that constitute the signalosome complex and this may play a significant role in neurodegeneration. Although some of the components of these platforms are starting to be identified, the complete picture is surely more complex, involving alterations in different signaling markers whose adverse and/or beneficial consequences remain to be clarified. An example of this is the identified interaction of ER, known to develop neuroprotective actions, and VDAC, recently identified as a contributor to Aβ neurotoxicity, both of which are assembled in a single multimolecular signaling complex in neuronal lipid rafts. There is no doubt that other, as yet unidentified, components of this signaling complex remain to be characterized. Furthermore, the altered composition of different lipid classes integrated in these membrane compartments appear to contribute to impaired signaling mechanisms and may underlie some neuropathological parameters [25]. Therefore, identification of important lipid markers and their interactions with target signaling proteins in lipid rafts are an important challenge to understand the mechanisms involved in neuronal pathophysiologies and will likely favor the development of new methods for early diagnosis or screening and of novel therapies against AD.

The role of estrogens in Alzheimer disease

Estrogens have neuroprotective cellular effects, which may contribute to their clinical benefits in delaying the onset and the development of AD. Animal studies have shown positive effects after estrogen treatment. It has been shown that a treatment with estrogens in rodents with mutations in APP decreases amyloid-β levels and thus its aggregation into plaques [66]. In rodents and cell cultures estradiol reduces the formation of Aβ [67] and tau hyperphosphorylation [68]. Estrogen exerts its action promoting the α-secretase non-amyloidogenic pathway of extracellular-regulated kinase 1 and 2 (ERK 1 and 2) and through the protein kinase C signalling pathway [69].

At the cellular level, estrogen binds to nuclear α and β receptor (ER α/β), and acts as a transcription factor. It enhances the expression of anti-apoptotic proteins—such as Bcl-2 and Bcl-xL—and down-regulates the expression of Bim, a pro-apoptotic factor, inhibiting apoptosis [70]. It has been reported that estrogen significantly increases the expression of the antiapoptotic protein Bcl-xL in cultured hippocampal neurons [71]. Estrogen-induced enhancement of Bcl-xL is associated with a reduction in Aβ-induced apoptosis, including inhibition of both caspase-mediated proteolysis and neurotoxicity [71].

Another protective mechanism against apoptosis modulated by estrogens is the activation of antioxidant defense systems by up-regulating the expression of specific enzymes (i.e., manganese superoxide dismutase and glutathione peroxidase) [72] Thus, estrogens have direct antioxidant effects increasing reduced glutathione levels and decreasing oxidative DNA damage in mitochondria [73].

Recent studies have observed that estradiol could enhance neurogenesis within the dentate gyrus of the hippocampus [74] and facilitate long-term potentiation in the hippocampus [75].

Other relevant properties by estradiol have been observed. It is neuroprotective in laboratory models of oxidative stress, excitatory neurotoxicity, apoptosis, and ischemia [76]. Estradiol promotes neurite growth and synapse formation [77] and it enhances glycolytic metabolism in the brain [78].

Clinical research has, however, failed to demonstrate convincing roles for these compounds in AD treatment or prevention [79, 80], but these studies also delineate areas of uncertainty and suggest further research opportunities.

Conclusions

It is well known that Aβ peptide, which is aggregated extracellularly in the neuritic plaques, generates a constant inflammatory environment and a prolonged activation of microglial and astroglial cells. This potentiates neuronal damage and it has been involved in the alteration of the BBB damaging the permeability of blood vessels [81]. Understanding the mechanisms of action of different species of Aβ peptide could lead to new therapeutic interventions directed to inhibit Aβ aggregation at the level of oligomers, which are much more toxic than the fibrillary form.

Moreover, recent evidence on the role of microglia-derived MVs, inflammasomes and signalosomes represent a step forward toward AD pathology comprehension. Indeed, this evidence confirms the importance of approaching AD as an immune and neuroinflammatory disease. In addition, the role of inflammatory chemokines, cytokines and other immune mediators in AD animal models still remains to be explored.

In conclusion, a detailed understanding of these concepts and molecular underpinnings of neuroinflammation and neuroimmune pathways in AD will need to be redefined and characterized clearly in order to truly understand the fundamental nature of this affliction.

Compliance with ethical standards

Conflict of interest On behalf of all Authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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