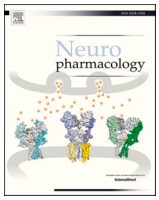
Neuropharmacology 252 (2024) 109941

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



NLRP3 inflammasome signalling in Alzheimer's disease

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ARTICLE INFO

Handling Editor: Bruno Frenguelli

Keywords:
NLRP3 inflammasome
Microglia
Alzheimer's disease
Neuroimmunology
Immune-targeted therapies
Preclinical models

ABSTRACT

Every year, 10 million people develop dementia, the most common of which is Alzheimer's disease (AD). To date, there is no way to prevent cognitive decline and therapies are limited. This review provides a neuro-immunological perspective on the progression of AD, and discusses the immune-targeted therapies that are in preclinical and clinical trials that may impact the development of this disease. Specifically, we look to the role of the NLRP3 inflammasome, its triggers in the brain and how its activation can contribute to the progression of dementia. We summarise the range of inhibitors targeting the NLRP3 inflammasome and its downstream pathways that are under investigation, and discuss future therapeutic perspectives for this devastating condition.

1. Introduction

Humans have developed a sophisticated immune system that is able to rapidly detect and respond to pathogenic organisms or tissue damage. This system has two branches, the innate and the adaptive immune system. The adaptive immune system consists of B and T cells, which express a huge variety of highly specific receptors that detect a unique antigen. This system is initially slower to respond than the innate immune system. During an infection for example, it can take a number of days for the adaptive immune response to take effect (Kumar et al., 2018). However, an immunological memory is created after the first response to the pathogen, thus on re-challenge the response is more rapid.

The innate immune system consists of cells such as macrophages, neutrophils and microglia that contain germline-encoded pattern recognition receptors (PRRs). PRRs detect set sequences denoting pathogen- or danger-associated molecular patterns (PAMPs and DAMPs), which contain either foreign or host-derived activating motifs respectively (Janeway and Medzhitov, 2002). The recognition of these molecular sequences is key for the innate immune system to mount a sufficient response. On activation, a PRR induces a signalling cascade within minutes, triggering pathway-specific transcription factors that facilitate the transcription of key inflammatory genes, which are translated and released e.g. Tumor necrosis factor α (TNFα) or Interferon-γ (IFN-γ). There are many different PRRs, including the Toll like receptor (TLR), NOD-like receptor (NLR) and RIG-like receptor (RLR) families, which differ in their cellular location, structure and the motif they recognise, meaning the cell is well equipped to detect any challenge or stress signal (Takeda et al., 2003; Heneka et al., 2018).

This first inflammatory response is central for the initial control of the trauma or challenge, such as invading pathogens (e.g. bacteria or viruses). The innate immune cells also coordinate with cells of the adaptive immune system and at later stages of the immune response, they secrete protective factors such as interleukin (IL)-10 to participate in wound healing and resolve the tissue damage. These immune responses are normally beneficial. Without a functioning immune response, humans are vulnerable to recurrent, severe infections and even death.

However, there are instances where the immune system can become maladaptive, and innate immune triggers induce an over-activated or non-resolving, chronic response. This heightened immune response is observed across many diseases such as atherosclerosis, gout and in neurodegenerative diseases such as Alzheimer's disease (AD). In this review, we will discuss the role of the innate immune system in the development and progression of AD. In particular, we will focus on the role of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, summarising the latest findings on NLRP3

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https://doi.org/10.1016/j.neuropharm.2024.109941
Received 31 January 2024; Received in revised form 20 March 2024; Accepted 29 March 2024
Available online 31 March 2024

Abbreviations

Aβ	Amyloid beta
ASC	Apoptosis-associated speck-like protein containing a caspase recruitment domain
AD	Alzheimer's disease
ADP	Adenosine diphosphate
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
ARIA	Amyloid-related imaging abnormalities
APOE	Apolipoprotein E
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase recruitment domain
CNS	Central nervous system
CR1	Complement receptor 1
CSF	Cerebrospinal fluid
DAMP	Danger associated molecular pattern
EOAD	Early onset AD
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
FTD	Frontotemporal dementia
GSDMD	Gasdermin D
GWAS	Genome-wide association studies
IFN-γ	Interferon-gamma
IL	Interleukin
LOAD	Late onset AD
LRR	Leucine-rich repeats
MCI	Mild cognitive impairment
NBD	Nucleotide binding domain
NFκB	Nuclear factor kappa B
NFT	Neurofibrillary tangle
NMDA	N-methyl-*d*-aspartate
NLR	NOD-like receptor
NLRP	NOD-, LRR-,and pyrin domain-containing protein
NOD	Nucleotide-binding oligomerization domain
PAMP	Pathogen-associated molecular pattern
PET	Positron emission tomography
PRR	Pattern recognition receptor
PTM	Post-translational modification
PYD	Pyrin domain
RLR	Retinoic acid-inducible gene I (RIG-I)-like receptors
siRNA	Small interfering RNA
TNFα	Tumor necrosis factor alpha
TLR	Toll like receptor
TREM2	Triggering receptor expressed on myeloid cells 2

inflammasome activation in the brain and the therapeutic potential of targeting this innate immune sensor.

2. Alzheimer's disease

In 2019, there were 55.2 million people with dementia worldwide, the most common of which is AD (WHO Global status report, 2021, https://www.who.int/health-topics/dementia#tab=tab_1). AD is broadly divided into two categories, early onset AD (EOAD) or late onset AD (LOAD). EOAD affects <1–5% of all cases, it is typically familial and occurs before the age of 65 (Tanzi, 2012). In contrast, LOAD is much more prevalent and the greatest risk factor is age. Currently over 33% of people aged 85 and over have AD (Rajan et al., 2021), and considering our aging population it is estimated that there will be over 78 million people with dementia and AD in 2030 (WHO Global status report, 2021). As the pathology and progression of disease is similar between EOAD and LOAD (Spina et al., 2021), the genetic factors that drive EOAD have been instrumental in understanding and modelling this condition.

2.1. *Aβ and Tau*

AD is characterised by a build-up of the protein amyloid-β (Aβ) and tau in plaques and neurofibrillary tangles (NFT) respectively. These are dense and insoluble deposits, causing physical disruption to the surrounding cells. Neuronal loss, especially cholinergic neurons, is also a

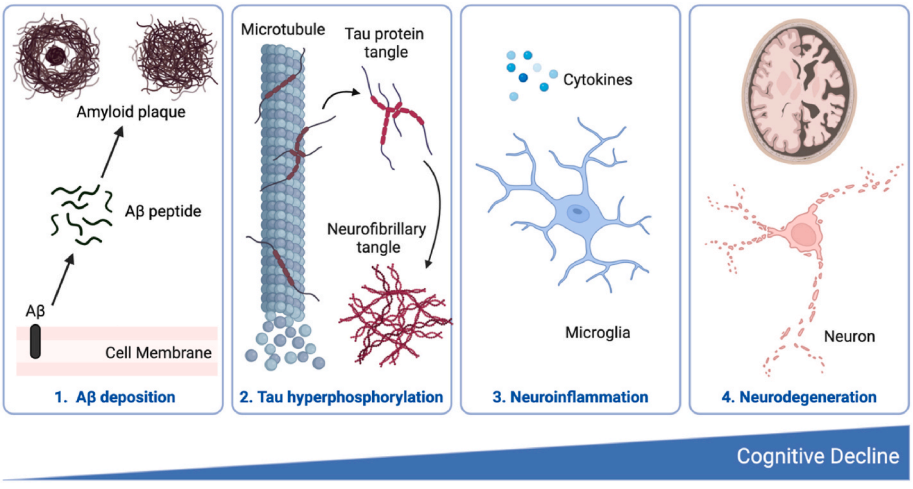

Fig. 1. Sequence of events that lead to the development of Alzheimer's disease. 1. The progression of AD has a long pre-clinical phase. Aβ is the first protein to become deposited in the brain, which forms oligomers, protofibrils and ultimately dense insoluble plaques. 2. The Aβ is followed by tau hyperphosphorylation, which then loses its capacity to stabilise microtubules, and forms intracellular neurofibrillary tangles. These neurons eventually die leaving extracellular tangles, often known as ghost tangles, behind. 3. The build-up of Aβ and tau activates microglia and astrocytes, triggering cytokine release and neuroinflammation. 4. Over time, these factors come together to cause neuronal death, inducing structural brain changes particularly at sites such as the hippocampus and frontal cortex. The progression to dementia and severe AD is more rapid.

common feature and is associated with the increasing cognitive decline (Fig. 1) (Mufson et al., 2008).

Aβ is formed from amyloid precursor protein (APP) by the sequential cleavage of β- and γ-secretase. To date, 102 mutations have been found in APP, with over 38 of them pathogenic (https://www.alzforum.org/mutations). Such mutations typically favour cleavage of APP towards the amyloidogenic as opposed to non-amyloidogenic pathway (Tcw and Goate, 2017). Strikingly, 346 mutations have been documented in PSEN1, which encodes for the protein presenilin-1 and forms the active cleavage site of γ-secretase. Of these mutations, 213 were classified as pathogenic or likely pathogenic (https://www.alzforum.org/mutations). Murine animal models of AD are often based on these mutations, such as the APPswe/PSEN1dE9 or the 5xFAD mice that mimic many features of AD, and are two of the more commonly used lines (Forner et al., 2021; McManus et al., 2014).

The progression of AD is initially quite slow, with a long pre-clinical phase. Aβ is the first protein to become deposited in the brain, which can begin approx. two decades before the emergence of cognitive issues (Villemagne et al., 2013). The Aβ is followed by tau hyperphosphorylation changes, inflammation, brain atrophy, then memory impairment and finally cognitive decline (Fig. 1) (Jack et al., 2010; Rajmohan and Reddy, 2017). Once the structural brain changes occur, the progression to dementia and severe AD is more rapid. Hippocampal atrophy and the following memory impairment occur on average 4 and 3 years respectively before the onset of dementia (Villemagne et al., 2013). The cognitive changes observed are divided into mild cognitive impairment (MCI), moderate and then severe AD, and range from general problems with thinking and memory to severe memory loss, personality changes and behavioural issues. Of those with MCI, 10–20% will develop AD within a year (Koepsell and Monsell, 2012; Petersen, 2016), and 68–80% will convert within 6 years (Mauri et al., 2012; Wilson et al., 2011; Lopez, 2013).

Tau is a neuronal microtubule-associated protein, that becomes hyperphosphorylated in AD and loses its capacity to stabilise microtubules. This results in impaired neuronal transport and further sensitises neurons to Aβ-induced toxicity (Rajmohan and Reddy, 2017; Ittner et al., 2011). These changes occur downstream of amyloid plaque formation, and Aβ has been shown to activate a number of kinases including CDK-5 and GSK-3β that drive tau hyperphosphorylation (Rajmohan and Reddy, 2017).

It is important to note that while Aβ is a classic feature of AD, there are older individuals who have significant levels of Aβ deposition throughout the brain but are cognitively intact (Mormino and Papp, 2018; Zolochevska and Taglialatela, 2016). It is not fully understood why that is the case. One possibility that might distinguish these people is in how the resident brain cells are responding to the Aβ stimulus, which may be different. Another idea is that the individuals will develop memory decline at later timepoints. Indeed, not all those who receive a diagnosis of MCI go on to develop AD (Koepsell and Monsell, 2012). Uncovering the mechanisms that promote resilience in these individuals is a topic of interest and will certainly help with the development of therapies to protect the brain against cognitive decline.

3. AD treatments

The first therapy to receive FDA approval for AD was a cholinesterase inhibitor, donepezil, in 1996. That was followed by rivastigmine in 1997 and galantamine in 2001 (which are also cholinesterase inhibitors), and the N-methyl-*d*-aspartate (NMDA) receptor antagonist memantine in 2003. These drugs act by either increasing the availability of acetylcholine to preserve cholinergic neuronal signalling or by blocking NMDA and thus reducing the glutamatergic neuronal hyperexcitability observed in AD. To date they are the only small molecule pharmaceutical therapies available for AD. Importantly, they are capable of managing the symptoms for a few months as opposed to slowing or even stopping disease pathology.

Due to the central role of amyloid in the development and progression of AD, as a novel therapeutic strategy many researchers have focused their efforts on targeting this protein directly. This resulted in the first FDA approved therapy in 15 years, Aducanumab (Aduhelm™) in 2021. Aducanumab is an Aβ-targeted monoclonal antibody that can bind Aβ aggregates, including soluble oligomers and insoluble fibrils (Budd Haeberlein et al., 2022). Lecanemab (Leqembi™) is a humanised monoclonal antibody that targets Aβ oligomers, protofibrils and insoluble fibrils (Swanson et al., 2021) and just received FDA approval in 2023. Finally, Donanemab is another humanised antibody that was submitted to the FDA for approval in 2023, but it recognises Aβ in established plaques, specifically by targeting an N-terminal pyroglutamate Aβ epitope (Sims et al., 2023; Mintun et al., 2021). Binding of these antibodies to Aβ interrupts the amyloid-aggregation process, while also targeting the Aβ for removal by microglia-mediated phagocytosis (Linse et al., 2020; Sevigny et al., 2016). While donepezil, galantamine, rivastigmine and memantine all received approval from the European Medicines Agency (EMA) shortly after FDA approval, that is not the case for any of the Aβ-targeted immunotherapies. To date, Aducanumab is only available to people in the USA and Lecanumab is available in the USA and Japan. There has been some controversy regarding the approval of these compounds, particularly Aducanumab. This is because the ENGAGE and EMERGE phase 3 clinical trials to test Aducanumab were terminated early in 2019 when an interim analysis predicted the studies would not meet their primary endpoints. A later re-analysis showed that those on the highest dose of Aducanumab had slowed cognitive decline in the EMERGE, but not the ENGAGE, trial (Budd Haeberlein et al., 2022). Based on these findings, the FDA granted accelerated approval for the use of Aducanumab in 2021, although this was met with mixed reactions from the AD community. In January 2024, it was announced that Aducanumab would be withdrawn from the market and production discontinued, likely due to the controversies and mixed results.

Targeting Aβ directly has revolutionised the treatments available for AD, and revitalised the amyloid hypothesis, which after decades of failed clinical trials was starting to be questioned. Indeed, these therapies also provide new treatment perspectives for those with Down Syndrome, who also develop Aβ-containing plaques as they age (Annus et al., 2016). However, there are important considerations before undertaking these therapies. While all three Aβ-targeted compounds can quickly remove Aβ in the brain, cognitive decline is slowed, rather than stopped completely (Budd Haeberlein et al., 2022; Sims et al., 2023; van Dyck et al., 2023). There is also the risk of amyloid-related imaging abnormalities (ARIA), which can be a brain edema or microhaemorrhage (ARIA-E or ARIA-H respectively) (Salloway et al., 2022). Indeed those with certain genetic predisposition such as apolipoprotein E4 (APOE) carriers appear more vulnerable to such effects (Salloway et al., 2022). As Aβ-targeted immunotherapies are not suitable for everyone, alternative treatments are still needed to prevent cognitive decline. Immune-modulating therapies (discussed below) may provide a new solution for how we treat MCI and AD.

4. The role of microglia in AD

Microglia are yolk-sac derived myeloid cells that carry out many critical functions in the brain from maintaining homeostasis, modulating neuronal activity and importantly, mediating the immune response of the central nervous system (CNS). These cells are the guardians of the brain. Microglia are highly phagocytic and by expressing an array of PRRs to detect PAMPs or DAMPs, along with constantly surveying their microenvironment, they are poised to respond to any threat or injury they encounter. However, unlike other tissues and organs, the brain is very sensitive to inflammation. Therefore, the microglial inflammatory response must be carefully regulated to limit the damage to neighbouring neurons and later support remodelling or repair. There are also number of receptor-ligand interactions

between microglia and astrocytes or neurons to maintain the microglia in a regulated state (Manich et al., 2019; Finneran and Nash, 2019). This is a very delicate balance, and the microglial response easily becomes disordered in diseases of the brain.

When Alois Alzheimer first described Alzheimer's disease in 1907, he already noted the presence of abnormal glial cells neighbouring the amyloid plaques (Alzheimer et al., 1995). We now know that microglia have a key role in the progression and development of AD. Genome-wide association studies (GWAS) have identified many microglial-specific genes that increase the risk for developing AD, such as Triggering receptor expressed on myeloid cells 2 (TREM2), CD33 and complement receptor 1 (CR1) (Naj et al., 2011; Bellenguez et al., 2022), many of which affect microglial phagocytosis (Butler et al., 2021; Ulrich et al., 2014). In 2008, Halle and colleagues first showed that when microglia phagocytose Aβ it triggers activation of the NLRP3 inflammasome (Halle et al., 2008), thus firmly establishing the role of innate immune signalling in this neurodegenerative disease.

5. NLRP3 inflammasome activation – what we know so far

The first inflammasome complex to be discovered was the NLRP1 inflammasome by the group of Jürg Tschopp in 2002 (Martinon et al., 2002). Inflammasomes are multi-protein complexes that contain a sensor protein (in this case NLRP1), the adaptor protein Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and the proteolytic enzyme caspase-1. This novel finding was quickly followed by the identification of the NLRP3 inflammasome and unravelling of the complex signalling pathways that regulate the assembly of this multi protein complex (Agostini et al., 2004; Martinon et al., 2006; Mariathasan et al., 2006). There are a number of PRRs that can form inflammasome complexes, including NLRP1, NLRP3, NLRC4 and AIM2 (Zheng et al., 2020). While the triggers of these PRRs are unique to each sensor protein, once activated, the complexes converge on similar signalling pathways, ultimately producing active IL-1β, as discussed in more detail below. The NLRP3 inflammasome is the best studied inflammasome complex, and to date, has the most well-defined role in AD. Therefore, NLRP3 will be the focus of this review.

Due to its inflammatory nature, activation of the NLRP3 inflammasome is a tightly regulated process with a number of steps, that need to occur in a specific order (Fig. 2). The first is a priming step, where a cytokine receptor or TLR signalling induces NF-κB activation and nuclear translocation resulting in the transcription and later translation of NLRP3 and pro-IL-1β (Bauernfeind et al., 2009). Post-translational

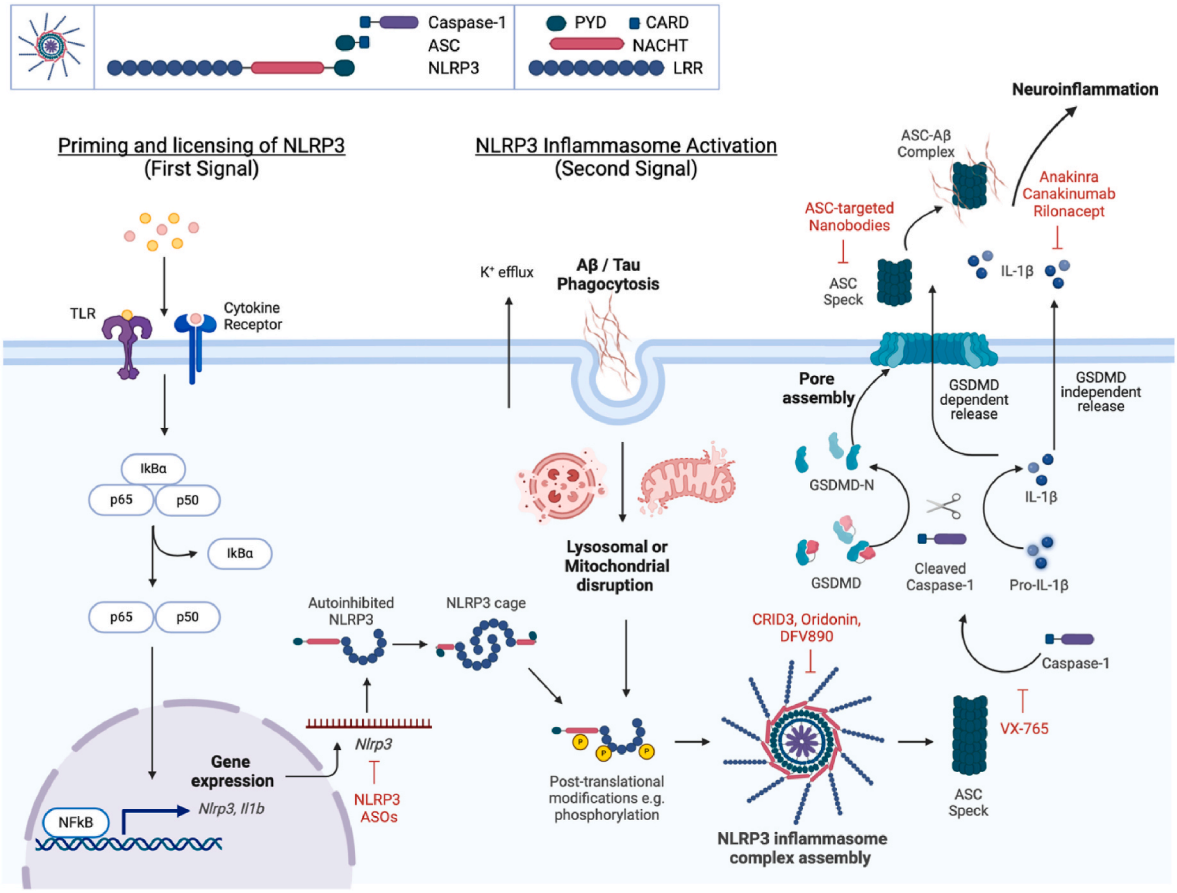

Fig. 2. NLRP3 inflammasome signalling pathway, activators and inhibitors. NLRP3 requires two signals for activation, a priming and licensing step (transcriptional regulation) and an activating step (post-transcriptonal regulation). TLR or cytokine receptor signalling can prime NLRP3 by increasing the mRNA levels of *Nlrp3 and Il1β*. Once translated, NLRP3 is in an autoinhibited form, and a second signal is required to release this and allow full assembly of the NLRP3 inflammasome complex. A number of post-translational modifications occur on NLRP3, such as phosphorylation, that facilitate this conformational change. NLRP3 also transitions from an oligomeric, cage like structure, into a disc-like shape, exposing its PYD domain and allowing ASC to bind. Aβ and tau can act as this second signal. Once assembled, ASC nucleates into a speck formation, providing a platform for the recruitment and auto-catalysis of caspase-1. Cleaved-caspase-1 in turn cleaves GSDMD, allowing GSDMD-N to oligomerize and form a pore in the plasma membrane. Pro-IL-1β is cleaved to its active form, which can leave through the GSDMD pores, and also through GSDMD-independent means. ASC-specks are also released, which can be taken up by neighbouring cells, or act as a seed for Aβ, perpetuating the inflammatory cycle. Current therapies are highlighted in red, namely NLRP3-targeted ASOs, to induced mRNA degradation. CRID3, Oridonin or DFV890, are specific NLRP3 inflammasome inhibitors. VX-765, a caspase-1 inhibitor. Nanobodies, to disrupt ASC specks. Anakinra, Canakinumab or Rilonacept to prevent IL-1β signalling.

modifications (PTMs) also occur during the first step, such as dephosphorylation, that contribute to the licensing of NLRP3 for downstream signalling (Akbal et al., 2022). NLRP3 is initially produced in an auto-inhibited and closed form, and a second signal is required to release this auto-inhibition allowing assembly of NLRP3 into an inflammasome complex.

Once the cell detects this second signal, other PTMs, including phosphorylation and ubiquitination occur on NLRP3 (see (Akbal et al., 2022) for a thorough review) that facilitate the transition of NLRP3 from an inactive oligomer to an active multimeric inflammasome. The NLRP3 protein contains a LRR, a NACHT domain containing ATP binding and hydrolysis activity, and a pyrin (PYD) domain, with PTMs identified on all three regions (Akbal et al., 2022). The NACHT domain is critical in the activation of NLRP3, where ATP binds the NACHT nucleotide binding domain (NBD) in the active state (Fu and Wu, 2023). In the inactive state, ADP is bound (Fu and Wu, 2023). It is important to note that unlike most cell types, human monocytes do not require two separate signalling steps to activate NLRP3, where engagement of TLR4 alone or presence of nigericin can drive NLRP3 inflammasome activation (Akbal et al., 2022; Gaidt et al., 2016; Gritsenko et al., 2020).

Recent studies have uncovered the exact structures formed by NLRP3 and its inflammasome complex that both influence and contribute to its activation. In the inactive state, NLRP3 oligomers are closed and can form a cage-like structure, where the PYD domain is hidden (Andreeva et al., 2021; Hochheiser et al., 2022a). The oligomers are formed though LRR-interactions between two NLRP3 proteins, and then back-to-back interactions with other NLRP3-LRR regions, ultimately forming a complex of 10 (human) or 12–16 (mouse) NLRP3 proteins in a double ring structure (Andreeva et al., 2021; Hochheiser et al., 2022a). Once activated, the NLRP3 complex undergoes a conformational change and opens up to a disc-like shape (Xiao et al., 2023). The PYD domain of NLRP3 becomes accessible, allowing the PYD domain of ASC to bind. ASC then oligomerizes in a prion-like manner (Hochheiser et al., 2022b), forming a speck and growing to approx. 1 μm in size (Fernandes-Alnemri et al., 2007). Along with its PYD domain, ASC also has a caspase recruitment domain (CARD), that binds pro-caspase-1. Facilitated by the molecular ASC platform, pro-caspase-1 dimerizes and undergoes an auto-catalytic reaction that produces active caspase-1 (Fig. 2). This is a crucial step in the pathway, the active caspase-1 can now cleave pro-IL-1β and pro-IL-18 to their active forms. Gasdermin D (GSDMD) is also cleaved by caspase-1, releasing its N-terminal fragment (He et al., 2015). This step allows GSDMD to oligomerize and form a pore in the plasma membrane, leading to the extracellular release of active IL-1β, IL-18, ASC specks and often, pyroptotic cell death. In the absence of GSDMD, GSDME can become cleaved and form a pore in the membrane, although this does not occur as quickly as GSDMD (Zhou and Abbott, 2021). Indeed, not all NLRP3-inflammasome triggers induce GSDMD-mediated pyroptosis (Rashidi et al., 2019). Some do not induce pyroptosis at all, even though they sufficiently induce NLRP3-dependent release of active IL-1β (Wolf et al., 2016). IL-1β can also be released without pyroptosis occurring (Monteleone et al., 2018). Cleaving pro-IL-1β to its active form changes the charge of this protein, allowing association with the negatively charged plasma membrane ruffles and therefore unconventional release (Monteleone et al., 2018).

Unlike other forms of cell death, such as apoptosis which is quite immunologically silent, pyroptosis is a highly inflammatory process and quickly alerts neighbouring cells (Man et al., 2017). These nearby cells sense the released IL-1β, IL-18 and ASC, but also the contents of the original dying cell that act as DAMPs, triggering a secondary response (Bertheloot et al., 2021). IL-1β release is highly inflammatory, and activates IL-1R1 and via the production of chemokines, causes neutrophil recruitment (Sadik et al., 2011; Miller et al., 2006). ASC specks are also released, and can be taken up providing neighbouring cells with the molecular platform to facilitate caspase-1 cleavage, without inflammasome activation (Franklin et al., 2014). As a large protein complex, ASC can also act as a seed for proteins to attach to thus contributing to its inflammatory activity (Venegas et al., 2017; Friker et al., 2020).

While it is well established that NLRP3 requires PTMs and structural conformation changes to become active, it also undergoes changes in its cellular location, such as from the cytosol to the *trans*-Golgi network or mitochondria (Andreeva et al., 2021; Wolf et al., 2016; Baik et al., 2023; Misawa et al., 2013). There are also species differences in the NLRP3 binding partners (e.g. NEK7) that can influence its activity (Y et al., 2016). How these factors come together and regulate NLRP3 activation in a time and location-dependent manner remain to be fully elucidated.

What makes NLRP3 a very interesting sensor and later inflammasome complex, is that many different kinds of signals trigger assembly of this protein, ranging from changes in potassium concentration (Muñ et al., 2013), crystals such as cholesterol (Duewell et al., 2010) or uric acid crystals (Martinon et al., 2006), and protein aggregates like Aβ (Halle et al., 2008; Sheedy et al., 2013; Heneka et al., 2013) that induce mitochondrial stress or lysosomal dysfunction. Due to this, NLRP3 inflammasome activation has been found across many different and seemingly unconnected diseases, from atherosclerosis (Duewell et al., 2010), gout (Goldberg et al., 2017), multiple sclerosis (Coll et al., 2015) and AD (Heneka et al., 2013). Together this demonstrates that NLRP3 is an overall sensor of cell stress and a key pharmaceutical target of interest in treating these different conditions.

6. NLRP3 inflammasome activation in AD

A number of inflammasome complexes have been examined in the development of AD, including NLRP1 and AIM2 (reviewed here (Heneka et al., 2018)). However, in the brain, NLRP3 is the most studied inflammasome complex to date. Microglial NLRP3 inflammasome activation occurs in a very similar fashion as that described for macrophages. Aβ and tau are first phagocytosed by TLR2, TLR4 and other scavenger receptors (Sheedy et al., 2013; Ries and Sastre, 2016; Meng et al., 2022; McManus, 2022). Aβ can trigger NLRP3 inflammasome activation via lysosomal disruption and the release of cathepsin D (Halle et al., 2008). Both oligomeric and fibrillar Aβ can activate NLRP3, inducing the production of cleaved-caspase-1 and active IL-1β (Fig. 2), although the intensity of this production is less than that observed for nigericin for example (Lučiūnaitė et al., 2020). Indeed, the signals used to prime and activate NLRP3 can induce different degrees of inflammasome activation and the production of downstream components, where microbial factors (e.g. nigericin) are much more potent and pyroptotic than sterile inflammatory triggers (e.g. ATP or Aβ) (Bezbradica et al., 2017). NLRP3 inflammasome activation has been found in both the brain and periphery of patients with AD pathology (Heneka et al., 2013; Saresella et al., 2016; Zhang et al., 2020). Specifically, the monocytes of AD patients have greater production of caspase-1 and IL-1β to NLRP3-activating stimuli (Saresella et al., 2016). Increased protein levels of cleaved caspase-1, active IL-1β, cleaved GSDMD and ASC have been found in the post mortem brain tissue of AD patients, in comparison with non-demented, age matched controls (Venegas et al., 2017; Heneka et al., 2013; Zhang et al., 2020; McManus et al., 2022; Ising et al., 2019). AD patients also have increased IL-1β and GSDMD in their cerebrospinal fluid (CSF) than healthy controls (Shen et al., 2021). A common product of NLRP3 inflammasome activation is the production of IL-18, although to date, this has not been studied much in relation to AD. It has been observed that IL-18 is increased in the post mortem brain tissue of those with AD (Ojala et al., 2009), and it can enhance neuronal production of Aβ *in vitro* (Sutinen et al., 2012).

In striking similarity to that observed in humans, activation of the NLRP3 inflammasome has been found by a number of research groups in various animal models of AD, including the APP/PS1 and 5xFAD models (Heneka et al., 2013; Zhang et al., 2020; McManus et al., 2022). Using the APP/PS1 mouse model, deletion of NLRP3 protected the mice from neuroinflammation, Aβ plaque deposition and the resulting cognitive impairment (Heneka et al., 2013). Although it is not known exactly when NLRP3 inflammasome activation occurs in patients, using the

APP/PS1 animal model, we have found that NLRP3 inflammasome activation directly parallels the initial Aβ deposition at 6 months (McManus et al., 2022), confirming how closely linked these phenomena are with each other. It is important to note a recent study where NLRP3 or caspase-1 deletion did not affect Aβ load in their APP/PS1 mouse model (Srinivasan et al., 2024). However, the authors did not assess the levels of any inflammasome components in the brains of these mice (e.g. cleaved caspase-1, IL-1β, GSDMD, ASC), nor learning or memory paradigms, therefore it is challenging to interpret the discrepancy in amyloid deposition across these studies.

Aβ also triggers microglial release of ASC specks, which act as a seed that Aβ can bind to, further enhancing amyloid pathology (Venegas et al., 2017). These ASC-Aβ composites are highly inflammatory, and cause greater IL-1β production than either ASC or Aβ alone in primed microglial cells (Friker et al., 2020).

Tau, which is found in AD and in frontotemporal dementia (FTD), also triggers activation of the NLRP3 inflammasome in patients (Ising et al., 2019) and in animal models of disease such as the Tau22 and Tau301S mouse models (Ising et al., 2019; Stancu et al., 2019). In striking similarity to that observed for Aβ, absence of NLRP3 or ASC in the Tau22 or 301S model of FTD rescues tauopathy, reduces neuroinflammation and improves cognitive performance (Ising et al., 2019; Stancu et al., 2019).

It has recently been demonstrated that myelin damage also occurs in AD, and in models of 5xFAD demyelination enhanced Aβ deposition (Depp et al., 2023). Similarly, western diet impairs lesion recovery in murine models, which was associated with cholesterol crystal-loaded microglial cells (Bosch-Queralt et al., 2021). Lipids can activate NLRP3 (Liang et al., 2021), as observed with western diet-induced cholesterol crystals that contribute to heart disease (Duewell et al., 2010). It is interesting to speculate whether impaired lipid signalling might also be inducing NLRP3 inflammasome activation in those with AD.

7. Therapeutic targeting of the NLRP3 inflammasome pathway

7.1. IL-1β

The first therapies to target the NLRP3 inflammasome pathway focused on IL-1β (Fig. 2). Three compounds have had great success: Anakinra (a recombinant IL-1 receptor antagonist) canakinumab (a human IgG monoclonal antibody that targets IL-1β) and rilonacept (a soluble decoy receptor containing domains of IL-1R1 and IL-1RAcP that also binds IL-1β) (Dubois et al., 2011). Anakinra and rilonacept also block IL-1α. These three compounds are FDA-approved to treat those with Cryopyrin-associated periodic syndromes (CAPS) who have a mutation in NLRP3, and to date are the only approved therapies for this condition (Booshehri and Hoffman, 2019). They have been used effectively in other diseases that have NLRP3 involvement, such as arthritis, gout or pericarditis (Mertens and Singh, 2009; Broderick and Hoffman, 2022).

In animal models, anakinra could reduce Aβ and Tau deposition, and lowered IL-1β together attenuating cognitive deficits in the 3xTg AD model (Kitazawa et al., 2011). Anakinra or IL-1r$^{-/-}$ also attenuated Aβ-induced mitochondrial and memory impairments *in vitro* and *in vivo* (Batista et al., 2021). In AD, there is currently an active, phase 2 clinical trial with canakinumab for those with MCI or AD (NCT04795466). These individuals receive canakinumab for 20 weeks, with later follow up visits where cognition and behaviour, and microglial activation by positron emission tomography (PET) will be assessed at various timepoints throughout the study. The estimated completion is spring 2024, therefore it will be very interesting to see the effectiveness of this therapy. Only a few studies have specifically targeted IL-1β in animal models or clinical trials of AD. This is perhaps due to issues crossing the Blood-brain barrier (BBB), where anakinra can pass more easily than canakinumab for example (Sjöström et al., 2021). Although as the BBB is more open in AD (Ryu and McLarnon, 2009), this should facilitate some treatments accessing the brain. However, IL-1β is only one downstream mediator, targeting NLRP3 directly or partners in the inflammasome complex would likely have a greater impact.

7.2. Caspase-1

Caspase-1 inhibition has also been examined in models of AD. Caspase-1 deficiency protected APP/PS1 (Heneka et al., 2013) and J20 mice (Flores et al., 2018) from developing amyloid pathology, neuroinflammation and cognitive deficits.

VX-765 is a well-characterised small molecule inhibitor of caspase-1 (also acting on caspase-4). It is a pro-drug, that upon uptake is acted upon by plasma esterases, converting VX-765 into an active peptidomimetic metabolite, VRT-043198, that bonds with the active site of caspase-1 thus blocking its activity. In the J20 mouse model of AD, VX-765 rescued spatial memory impairment, reduced microgliosis and significantly attenuated Aβ deposition in the 5–8-month-old mice (Flores et al., 2018). It is important to note that the memory deficits returned when the VX-765 treatment was stopped, indicating the need to regularly inhibit this pathway to maintain protective effects. In older (12–15 month) J20 mice, VX-765 was still able to rescue memory impairments by boosting performance in the novel objection recognition and Barnes maze tests (Flores et al., 2022). Interestingly VX-765 was no longer able to reduce microglial activity in the older J20 animals (assessed by Iba1-staining), nor did it reduce neuroinflammation or Aβ deposition, suggesting a divergence in pathways contributing to the age-related memory loss in this model (Flores et al., 2022).

7.3. NLRP3

Due to its involvement across so many different diseases, pharmacological inhibition of the NLRP3 inflammasome is of key interest. CRID3 (or CP-456773, MCC950) is a specific, commercially available inhibitor of NLRP3. Interestingly, it was known for many years that CRID3 could block IL-1β, but it was only in 2015 that CRID3 was identified as a specific NLRP3 inflammasome inhibitor (Coll et al., 2015). We now understand that CRID3 acts within the NACHT domain of NLRP3 to prevent its activity (Coll et al., 2019). Using cryo-electron microscopy, Hochheiser and colleagues recently demonstrated that CRID3 binds into a specific cleft within NLRP3, that connects four NACHT subdomains with an LRR transition segment. Having unravelled the precise site of action of CRID3, this will undoubtedly facilitate the design of better, more potent inhibitors moving forward (Hochheiser et al., 2022a). While CRID3 has been used in clinical trials, it has high plasma protein binding (Primiano et al., 2016) and it unfortunately can induce liver toxicity in humans at higher concentrations (Torres et al., 2021; Mangan et al., 2018; Cross, 2020). This makes the compound ideal for mechanistic studies *in vitro* and *in vivo*, but obviously less so as a therapy for patients.

In terms of AD, CRID3 effectively blocked the Aβ (Lučiūnaitė et al., 2020; McManus et al., 2022; Dempsey et al., 2017) or Tau (Ising et al., 2019; Stancu et al., 2019)-induced activation of the NLRP3 inflammasome *in vitro*, preventing cleavage and release of caspase-1 and IL-1β. In animal models, CRID3 could prevent the production of IL-1β, reduced Aβ deposition and rescued memory impairments in the APP/PS1 model of AD (Dempsey et al., 2017). CRID3 also reduced Tau seeding in the P301S model of Tauopathy, and attenuated the Tau-induced microgliosis (Stancu et al., 2019).

There are a number of other NLRP3 inflammasome inhibitors at various stages of pre-clinical (IFM-514 (Torres et al., 2021), CY-09 (Wang et al., 2021)) or clinical trials such as DFV890 (IFM-2427) (Madurka et al., 2023), OLT1177 (Marchetti et al., 2018), Tranilast (Huang et al., 2018), Oridonin (He et al., 2018), Selnoflast (McFarthing et al., 2023) or Inzomelid (IZD174) (Coll et al., 2022), see Table 1 for details. OLT1177 is one of the few NLRP3 inhibitors where the structure

Table 1
List of NLRP3 inflammasome inhibitors in clinical trials.

Name	Alternative Names	Company	BBB Penetrant?	Clinical Trial Completed	Recruiting
DFV890	IFM-2427	Novartis Pharmaceuticals		NCT04868968, phase 2, FCAS NCT04382053, phase 2, SARS-CoV-2 infected patients with COVID-19 pneumonia	NCT04886258, phase 2, knee osteoarthritis NCT06031844, phase 2a, Coronary Heart Disease NCT06097663, phase 2a, Coronary Heart Disease and TET2 or DNMT3A CHIP NCT05552469, phase 1b, patients with myeloid diseases
INZOMELID	IZD174	Roche	Yes	NCT04015076, phase 1, Safety and Tolerability study in healthy and CAPS	
NT-0796		NodThera Limited	Yes	NCT06129409, phase 1/2, Obese Participants at Risk of Cardiovascular Disease	
OLT1177	Dapansutrile	Olatec Therapeutics LLC	Yes	NCT02104050, phase 2b, pain associated with osteoarthritis of the knee NCT03534297, phase 1b, Heart Failure	NCT05658575, phase 2/3, acute gout flare
Oridonin		Wuhan Union Hospital, China		NCT05130892, Phase 4, Coronary artery disease with percutaneous coronary intervention	
RRX-001		EpicentRx, Inc.	Yes	NCT01359982, phase 1, Cancer Subjects NCT02215512, phase 1, Brain Metastases	
SELNOFLAST	SOMALIX/RG6418/ IZD334/RO7486967	Roche		NCT04086602, phase 1, Safety and Tolerability	NCT05924243, phase 1b, Parkinson's disease
Tranilast		Nuon Therapeutics, Inc. and others	Yes	NCT05130892, Phase 4, Coronary artery disease with percutaneous coronary intervention NCT01109121, phase 2, Hyperuricemia and moderate to severe gout NCT00882024, phase 2, Patients With Active Rheumatoid Arthritis (RA)	NCT03923140, phase 2, CAPS
VTX2735		Ventyx Biosciences, Inc, Zomagen Biosciences, Ltd			NCT05812781, phase 1, CAPS
ZYIL1		Zydus Lifesciences Limited		NCT05186051, phase 2a, CAPS	NCT05981040, phase 2, Amyotrophic lateral sclerosis

has been shared and as OLT1177 reduces the ATPase activity of NLRP3, it likely binds to that region (Marchetti et al., 2018). A recent study found that OLT1177 does not directly bind to NLRP3 (Teske et al., 2024), however, this does not detract from the effectiveness of OLT1177 at preventing the release of active IL-1β (Marchetti et al., 2018; Tengesdal et al., 2021; Oizumi et al., 2022; Lonnemann et al., 2020) and the promising findings from the ongoing clinical trials (Table 1). The structures of most of the compounds are not yet publicly available, thus it is unknown where they precisely bind to prevent NLRP3 inflammasome signalling. However, as some of these inhibitors are in clinical trials for those with Parkinson's disease or ALS (Selnoflast and ZYIL1) and many compounds are additionally BBB penetrant (Table 1), the results of these trials will be of interest to many with strong implications for future treatments of AD.

7.4. ASOs or siRNA

An alternative approach to prevent NLRP3 inflammasome signalling has been to reduce the mRNA levels of NLRP3, thus affecting the amount translated to protein. Anti-sense oligonucleotides (ASOs) function by targeting a specific mRNA for degradation and SOD1-targeted ASOs have recently been used in clinical trials to treat Amyotrophic lateral sclerosis (ALS) (Miller et al., 2022). Interestingly, NLRP3-targeted ASOs were successfully able to reduce the protein levels of NLRP3, thus also significantly reducing NLRP3 inflammasome activation to nigericin and Aβ stimulation, in both human and murine *in vitro* models of disease (Braatz et al., 2023). NLRP3-specific ASOs were also protective in murine models of CAPS, which reduced the production of NLRP3 and active IL-1β, prolonging the survival time of these mice (Kaufmann et al., 2023).

In a similar fashion, small interfering RNA (siRNA) also targets mRNA for degradation. Injecting caspase-1-targeted siRNA (under a AAV) into APP/PS1 mice significantly reduced neuroinflammation and rescued behavioural function in these animals (Han et al., 2020). *In vitro*, siRNA targeting of caspase-1 and GSDMD successfully reduced the amount of IL-1β released to the supernatant after Aβ exposure (Han et al., 2020), although GSDMD inhibition or depletion has not yet been examined in murine models of AD.

7.5. Nanobodies

Camelids (e.g. camels or alpacas) generate single domain antibodies that are derived from heavy chain peptides. They are approx. 15 kDa in size, one tenth that of a conventional antibody. Nanobodies bind with high affinity and specificity, are very stable and due to their small size, they can overcome limitations posed by conventional antibodies allowing them to bind previously inaccessible epitopes (Wouters et al., 2020). Nanobodies against ASC have been developed, which successfully blocked ASC-CARD interactions *in vitro* thus reducing the nigericin-induced cleaved-caspase-1 and active IL-1β production (Schmidt et al., 2016). *In vivo*, these ASC-targeted nanobodies were able to prevent ASC prion activity, disassemble ASC specks and were

protective against disease progression in murine models of gout and arthritis (Bertheloot et al., 2022). GSDMD nanobodies have also been developed that inhibit the formation of GSDMD pores *in vitro* (Hochheiser et al., 2022b).

To date, nanobodies have largely targeted extracellular components of the inflammasome pathways, due to the challenge of entering the cell. Another challenge that occurs in these approaches for neurodegenerative diseases is that the nanobody would also need to cross the BBB to access the brain parenchyma. Interestingly, brain-penetrant nanobodies have been developed. In this case, Wouters and colleagues developed a nanobody to target the transferrin receptor (that is highly expressed on endothelial cells) and coupled this nanobody to their target of interest, which successfully accessed the CNS after receptor-mediated endocytosis (Wouters et al., 2020). This Trojan-horse like system is a novel way to access the brain and as nanobodies can be readily linked to other nanobodies or peptides (Bannas et al., 2017; Abskharon et al., 2023), their use has the potential to revolutionize therapies in many diseases.

8. Conclusions

There is a clinically unmet need to protect against age-related diseases, particularly AD and dementia. As we live in an aging society, this situation is confounded by the number of people who will develop dementia and require enhanced care in the coming years. A key reason for this has been the lack of understanding on the mechanisms that lead to the development of AD, and importantly, the immune mediated aspects. We now know that the NLRP3 inflammasome is triggered by the growing presence of Aβ and tau in the brain, inducing production of active IL-1β and ASC specks. This causes a feed-forward inflammatory cycle, where IL-1β and ASC act on neighbouring brain cells or in the case of ASC, provide a platform to further enhance Aβ aggregation, thus causing physical disruption along with additional DAMP-signals to cells. Under these inflammatory conditions, the microglial phagocytic capacities reduce (Koenigsknecht-Talboo and Landreth, 2005), which also contributes to the worsening pathology.

Preclinical studies have shown the benefits of preventing NLRP3 inflammasome signalling, by genetic depletion of NLRP3 components and binding factors or pharmacological inhibition. Indeed there are now a number of approaches under investigation to block this complex, from mRNA-targeted therapies, nanobodies and novel small molecule inhibitors as discussed in this review. An important factor is that the inhibitors need to reach the brain by crossing the BBB, which adds a layer of complexity that is not required for other NLRP3-mediated diseases. With the recent introduction of Aβ-targeted therapies, a combination approach with NLRP3 inhibition would be relevant, with the potential to boost the effects of either treatment alone.

Funding

R.M. is supported by grants from the Alzheimer's Forschung Initiative (#20043), Deutsche Demenzhilfe, Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC2151 – 390873048 and Project-ID 432325352 – SFB 1454.

E.L. is supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC2151 – 390873048 and Project-ID 432325352 – SFB 1454, Helmholtz Association, under the project title 'Immunology&Inflammation', #ZT-0027.

CRediT authorship contribution statement

Róisín M. McManus: Conceptualization, Writing – original draft, Writing – review & editing. **Eicke Latz:** Conceptualization, Writing – review & editing.

Declaration of competing interest

E.L. is a Co-founder and adviser at IFM Therapeutics, Dioscure Therapeutics, a 'Stealth' Biotech and Odyssey Therapeutics. R.M.M. has no competing interests.

Data availability

No data was used for the research described in the article.

Acknowledgments

All figures were created using BioRender.com, using their images and parts of schematics.

References

Abskharon, R., et al., 2023. Structure-based design of nanobodies that inhibit seeding of Alzheimer's patient-extracted tau fibrils. Proc. Natl. Acad. Sci. U. S. A. 120, e2300258120.
Agostini, L., et al., 2004. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20, 319–325.
Akbal, A., et al., 2022. How location and cellular signaling combine to activate the NLRP3 inflammasome. Cell. Mol. Immunol. 19, 1201–1214.
Alzheimer, A., Stelzmann, R.A., Schnitzlein, H.N., Murtagh, F.R., 1995. An English translation of Alzheimer's 1907 paper, 'Uber eine eigenartige Erkankung der Hirnrinde'. Clin. Anat. N. Y. N 8, 429–431.
Andreeva, L., et al., 2021. NLRP3 cages revealed by full-length mouse NLRP3 structure control pathway activation. Cell 184, 6299–6312.e22.
Annus, T., et al., 2016. The pattern of amyloid accumulation in the brains of adults with Down syndrome. Alzheimers Dement. J. Alzheimers Assoc. 12, 538–545.
Baik, S.H., et al., 2023. Hexokinase dissociation from mitochondria promotes oligomerization of VDAC that facilitates NLRP3 inflammasome assembly and activation. Sci. Immunol. 8, eade7652.
Bannas, P., Hambach, J., Koch-Nolte, F., 2017. Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics. Front. Immunol. 8, 1603.
Batista, A.F., et al., 2021. Interleukin-1β mediates alterations in mitochondrial fusion/fission proteins and memory impairment induced by amyloid-β oligomers. J. Neuroinflammation 18, 54.
Bauernfeind, F.G., et al., 2009. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J. Immunol. Baltim. Md 1950 183, 787–791.
Bellenguez, C., et al., 2022. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat. Genet. 54, 412–436.
Bertheloot, D., Latz, E., Franklin, B.S., 2021. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell. Mol. Immunol. 18, 1106–1121.
Bertheloot, D., et al., 2022. Nanobodies dismantle post-pyroptotic ASC specks and counteract inflammation in vivo. EMBO Mol. Med. 14, e15415.
Bezbradica, J.S., Coll, R.C., Schroder, K., 2017. Sterile signals generate weaker and delayed macrophage NLRP3 inflammasome responses relative to microbial signals. Cell. Mol. Immunol. 14, 118–126.
Booshehri, L.M., Hoffman, H.M., 2019. CAPS and NLRP3. J. Clin. Immunol. 39, 277–286.
Bosch-Queralt, M., et al., 2021. Diet-dependent regulation of TGFβ impairs reparative innate immune responses after demyelination. Nat. Metab. 3, 211–227.
Braatz, C., et al., 2023. NLRP3-directed antisense oligonucleotides reduce microglial immunoactivities in vitro. J. Neurochem. https://doi.org/10.1111/jnc.15778.
Broderick, L., Hoffman, H.M., 2022. IL-1 and autoinflammatory disease: biology, pathogenesis and therapeutic targeting. Nat. Rev. Rheumatol. 18, 448–463.
Budd Haeberlein, S., et al., 2022. Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. J. Prev. Alzheimers Dis. 9, 197–210.
Butler, C.A., Thornton, P., Brown, G.C., 2021. CD33M inhibits microglial phagocytosis, migration and proliferation, but the Alzheimer's disease-protective variant CD33m stimulates phagocytosis and proliferation, and inhibits adhesion. J. Neurochem. 158, 297–310.
Coll, R.C., et al., 2015. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat. Med. 21, 248–255.
Coll, R.C., et al., 2019. MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. Nat. Chem. Biol. 15, 556–559.
Coll, R.C., Schroder, K., Pelegrín, P., 2022. NLRP3 and pyroptosis blockers for treating inflammatory diseases. Trends Pharmacol. Sci. 43, 653–668.
Cross, R., 2020. Could an NLRP3 inhibitor be the one drug to conquer common diseases? Chem. Eng. News 98 (7). https://cen.acs.org/pharmaceuticals/drug-discovery/Could-an-NLRP3-inhibitor-be-the-one-drug-to-conquer-common-diseases/98/i7.
Dempsey, C., et al., 2017. Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-β and cognitive function in APP/PS1 mice. Brain Behav. Immun. 61, 306–316.
Depp, C., et al., 2023. Myelin dysfunction drives amyloid-β deposition in models of Alzheimer's disease. Nature 618, 349–357.
Dubois, E.A., Rissmann, R., Cohen, A.F., 2011. Rilonacept and canakinumab. Br. J. Clin. Pharmacol. 71, 639–641.

Duewell, P., et al., 2010. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 464, 1357–1361.
Fernandes-Alnemri, T., et al., 2007. The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. Cell Death Differ. 14, 1590–1604.
Finneran, D.J., Nash, K.R., 2019. Neuroinflammation and fractalkine signaling in Alzheimer's disease. J. Neuroinflammation 16, 30.
Flores, J., et al., 2018. Caspase-1 inhibition alleviates cognitive impairment and neuropathology in an Alzheimer's disease mouse model. Nat. Commun. 9, 3916.
Flores, J., Fillion, M.-L., LeBlanc, A.C., 2022. Caspase-1 inhibition improves cognition without significantly altering amyloid and inflammation in aged Alzheimer disease mice. Cell Death Dis. 13, 864.
Forner, S., et al., 2021. Systematic phenotyping and characterization of the 5xFAD mouse model of Alzheimer's disease. Sci. Data 8, 270.
Franklin, B.S., et al., 2014. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. Nat. Immunol. 15, 727–737.
Friker, L.L., et al., 2020. β-Amyloid clustering around ASC fibrils boosts its toxicity in microglia. Cell Rep. 30, 3743–3754.e6.
Fu, J., Wu, H., 2023. Structural mechanisms of NLRP3 inflammasome assembly and activation. Annu. Rev. Immunol. 41, 301–316.
Gaidt, M.M., et al., 2016. Human monocytes engage an alternative inflammasome pathway. Immunity 44, 833–846.
Goldberg, E.L., et al., 2017. β-Hydroxybutyrate deactivates neutrophil NLRP3 inflammasome to relieve gout flares. Cell Rep. 18, 2077–2087.
Gritsenko, A., et al., 2020. Priming is dispensable for NLRP3 inflammasome activation in human monocytes in vitro. Front. Immunol. 11, 565924.
Halle, A., et al., 2008. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat. Immunol. 9, 857–865.
Han, C., et al., 2020. New mechanism of nerve injury in Alzheimer's disease: β-amyloid-induced neuronal pyroptosis. J. Cell Mol. Med. 24, 8078–8090.
He, W., et al., 2015. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res. 25, 1285–1298.
He, H., et al., 2018. Oridonin is a covalent NLRP3 inhibitor with strong anti-inflammasome activity. Nat. Commun. 9, 2550.
Heneka, M.T., et al., 2013. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493, 674–678.
Heneka, M.T., McManus, R.M., Latz, E., 2018. Inflammasome signalling in brain function and neurodegenerative disease. Nat. Rev. Neurosci. 19, 610–621.
Hochheiser, I.V., et al., 2022a. Structure of the NLRP3 decamer bound to the cytokine release inhibitor CRID3. Nature 604, 184–189.
Hochheiser, I.V., et al., 2022b. Directionality of PYD filament growth determined by the transition of NLRP3 nucleation seeds to ASC elongation. Sci. Adv. 8, eabn7583.
Huang, Y., et al., 2018. Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. EMBO Mol. Med. 10, e8689.
Ising, C., et al., 2019. NLRP3 inflammasome activation drives tau pathology. Nature 575, 669–673.
Ittner, L.M., Götz, J., 2011. Amyloid-β and tau–a toxic pas de deux in Alzheimer's disease. Nat. Rev. Neurosci. 12, 65–72.
Jack, C.R., et al., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 9, 119–128.
Janeway, C.A., Medzhitov, R., 2002. Innate immune recognition. Annu. Rev. Immunol. 20, 197–216.
Kaufmann, B., et al., 2023. Antisense oligonucleotide therapy decreases IL-1β expression and prolongs survival in mutant Nlrp3 mice. J. Immunol. Baltim. Md 1950 211, 287–294.
Kitazawa, M., et al., 2011. Blocking IL-1 signaling rescues cognition, attenuates tau pathology, and restores neuronal β-catenin pathway function in an Alzheimer's disease model. J. Immunol. Baltim. Md 1950 187, 6539–6549.
Koenigsknecht-Talboo, J., Landreth, G.E., 2005. Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J. Neurosci. Off. J. Soc. Neurosci. 25, 8240–8249.
Koepsell, T.D., Monsell, S.E., 2012. Reversion from mild cognitive impairment to normal or near-normal cognition: risk factors and prognosis. Neurology 79, 1591–1598.
Kumar, B.V., Connors, T., Farber, D.L., 2018. Human T cell development, localization, and function throughout life. Immunity 48, 202–213.
Liang, J.J., Fraser, I.D.C., Bryant, C.E., 2021. Lipid regulation of NLRP3 inflammasome activity through organelle stress. Trends Immunol. 42, 807–823.
Linse, S., et al., 2020. Kinetic fingerprints differentiate the mechanisms of action of anti-Aβ antibodies. Nat. Struct. Mol. Biol. 27, 1125–1133.
Lonnemann, N., et al., 2020. The NLRP3 inflammasome inhibitor OLT1177 rescues cognitive impairment in a mouse model of Alzheimer's disease. PNAS 117 (50), 32145–32154. https://doi.org/10.1073/pnas.2009680117. Epub 2020 Nov 30.
Lopez, O.L., 2013. Mild cognitive impairment. Contin. Lifelong Learn. Neurol. 19, 411–424.
Lučiūnaitė, A., et al., 2020. Soluble Aβ oligomers and protofibrils induce NLRP3 inflammasome activation in microglia. J. Neurochem. 155, 650–661.
Madurka, I., et al., 2023. DFV890: a new oral NLRP3 inhibitor-tested in an early phase 2a randomised clinical trial in patients with COVID-19 pneumonia and impaired respiratory function. Infection 51, 641–654.
Man, S.M., Karki, R., Kanneganti, T.-D., 2017. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol. Rev. 277, 61–75.
Mangan, M.S.J., et al., 2018. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat. Rev. Drug Discov. 17, 588–606.
Manich, G., et al., 2019. Role of the cd200-cd200r Axis during homeostasis and neuroinflammation. Neuroscience 405, 118–136.
Marchetti, C., et al., 2018. OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. Proc. Natl. Acad. Sci. U. S. A. 115, E1530–E1539.
Mariathasan, S., et al., 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440, 228–232.
Martinon, F., Burns, K., Tschopp, J., 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol. Cell 10, 417–426.
Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., Tschopp, J., 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440, 237–241.
Mauri, M., Sinforiani, E., Zucchella, C., Cuzzoni, M.G., Bono, G., 2012. Progression to dementia in a population with amnestic mild cognitive impairment: clinical variables associated with conversion. Funct. Neurol. 27, 49–54.
McFarthing, K., et al., 2023. Parkinson's disease drug therapies in the clinical trial pipeline: 2023 update. J. Park. Dis. 13, 427–439.
McManus, R.M., 2022. The role of immunity in Alzheimer's disease. Adv. Biol. 6, e2101166.
McManus, R.M., Higgins, S.C., Mills, K.H.G., Lynch, M.A., 2014. Respiratory infection promotes T cell infiltration and amyloid-β deposition in APP/PS1 mice. Neurobiol. Aging 35, 109–121.
McManus, R., et al., 2022. NLRP3-Mediated glutaminolysis regulates microglia in Alzheimer's disease. SSRN Scholarly. https://doi.org/10.2139/ssrn.4178538. Paper at.
Meng, J.X., et al., 2022. Hyperphosphorylated tau self-assembles into amorphous aggregates eliciting TLR4-dependent responses. Nat. Commun. 13, 2692.
Mertens, M., Singh, J.A., 2009. Anakinra for rheumatoid arthritis: a systematic review. J. Rheumatol. 36, 1118–1125.
Miller, L.S., et al., 2006. MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity 24, 79–91.
Miller, T.M., et al., 2022. Trial of antisense oligonucleotide tofersen for SOD1 ALS. N. Engl. J. Med. 387, 1099–1110.
Mintun, M.A., et al., 2021. Donanemab in early Alzheimer's disease. N. Engl. J. Med. 384, 1691–1704.
Misawa, T., et al., 2013. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat. Immunol. 14, 454–460.
Monteleone, M., et al., 2018. Interleukin-1β maturation triggers its relocation to the plasma membrane for gasdermin-D-dependent and -independent secretion. Cell Rep. 24, 1425–1433.
Mormino, E.C., Papp, K.V., 2018. Amyloid accumulation and cognitive decline in clinically normal older individuals: implications for aging and early Alzheimer's disease. J. Alzheimers Dis. JAD 64, S633–S646.
Mufson, E.J., Counts, S.E., Perez, S.E., Ginsberg, S.D., 2008. Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert Rev. Neurother. 8, 1703–1718.
Muñoz-Planillo, R., et al., 2013. K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 38, 1142–1153.
Naj, A.C., et al., 2011. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat. Genet. 43, 436–441.
Oizumi, T., et al., 2022. NLRP3 inflammasome inhibitor OLT1177 suppresses onset of inflammation in mice with dextran sulfate sodium-induced colitis. Dig. Dis. Sci. 67 (7), 2912–2921. https://doi.org/10.1007/s10620-021-07184-y.
Ojala, J., et al., 2009. Expression of interleukin-18 is increased in the brains of Alzheimer's disease patients. Neurobiol. Aging 30, 198–209.
Petersen, R.C., 2016. Mild cognitive impairment. Contin. Minneap. Minn 22, 404–418.
Primiano, M.J., et al., 2016. Efficacy and pharmacology of the NLRP3 inflammasome inhibitor CP-456,773 (CRID3) in murine models of dermal and pulmonary inflammation. J. Immunol. Baltim. Md 1950 197, 2421–2433.
Rajan, K.B., et al., 2021. Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020-2060). Alzheimers Dement. J. Alzheimers Assoc. 17, 1966–1975.
Rajmohan, R., Reddy, P.H., 2017. Amyloid-beta and phosphorylated tau accumulations cause abnormalities at synapses of Alzheimer's disease neurons. J. Alzheimers Dis. JAD 57, 975–999.
Rashidi, M., et al., 2019. The pyroptotic cell death effector gasdermin D is activated by gout-associated uric acid crystals but is dispensable for cell death and IL-1β release. J. Immunol. Baltim. Md 1950 203, 736–748.
Ries, M., Sastre, M., 2016. Mechanisms of Aβ clearance and degradation by glial cells. Front. Aging Neurosci. 8, 160.
Ryu, J.K., McLarnon, J.G., 2009. A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J. Cell Mol. Med. 13, 2911–2925.
Sadik, C.D., Kim, N.D., Luster, A.D., 2011. Neutrophils cascading their way to inflammation. Trends Immunol. 32, 452–460.
Salloway, S., et al., 2022. Amyloid-related imaging abnormalities in 2 phase 3 studies evaluating aducanumab in patients with early alzheimer disease. JAMA Neurol. 79, 13–21.
Saresella, M., et al., 2016. The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer's disease. Mol. Neurodegener. 11, 23.
Schmidt, F.I., et al., 2016. A single domain antibody fragment that recognizes the adaptor ASC defines the role of ASC domains in inflammasome assembly. J. Exp. Med. 213, 771–790.
Sevigny, J., et al., 2016. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature 537, 50–56.

Sheedy, F.J., et al., 2013. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nat. Immunol. 14, 812–820.
Shen, H., et al., 2021. Pyroptosis executive protein GSDMD as a biomarker for diagnosis and identification of Alzheimer's disease. Brain Behav 11, e02063.
Sims, J.R., et al., 2023. Donanemab in early symptomatic alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. JAMA 330, 512–527.
Sjöström, E.O., et al., 2021. Transport study of interleukin-1 inhibitors using a human in vitro model of the blood-brain barrier. Brain Behav. Immun. - Health 16, 100307.
Spina, S., et al., 2021. Comorbid neuropathological diagnoses in early versus late-onset Alzheimer's disease. Brain J. Neurol. 144, 2186–2198.
Srinivasan, S., et al., 2024. Inflammasome signaling is dispensable for ß-amyloid-induced neuropathology in preclinical models of Alzheimer's disease. Front. Immunol. 15, 1323409.
Stancu, I.-C., et al., 2019. Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo. Acta Neuropathol. 137, 599–617.
Sutinen, E.M., Pirttilä, T., Anderson, G., Salminen, A., Ojala, J.O., 2012. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-β production in human neuron-like cells. J. Neuroinflammation 9, 199.
Swanson, C.J., et al., 2021. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril antibody. Alzheimer's Res. Ther. 13, 80.
Takeda, K., Kaisho, T., Akira, S., 2003. Toll-like receptors. Annu. Rev. Immunol. 21, 335–376.
Tanzi, R.E., 2012. The genetics of alzheimer disease. Cold Spring Harb. Perspect. Med. 2, a006296.
Tcw, J., Goate, A.M., 2017. Genetics of β-amyloid precursor protein in Alzheimer's disease. Cold Spring Harb. Perspect. Med. 7, a024539.
Tengesdal, I.W., et al., 2021. Targeting tumor-derived NLRP3 reduces melanoma progression by limiting MDSCs expansion. PNAS 118 (10), e2000915118. https://doi.org/10.1073/pnas.2000915118.
Teske, K.A., et al., 2024. Interrogating direct NLRP3 engagement and functional inflammasome inhibition using cellular assays. Cell Chem. Biol. 31, 349–360.e6.
Torres, S., et al., 2021. The specific NLRP3 antagonist IFM-514 decreases fibrosis and inflammation in experimental murine non-alcoholic steatohepatitis. Front. Mol. Biosci. 8, 715765.
Ulrich, J.D., et al., 2014. Altered microglial response to Aβ plaques in APPPS1-21 mice heterozygous for TREM2. Mol. Neurodegener. 9, 20.
van Dyck, C.H., et al., 2023. Lecanemab in early Alzheimer's disease. N. Engl. J. Med. 388, 9–21.
Venegas, C., et al., 2017. Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease. Nature 552, 355–361.
Villemagne, V.L., et al., 2013. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol. 12, 357–367.
Wang, X., et al., 2021. NLRP3 inflammasome inhibitor CY-09 reduces hepatic steatosis in experimental NAFLD mice. Biochem. Biophys. Res. Commun. 534, 734–739.
Wilson, R.S., Leurgans, S.E., Boyle, P.A., Bennett, D.A., 2011. Cognitive decline in prodromal alzheimer disease and mild cognitive impairment. Arch. Neurol. 68.
Wolf, A.J., et al., 2016. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. Cell 166, 624–636.
Wouters, Y., Jaspers, T., De Strooper, B., Dewilde, M., 2020. Identification and in vivo characterization of a brain-penetrating nanobody. Fluids Barriers CNS 17, 62.
Xiao, L., Magupalli, V.G., Wu, H., 2023. Cryo-EM structures of the active NLRP3 inflammasome disc. Nature 613, 595–600.
Y, H., My, Z., D, Y., B, M., G, N., 2016. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature 530.
Zhang, X., et al., 2020. Oligodendroglial glycolytic stress triggers inflammasome activation and neuropathology in Alzheimer's disease. Sci. Adv. 6, eabb8680.
Zheng, D., Liwinski, T., Elinav, E., 2020. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. Cell Discov 6, 36.
Zhou, B., Abbott, D.W., 2021. Gasdermin E permits interleukin-1 beta release in distinct sublytic and pyroptotic phases. Cell Rep. 35, 108998.
Zolochevska, O., Taglialatela, G., 2016. Non-demented individuals with Alzheimer's disease neuropathology: resistance to cognitive decline may reveal new treatment strategies. Curr. Pharm. Des. 22, 4063–4068.