PhD-FSTC-2015-56
The Faculty of Sciences, Technology and Communication

DISSERTATION

Defense held on 26/1/2015 in Luxembourg

to obtain the degree of

DOCTEUR DE L'UNIVERSITÉ DU LUXEMBOURG

EN BIOLOGIE

by

Audrey GUSTIN

Born on 12th August in Namur (Belgium)

INFLAMMASOME IMPLICATION IN NEURODEGENERATIVE DISEASES.

- A role for inflammasome in Parkinson's disease -

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INFLAMMASOME IMPLICATION IN NEURODEGENERATIVE DISEASES.

- *A role for inflammasome in Parkinson's disease?* –

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To my family and friends

I hereby declare that this dissertation has been written only by the undersigned and that no sources have been used in preparation of this thesis other than those cited.

Luxembourg, October 2015

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Abstract

Neuroinflammation is the local reaction of the brain to infection, trauma, toxic molecules or protein aggregates. The brain resident macrophages, microglia, are able to trigger an appropriate response involving secretion of cytokines and chemokines, resulting in the activation of astrocytes and recruitment of peripheral immune cells. The activated astrocytes also produce pro-inflammatory molecules that can lead to a vicious inflammatory circle in the brain. This toxic environment produced by both cell types seems to be one of the causes of neuronal death in neurodegenerative diseases. IL-1β plays an important role in this response; yet its expression and mode of action in the brain are not fully understood and its precise implication in neurodegenerative diseases needs further characterization.

Therefore, the aim of this study was to obtain a better understanding of the role of the inflammasome-dependent IL-1β signalling within the brain as well as its possible implication in neurodegenerative diseases. The main objectives were *i)* to profile the expression and regulation of inflammasome in microglia and astrocytes, *ii)* to test the reactivity of inflammasome to compounds-related to neurodegenerative diseases *iii)* to investigate the implication of inflammasome in Parkinson's disease (PD).

Our results indicate that the capacity to form a functional NLRP3 inflammasome and to secrete IL-1β is limited to the microglial compartment in the mouse brain. Indeed, we were not able to observe IL-1β secretion from astrocytes, nor do they express all NLRP3 inflammasome components. The capacity of these cells to express others inflammasome complexes also seems compromised, contrarily to microglia which exhibit the potentiality to express AIM2, NLRC4 or non-canonical inflammasome.

In addition to IL-1β, microglia were able to secrete IL-18, IL-1α and HMGB1 in an NLRP3 inflammasome-dependent way and through mechanisms similar to those observed in macrophages. Moreover, microglia stimulation with neurodegeneration-related compounds, such as amyloid-β peptide, rotenone or ATP resulted in the inflammasome activation and IL-1β release, suggesting that the microglial inflammasome can play a role in the neuroinflammation observed during neurodegenerative disease. Finally, if NLRP3 is linked to Alzheimer's pathogenesis, our preliminary results tend to indicate that genetic ablation of NLRP3 do not exert any significant impact on the neurodegenerative processes occurring in an *in vivo* model of Parkinson's disease. However, deeper investigations will be needed to better define the role of the inflammasome and its targets in PD.

Key words:

Microglia – Astrocytes – Neuroinflammation - Inflammasome – IL-1 – Parkinson's disease

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Abbreviations

6-OHDA	6-hydroxydopamine
α-syn	Alpha-synuclein
AD	Alzheimer's disease
ADP	Adenosine di-phosphate
AEC-M1	Astrocyte-enriched cultures (once sorted)
AEC-M2	Astrocyte-enriched cultures (twice sorted)
AIM2	Absent In Melanoma 2
ALR	AIM2-like receptors
ALS	Amyotrophic lateral sclerosis
AP1	Activator protein 1
APCs	Astrocyte progenitor cells
APP	Amyloid precursor protein
ASC	Apoptosis-associated speck like protein containing a CARD
ATP	Adenosine tri-phosphate
Aβ	Amyloid-beta
BACE-1	Beta-site amyloid precursor protein cleavage enzyme-1
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BMDM	Bone marrow-derived macrophages
BMPs	Bone morphogenetic proteins
BSA	Bovine serum albumin
CAPS	Cryopyrin-associated periodic syndromes
CARD	Caspase activation and recruitment domain
CCM	Complete cytokine mix
cDNA	complementary Desoxyribonucleic acid
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL10	Chemokine (C-X-C motif) ligand 10
Cytod	Cytochalasin D
Da	Dalton
DAMPs	Danger/Damage-associated molecular patterns
DMEM	Dulbecco's modified Eagle medium
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleic acid
DTT	Dithiothreitol

DV	Dorso-ventral
E~	Embryonic day ~
EAE	Experimental autoimmune encephalomyelitis
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EMPs	Erythromyeloid progenitors
FBS	Fetal bovine serum
FCAS	Familial cold autoinflammatory syndrome
FGF	Fibroblast Growth Factor
GABA	gamma-Aminobutyric acid
GBA	Glucocerebrosidase
GFAP	Glial fibrillary acidic protein
HD	Huntington's disease
HMGB1	High mobility group box 1
HRP	Horseradish peroxidase
ICE	IL-1beta converting enzyme
IFI16	Interferon gamma-inducible protein 16
IFNγ	Interferon gamma
IL-18bp	Interleukin 18 binding protein
IL-18R	Interleukin 18 receptor
IL-1R	Interleukin 1 receptor
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
JAK/STAT	Janus kinase/Signal transducer and activator of transcription
KCl	Potassium chloride
KO	Knockout
LDH	Lactate deshydrogenase
LPS	Lipopolysaccharide
LRR	Leucin-rich repeat
LRRK2	Leucine-rich repeat kinase 2
MACS	Magnetic cell sorting
MAP	Mitogen-activated protein
M-CSF	Macrophages colony-stimulating factor
MDP	Muramyl dipeptide
MGC P0	Mixed glial cell cultures (passage 0)
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRs	Mannose receptors
MS	Multiple sclerosis
MSU	Monosodium urate crystals

MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
MW	Multiwell
MWS	Muckle-Wells Syndrome
NAC	N-acetyl-L-cysteine
NAIPs	NLR-family apoptosis-inhibiting proteins
NBD	Nucleotide-binding domain
NF-κB	Nuclear factor kappa B
NLR	Nucleotide-binding domain and leucin-rich repeat containing receptor
NO	Nitric oxide
NSC	Neural stem cell
OPC	Oligodendrocyte progenitor cell
P3C	Pam3CSK4
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate buffer saline
PD	Parkinson's disease
PGE2	Prostaglandin E2
PNS	Peripheral nervous system
PRRs	Pattern recognition receptors
PYD	Pyrin domain
PYHIN	Pyrin and HIN domain-containing protein
RAGE	Receptor for advanced glycation end-products
RGC	Radial glial cell
RIG-I	Retinoic acid-inducible gene I
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Real-Time Polymerase chain reaction
SCI	Spinal cord injury
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNpc	Substantia nigra pars compacta
SOD-1	Superoxide dismutase 1
SRs	Scavenger receptors
T3SS	Type III secretory system
TBI	Traumatic brain injury
TLRs	Toll-Like receptors
TNFα	Tumor necrosis factor alpha
WB	Western blot
WT	Wild-type

I. Introduction

Part I: The Central Nervous System

The nervous system is divided into two parts: the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). The CNS includes the brain and the spinal cord. It integrates the information and coordinates the activity of all parts of the body. The PNS includes ganglia (spinal and autonomic) and nerves. It is in charge of the communication between the brain and the sensory organs, the muscles, the glands and the blood vessels. In this introduction, we will focus our attention on the CNS.

1. Development of the CNS

In mammals, the CNS development begins at the end of the gastrula stage with the induction of the neuroectoderm and the neural plate formation. This structure is arising from a layer of primary progenitors known as neural stem cells (NSCs). During the CNS development, NSCs undergo cellular differentiation which restricts the potentiality of progenitor cells to finally give rise to mature neurons, astrocytes and oligodendrocytes (Fig. 1; Paridaen & Huttner, 2014).

Two important steps drive CNS development: the neurogenesis and the gliogenesis. At the onset of neurogenesis, around embryonic day 12 (E12) in mice, the NSC switches from a symmetric to an asymmetric division mode, giving rise to a NSC daughter cell in addition to an immature neuron (Miller & Gauthier, 2007). Interestingly, during this phase, NSCs also undergo a phenotypic change to become Radial Glial cells (RGCs) which support the migration of the neurons (Götz & Huttner, 2005). Once their final location reached, the immature neurons integrate the complex neuronal network by developing their projections and their neurotransmitter secretion capacity.

The switch from neurogenesis to gliogenesis occurs around E16-18 (in mice cortex) and is controlled by both extrinsic and intrinsic signals. Most of these embryonic developmental signals include bone morphogenetic proteins (BMPs), JAK (Janus kinase)/ STAT (Signal Transducer and Activator of Transcription), Notch, Noggin and Sonic hedgehog signalling pathways (Jiang & Nardelli, 2015). During gliogenesis, NSCs successively give rise to astrocyte progenitor (APCs) and oligodendrocyte progenitor cells (OPCs ; Xing et al, 2014). As with the development of any cell lineage, astrocyte and oligodendrocyte differentiation requires a sequential series of events that result in the

generation of a mature cell population that actively participates in CNS physiology, as presented hereafter.

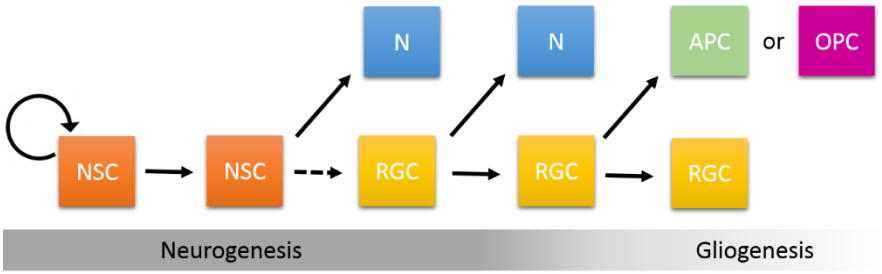


Fig. 1: Simplistic view of brain cell development.

Neurons and macroglia derive from a common progenitor, the neural stem cell (NSC), and differentiate trough two successive steps: neurogenesis and gliogenesis. RGC = Radial Glial Cell, N= immature neuron, APC = Astrocyte Progenitor Cell, OPC = Oligodendrocyte Progenitor Cell. Adapted from (Xing *et al*, 2014)

2. Cell populations

In the adult brain, three major cell populations compose the CNS: Neurons, Macroglia (Oligodendrocytes and Astrocytes) and Microglia. Nowadays, their numerical repartition in the adult brain is always debated. A widespread belief is that glia makes up more than 80% of the cells in the human brain. However, recent reports suggest that, at least in the human brain, this ratio is much closer to 1:1(Lent *et al*, 2012). In any case, all brain cell types deserve attention considering their important roles and specificities.

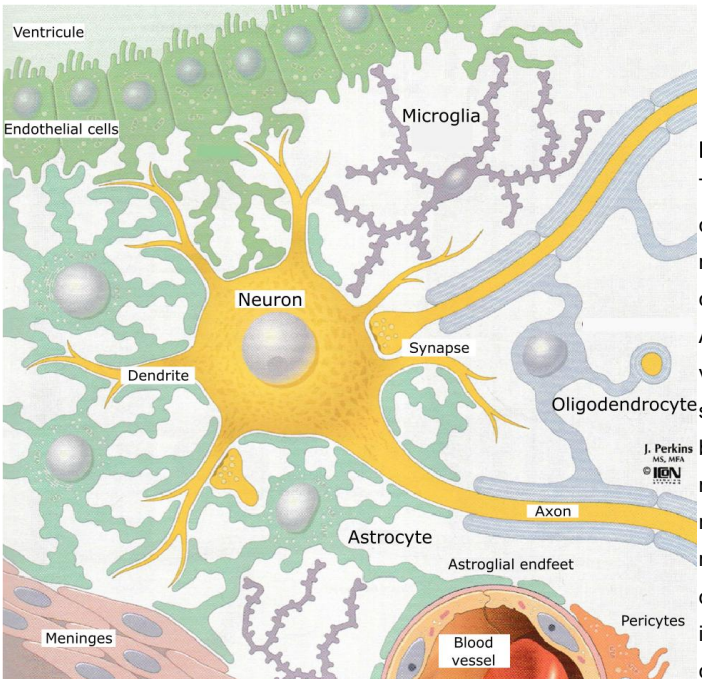


Fig. 2: The different cells of the CNS.

The brain is composed of neurons and glial cells. Glia can be divided into two classes: macroglia and microglia. The macroglial cells comprise astrocytes and oligodendrocytes. Astrocytes make the connection between blood vessels and neurons, participate to the neuronal synaptic communication and contribute to the blood brain barrier. Oligodendrocytes form the myelin sheath around the axon to speed the neuronal signal transmission. Microglia are responsible for the monitoring of the brain for damage or infections. They are also implicated in neuronal development and neural connectivity.

Adapted from (Felten, 2006).

2.1. Neurons

Neurons are about 100 billion in the human adult brain (Lent *et al*, 2012). They are the excitable cells that process and transmit the information across the body.
Neurons represent a heterogeneous cell population. They can differ by their size, by their shape (bipolar, multipolar or unipolar) or by their functions. However, all mature neurons share common characteristics. They possess a cellular body (or soma), representing the metabolic centre, from which extend two different types of processes: dendrites and axons (Fig. 2). The dendrites collect and transport the afferent information to the cellular body by a modification of the cell membrane polarization. The stimuli, which have traveled down the dendrites, converge at the axon hillock where they are summed to determine the neuronal response. If the sum of the stimuli reaches a certain voltage, known as the threshold potential, depolarization will be transmitted to the axon. Most of the axons are insulated at regular intervals by the myelin sheaths produced by oligodendrocytes (see below) in view to accelerate signal transmission (Zoupi *et al*, 2011).

Each neuron is connected to another one by a structural cleft called synapse. A synapse includes the pre-synaptic neuron membrane, the post-synaptic neuron/organ/cell membrane and the intercellular space between them. The information exchange taking place in the synapse is called neurotransmission (or synapse signalling). It aims to propagate information. The neurotransmission can be electrical or chemical. The electrical one transmits directly the impulse via gap junctions established between both neurons. On the other hand, in the chemical synapse, the pre-synaptic neurons are in charge of the release of neurotransmitters into the cleft to spread the information. The neurotransmitters will interact with post-synaptic membrane receptors to initiate the membrane depolarization and are quickly removed from the synaptic space to avoid excessive activation (Nelson, 1993). The most common neurotransmitters are γ-aminobutyric acid (GABA – inhibitory) and glutamate (excitatory). However, other neurotransmitters exist and determine six main neuronal systems: Noradrenaline/Noradrenergic, Adrenaline/Adrenergic, Dopamine/Dopaminergic, Serotonine/Serotonergic, Acetylcholine/Cholinergic and Histamine/Histaminergic neurons (Pradhan *et al*, 2014).

2.2. Oligodendrocytes

Oligodendrocytes are the myelinating glial cells of the CNS: they electrically insulate the axons by forming myelin sheaths around them. This myelinisation process is a remarkably specialized cellular interaction specific to vertebrates. It enables the saltatory

conduction, in which a neuronal action potential is propagated between myelin sheet gaps (nodes of Ranvier) to increase both the speed and energy efficiency of nerve conduction. A single oligodendrocyte is able to myelinate up to 60 segments of different axons (Scholze & Barres, 2012). In addition to the electrically insulation of axons, oligodendrocytes also provide them trophic support and promote the neuron viability via a metabolic coupling, where oligodendrocytes provide lactate to axons as an energy source (Lee *et al*, 2012).

2.3. Astrocytes

Astrocytes are the major glial cell type in the brain. They represent a heterogeneous population of cells that exhibit different structural, functional, chemical and molecular characteristics.

2.3.1. Astrocytes: a heterogeneous population

Classically, astrocytes are classified into two major types: the protoplasmic astrocytes and the fibrous astrocytes. Protoplasmic astrocytes, found in the grey matter, are spongiform and possess a lot of extensive fine branching. They particularly ensure the blood-brain barrier (BBB), the homeostatic and the metabolic astrocytic functions (see below). The fibrous astrocytes are located in the white matter and are characterized by fewer long processes which form an axonal support network. However, over the years, studies have revealed that astrocytes are much more heterogeneous than the classical and simple distinction between fibrous and protoplasmic astrocytes (Bribian *et al*, 2015). Indeed, the astrocytic population encloses different groups of cells which differ in their morphology, developmental, origin, gene expression profile, electrophysiological properties, function and response to injury and disease (Zhang & Barres, 2010; Oberheim et al, 2012).

Currently, astrocytes are defined by a the expression of different markers such as Glial Fibrillary Acidic Protein (GFAP), the glutamate transporters GLAST/EATT1 and GLT-1/EAAT2 or the calcium binding protein S100β among others (Schitine *et al*, 2015). However, the expression of these markers depends on the considered brain structure, varies over time and is also observed in others cells, without clear evidence if they are astrocytes or not (*e.g.* radial glial cells, retinal Muller cells or Bergmann glia ; Khakh & Sofroniew, 2015; Rossi, 2015). Therefore, deeper investigations are needed to better

define the astrocyte notion, especially by the characterization of type-specific markers (Bayraktar et al. 2015; A. V. Molofsky et al. 2012).

2.3.2. Astrocyte functions

Consistent with their heterogeneity, astrocytes ensure a large variety of functions in order to preserve the brain homeostasis.

The Blood Brain Barrier (BBB). An important characteristic of the brain parenchyma is that its communication with the circulatory system is tightly regulated. The BBB, a physical and functional separation between the CNS and the peripheral blood circulation, plays this role by preventing the diffusion of harmful elements into the brain (Abbott *et al*, 2006). The main members of BBB are the endothelial cells, the pericytes, the astrocytes and the extracellular matrix (Obermeier *et al*, 2013). Astrocytes possess specialized endfeets that cover about 99% of the abluminal surface of cerebral vessels. Their role is to surround the endothelial cells by providing a structural support (Prat *et al*, 2001; Abbott *et al*, 2006). Astrocytes also release morphogens and growth factors, such as fibroblast growth factor (FGF) 2 and 5, that modulate pathways involved in the regulation of BBB integrity by maintaining the endothelial tight junctions (Reuss *et al*, 2003; Obermeier *et al*, 2013).

The neurovascular unit is the functional association of neurons and blood vessels by the intermediate of astrocytes. Astrocytes are responsible for the energy supply of neurons. When neurons increase their energy need, they release some factors sensed by astrocytes (such as glutamate). These signals lead astrocytes to modify the blood stream in order to upregulate the glucose uptake. Astrocytes can convert this glucose to glycogen for storage (major brain energy store) or can transform glucose into lactate which will in turn be delivered to neurons to satisfy their increased metabolic demands (Tsacopoulos & Magistretti, 1996).

Tripartite synapse. Astrocytes play a fundamental role in the homeostasis of synapses by enwrapping the pre- and post-synaptic cleft to form a tripartite system. The main role of this organization is to control the water quantity as well as the concentration of metabolites and neurotransmitters in the synapse. They ensure, for example, the clearance of potassium or glutamate, two substances becoming neurotoxic if present in excess (Oliveira Da Cruz et al. 2015; Sibille, Pannash, and Rouach 2013). Astrocytes are also able to sense the synaptic activity through the stimulation of their ion channels,

neurotransmitter transporters and receptors. Once stimulated, calcium signalling takes place in astrocytes with a subsequent uptake or release of gliotransmitters such as glutamate, ATP, and D-serine or adenosine. These transmitters can directly act on post-synaptic neurons or can modulate the release of neurotransmitters by pre-synaptic neurons (Halassa *et al*, 2007; Perea *et al*, 2014). In addition, astrocytes also play a role in synapse maintenance and neurite outgrowth (Allen, 2014; Rossi, 2015).

Reactive astrogliosis. Astrocytes respond to all forms of CNS insult such as infection, trauma, ischemia and neurodegeneration by a process called “reactive astrogliosis” (Burda & Sofroniew, 2014). The major roles of reactive astrogliosis are to protect the brain parenchyma, seal off damaged areas, reconstruct the BBB and promote the correct remodelling of the neural circuitry. This aspect of the astroglial function will be detailed in the second part of this introduction (Part II: “The neuroinflammation”).

2.4. Microglia

Microglia are the resident macrophages of the CNS. In the mature brain, microglia are dispersed throughout the whole CNS, although their density can be higher in some brain regions such as in the hippocampus and the substantia nigra (Lawson *et al*, 1990).

2.4.1. Origin

In contrast to the previously mentioned brain cells, microglia do not have an ectodermal origin.

For decades, microglial cells have been described as having a mesodermal origin, derived from hematopoietic stem cells in the bone marrow. This dogma has recently been rebutted by elegant experiments demonstrating that microglia derive from primitive macrophages produced in the Yolk sack (Fig. 3, Ginhoux *et al*, 2010; Schulz *et al*, 2012; Lourbopoulos *et al*, 2015). These progenitors infiltrate the brain through blood vessels between E8.5 and E9.5 (Ginhoux *et al*, 2010; Ransohoff *et al*, 2015), just before the onset of neurogenesis and the BBB implementation (Dahlstrand *et al*, 1995). Then, the isolation of microglia from the periphery contribute to the maintenance of their difference from circulating immune cells (Schulz *et al*, 2012; Ginhoux *et al*, 2013) and to their unique transcriptional signature (Butovsky *et al*, 2014). Thus, even if microglia are classically defined as macrophages of the brain, recent evidence suggests that they differ considerably from the macrophages residing in other tissues.

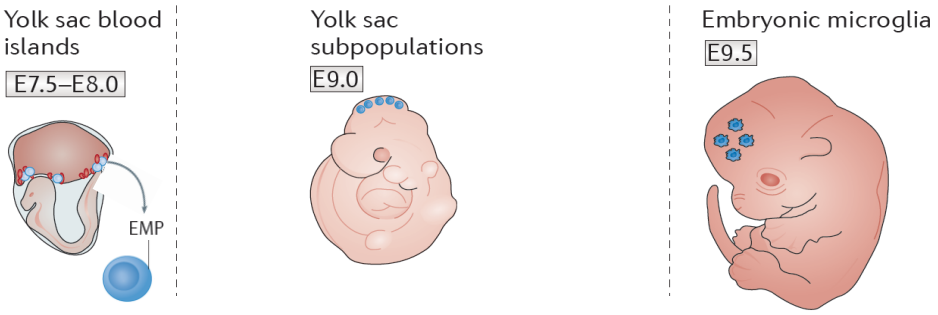


Fig. 3: Microglial ontogeny.

A transient early wave of myeloid cell development called primitive hematopoiesis takes place at E7.5–E8.0 in the yolk sac in mice. Around E9, the erythromyeloid progenitors (EMPs) populate the brain parenchyma and give rise to the embryogenic microglia. These early microglia reside in the brain throughout life. Adapted from (Prinz & Priller, 2014).

The microglial turnover in the healthy adult brain is ensured by their self-renewal capacity and is totally independent from the bone-marrow derived macrophages (Elmore *et al*, 2014). The understanding of microglial origin is experiencing a revolution, and many aspects of their physiology are being redefined.

2.4.2. Roles of Microglia

Microglia are often described as the macrophages of the brain as they form the first line of defence against pathogen invasion, injury or disease. Their implication in immunity is indeed well characterized. Phagocytic microglia can detect and quickly remove pathogens, damaged or dying cells and clear the debris.

However, it is now outdated to consider microglia as being only the brain's immune cells (Fig. 4). There is increasing evidence that resting microglia play an important role during brain development and the establishment of neuronal connectivity. Microglia participate in removing apoptotic neurons during embryogenesis but also in the adult brain (Caldero *et al*, 2009; Sierra *et al*, 2010). Microglia may also phagocyte unwanted dendrites and synapses, thus participating in the axon pruning process (Nimmerjahn *et al*, 2005; Wake *et al*, 2009; Tremblay *et al*, 2010). In addition, they are able to secrete brain-derived neurotrophic factor (BDNF), which is implicated in the learning-dependent synapse formation (Parkhurst *et al*, 2013). Microglia also promote the neuron survival and axonal growth by providing other growth factors to neurons (Ueno *et al*, 2013).

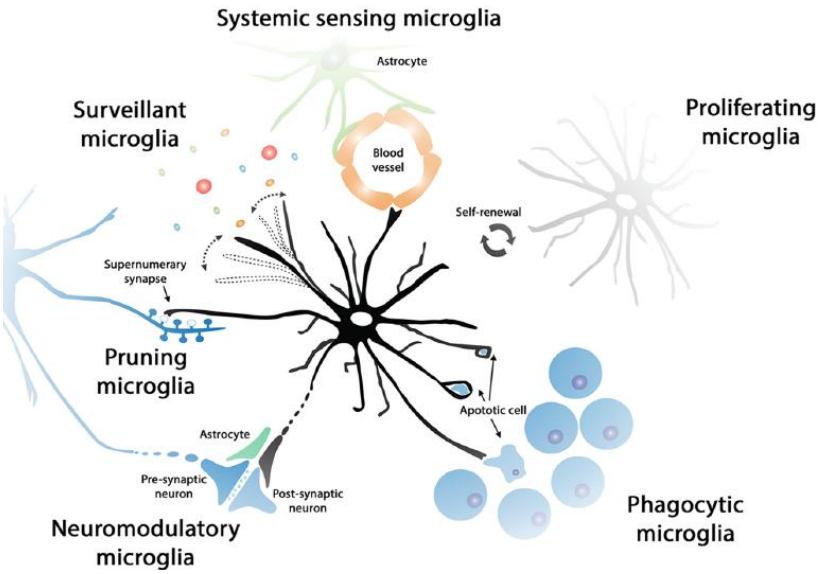


Fig. 4: Functional roles of microglia in healthy brain.

The population of microglial cells is maintained by self-renewal. Surveillant microglial cells constantly scan the brain microenvironment, in order to detect perturbations of CNS homeostasis. Phagocytic microglia can detect and quickly remove damaged or dying neurons, preventing the injury of neighbouring cells. The phagocytic capacity of microglia is particularly important in development (pruning microglia), when they can contribute to the removal of supernumerary synapses in neuronal pathways. Moreover, it has been suggested that microglia can have a direct or indirect modulatory role at the synapse, influencing neuronal activity (neuromodulatory microglia ; Gomez-Nicola & Perry, 2015).

3. Neurodegenerative diseases

Dysfunction of the different brain cell types can lead to neurological disorders, in particular to neurodegenerative diseases. These disorders characterized by a strong neuronal loss in specific brain areas, include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease and Amyotrophic Lateral Sclerosis (ALS). In the following sections, we will focus our attention on AD and PD.

3.1. Alzheimer's disease

AD is characterized by memory loss, progressive cognitive impairment, and neuropsychiatric disturbances. The histopathological hallmarks of AD comprise amyloid-β (Aβ) plaques, formation of neurofibrillary tangles and neuroinflammation. Together, they lead to the death of glutaminergic neurons, cholinergic neurons and interneurons in cortical and subcortical regions (Rubio-Perez & Morillas-Ruiz, 2012; Heneka *et al*, 2013).

The vast majority of AD cases (95%) are sporadic forms while the remaining cases (5%) are caused by mutations conferring an hereditary profile to the disease (Calero *et al*, 2015). Interestingly, these AD-linked mutations often lead to an Aβ overproduction.

The Aβ peptide is formed from the sequential proteolysis of the amyloid precursor protein (APP) present on the neuronal cell surface. APP can be processed by two different mechanisms: the non-amyloidogenic (physiological) and the amyloidogenic (pathological) pathways (Fig. 5). The non-amyloidogenic pathway involves a first cleavage of APP by a α-secretase and a second cleavage within the membrane by a γ-secretase. The α-secretase cleaves APP in the Aβ domain, inhibiting the production of the Aβ peptide. In contrast, the amyloidogenic pathway is directly responsible for the formation of the Aβ peptide. In this case, APP is first cleaved by BACE-1 (β-site amyloid precursor protein cleavage enzyme-1), which liberates a sAPPβ fragment. This one is further processed by the γ-secretase which releases the 40 or 42 amino acids long Aβ peptides into the extracellular compartment (Haass *et al*, 2012).

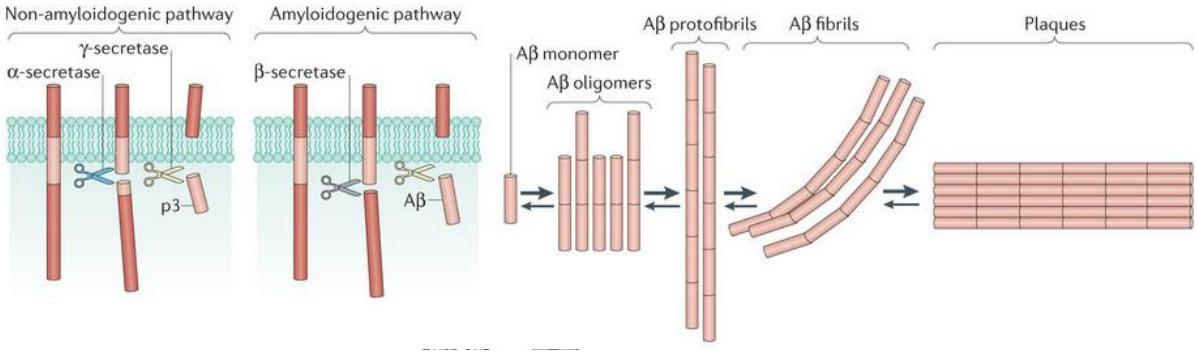


Fig. 5: Aβ processing.

The processing of the APP can be achieved through two pathways: amyloidogenic and non-amyloidogenic pathways. The non-amyloidogenic pathway is accomplished by the successive action of the α-secretase and γ-secretase. The amyloidogenic pathway is performed by the action of BACE-1 followed by the γ-secretase. This pathway leads to the production of the Aβ and the sAPPβ fragment. Aβ monomers may then go on to form oligomers, protofibrils, fibrils and amyloid plaques in case of pathology (Querfurth & LaFerla, 2010).

In the healthy brain, Aβ is released within synapses to dampen excitatory transmission and to prevent neuronal hyperactivity (Kamenetz *et al*, 2003). The imbalance between the production and the clearance of Aβ is one of the causes of the aggregation of the peptide. Aβ is able to self-associated and form soluble oligomers which can further aggregate and give rise to insoluble fibrillary plaques (Querfurth & LaFerla, 2010).

Once Aβ peptides accumulated, as observed in AD, they can be further proteolytically processed, chemically modified, or cross-linked, thereby modifying their

relative insolubility, stability, and toxicity. This is why the Aβ peptides identified in amyloid plaques
vary in their amino acid composition, ranging from the full-length Aβ (1-40/42) to shorter carboxy-terminal or amino-terminal Aβ peptides (Wiltfang *et al*, 2002). Among the shorter Aβ peptides, the 25-35 fragment is endogenously found in elderly people and has been described to be more toxic form (Millucci *et al*, 2009). Moreover, the $A\beta_{25-35}$ affects the cognitive processes when injected in rat CNS (Stepanichev *et al*, 2005; Limón *et al*, 2009).

Currently, it is still debated whether Aβ plaques are part of the neurodegenerative process itself or whether they are the consequence of neuronal loss. In both cases, the accumulation of Aβ peptide is thought to contribute to the development of the pathology at least by enhancing inflammation and neuronal disturbances.

3.2. Parkinson's disease

PD is the second most frequent neurodegenerative disorder. Similar to AD, PD affects predominantly older people and its prevalence increases with age from 428 at 60-69 years to 1,903 per 100,000 in 80 years old people (Pringsheim *et al,* 2015). PD is mainly characterized by a triad of motor impairments, *i.e.* bradykinesia, resting tremor and rigidity but also by non-motor symptoms such as depression, anxiety and dementia. The principal neuropathological hallmark of PD is the progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) which is responsible for the motor dysfunction. Other PD characteristics are the presence of protein inclusions called Lewy Bodies and the chronic neuroinflammation (Phani *et al*, 2012).

The aetiology of PD is thought to result from an interaction between aging, environmental and genetic factors (Herrero *et al*, 2015). The exposure to pesticides (*i.e.* rotenone), herbicides (*i.e.* paraquat), air pollutants or metal iron has been identified as a key risk factor to develop sporadic PD (Litteljohn *et al*, 2010). The genetic forms of the disease (5%) are linked to rare mutations which increase the susceptibility to develop the disease. To this date, mutations associated to PD have been identified in several genes: leucine-rich repeat kinase 2 (*Lrkk2*), glucocerebrosidase (*Gba*), *Park2* and *Parkin7* (coding respectively for Parkin and DJ-1), *Pink1*, *Mapt* genes and more importantly *Snca* gene (Xu & Chan, 2015). In human, six point mutations in *Snca* have been identified and are linked to PD. They are listed in table 1 (Xu & Chan, 2015).

Table 1: Missense mutations in *Snca* gene causing autosomal dominant Parkinson's disease.

Point mutations		References
Ala30Pro	A30P	(Jensen *et al*, 1998)
Ala53Thr	A53T	(Farrer *et al*, 1998)
Glu46Lys	E46K	(Zarranz *et al*, 2004)
His50Gln	H50Q	(Proukakis *et al*, 2013)
Gly51Asp	G51D	(Kiely *et al*, 2013)
Ala53Glu	A53E	(Pasanen *et al*, 2014)

Snca gene encodes for a small protein named α-synuclein (α-syn). α-syn is an abundant protein in the brain, and notably in the substantia nigra. Until now, the precise role of α-syn remains unknown. However, consistent with its initial description in neuronal nuclei and presynaptic terminals (Maroteaux *et al*, 1988), it has been proposed that α-syn associates with vesicular and membranous structures in order to play a role in neurotransmitter management (Bendor *et al*, 2013). α-syn proteins can adopt an altered conformation under pathological conditions, leading to the cytotoxic formation of oligomers, amyloid fibrils and finally Lewy bodies (Fig. 6 ; Conway *et al*, 2000; Fujiwara *et al*, 2002).

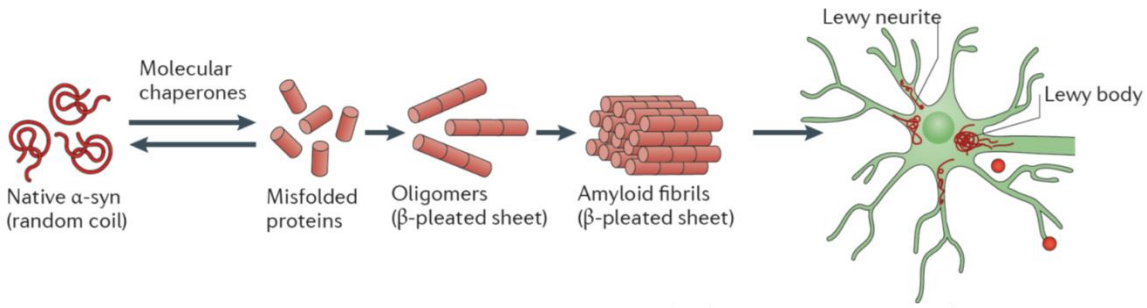


Fig. 6: Hypothetical model of α-syn toxicity in PD.

Under physiological conditions, α-syn exists in a soluble random coil state. Under pathological conditions, the native protein undergoes misfolding into pathogenic α-syn oligomers that further aggregate into amyloid fibrils. Ultimately, these structures are the building blocks for the pathological inclusions of α-syn called Lewy bodies. Adapted from (Irwin *et al*, 2013).

It is still not well understood how these α-syn aggregates harm neurons. Nevertheless, different hypotheses have been proposed such as their ability to induce an

oxidative stress, to inhibit the protein degradation system or to impair the normal dopamine neurotransmission (Xu & Chan, 2015). In addition, α-syn as well as its mutant forms, can activate microglia and thus promote the neuroinflammation (Zhang *et al*, 2005; Lee *et al*, 2010; Roodveldt *et al*, 2010).

Part II: The neuroinflammation

1. The inflammatory response

1.1. Generalities

Inflammation is a normal, immediate and transient response to external aggressions compromising the integrity of the body. It is defined by the four cardinal signs of inflammation: redness, heat, swelling and pain described over 2,000 years ago by Celsus.

However, the inflammation process is much more complex and involves specific molecular and cellular responses to infection, injury or stress. It aims to defend the body against insults, to clean dead and damaged cells and to restore the homeostasis.

Inflammation is classically viewed as an immediate and transient response of the immune system. However, it happens that inflammation becomes chronic when the injury or infection persists or during autoimmune diseases. Both acute and chronic inflammation differ by the rapidity of their onset (fast / slow), the types of involved immune cells (granulocytes / monocytes and lymphocytes), or the resulting tissue injury (Mild / Severe and progressive).

1.2. Inflammatory signalling pathways

Inflammation is classically triggered when immune cells detect the presence of specific signals by the germ-line encoded receptors also known as Pattern Recognition Receptors (PRRs). PRRs specifically recognize either pathogen-associated molecular pattern (PAMPs) or sterile danger/damage-associated molecular patterns (DAMPs). PAMPs include for example bacterial- and viral-derived carbohydrates, nucleic acids or lipoproteins whereas DAMPs comprise molecules such as ATP (Adenosine tri-phosphate), uric acid, HMGB1 (High mobility group box 1) or heat-shock proteins (Kigerl *et al*, 2014). Under normal, non-inflammatory conditions, DAMPs exhibit cellular compartmentalization preventing a physiological interaction with their specific PRRs. Under pathological conditions, however, DAMPs are either passively released from injured/inflamed tissues, dying cells or actively secreted (Feldman *et al*, 2015).

The most common PRRs family is represented by the Toll-like receptors (TLRs). Besides, PRRs also include the intracellular Nod-like Receptors (NLRs, described below),

Scavenger receptors (SRs), Mannose Receptors (MRs) and complement receptors (Farina *et al*, 2007; Kigerl *et al*, 2014). Following the recognition of PAMPs/DAMPs, PRRs trigger intracellular signalling pathways including NF-κB (Nuclear Factor-κB), MAP (Mitogen-Activated Protein) kinase, JAK/STAT and AP1 (Activator Protein 1) pathways. These signalling pathways lead to the production of a large variety of pro-inflammatory factors that can induce a positive regulatory loop to amplify their inflammatory responses.

Among these pro-inflammatory products, the most notable are nitric oxide (NO), reactive oxygen species (ROS), cytokines and chemokines. An important group of inflammatory mediators is the family of cytokines which are small proteins including interleukins, interferons, tumor necrosis factor, adipokines and lymphokines. Interleukin-1beta (IL-1β) and tumor necrosis factor alpha (TNFα) are two members of cytokines which have gained much attention in the literature and play pivotal roles during inflammation.

1.3. Sterile inflammation

Inflammation is often considered as the consequence of an infection. However, this process can also be trigger in the absence of pathogen and is then called “sterile inflammation”. It can be caused by mechanical trauma, ischemia, stress or environmental conditions. These damage-related stimuli induce the release of DAMPs which in turn activate the PRRs (*e.g.* TLRs or NLRs) and drive the sterile inflammatory reaction. Sterile inflammation is implicated in disease processes such as gout, atherosclerosis, cancer or neuroinflammatory diseases including AD and PD (Tsung *et al*, 2014; Feldman *et al*, 2015).

2. Neuroinflammation

Even if the access of peripheral immune cells to the CNS is restricted and tightly regulated, it is now commonly accepted that the CNS is capable of dynamic immune and inflammatory responses to a variety of insults (for review: Heneka, Kummer, and Latz 2014; Lyman et al. 2014; Rivest 2009).

Astrocytes and microglia represent the key effectors of the neuroinflammatory response. However, additional cell types are also implicated in this process: 1) CNS intrinsic neural cells (neurons, oligodendrocytes, NG2+ cells, NSCs progenitors), 2) CNS intrinsic non neural cells (pericytes or endothelial cells) and 3) CNS extrinsic non neural

cells (monocytes, macrophages, eosinophils, natural killer cells, T and B cells, among others ; Ransohoff & Brown, 2012).

2.1. Reactive Microglia

The on/off system. In the healthy brain, microglia exhibit a highly ramified shape and are called “resting microglia”. This term is misleading as these cells are not really quiescent. Indeed, microglia constantly scan the brain parenchyma with their long and motile processes in order to detect any homeostasis perturbation. This “resting” state is, in part, actively maintained by surrounding cells, in particular by healthy neurons which provide so called “off signals” such as CX3CL1, CD22, various neurotransmitters or neurotrophins (Biber *et al*, 2007). However, these “off signals” can be altered or missing, for example after neuronal loss. In addition, microglia can also detect specific “on” signals via its PRRs such as the presence of pathogens, cellular debris, critical concentrations of physiological molecules (*e.g.* glutamate, ATP) or presence of misfolded proteins (*e.g.* Aβ or α-syn peptides ; Long-smith *et al*, 2009). In case of “off” signals disruption and/or “on” signals detection, microglia react promptly and shift toward an “activated state”.

Activation state of microglia. This activated state is characterized by an impressive change in proliferation and microglia morphology (*i.e.* the cells are adopting an amoeboid shape). Microglia become also highly motile and migrate to the site of injury. There, they phagocyte damaged cells or debris and generate a particular environment by secreting a large variety of factors: cytokines, chemokines, NO, ROS or growth factors. They also release chemoattractant factors to recruit peripheral immune cells (Prinz *et al*, 2011).

Until recently, the profile of microglial activation was essentially characterized by two extreme phenotypes: the M1 state, which is highly pro-inflammatory, and the M2 state, an alternative activation state rather dedicated to the tissue repair. However, this concept has recently been reappraised, because these two phenotypes were not sufficient to characterise all the activation states of microglia. It is now evident that they have to be considered as a large continuous spectrum of phenotypes with a large variety of functions (Heppner *et al*, 2015). The context, the environment and the surrounding cells (especially astrocytes and neurons) influence and determine all these specific phenotypes (Hanisch & Kettenmann, 2007).

Short and moderate activating signals direct microglia toward a neuroprotective phenotype (Fig. 7). In this context, microglia clear debris by phagocytosis, secrete growth factors and support regeneration. In contrast, intensive acute or chronic activation can render microglia neurotoxic. In this case, they produce ROS, NO, proteases but also a

large plethora of pro-inflammatory cytokines such as IL-1, IL-6 and TNFα. All these factors endanger neuronal activity (London *et al*, 2013).

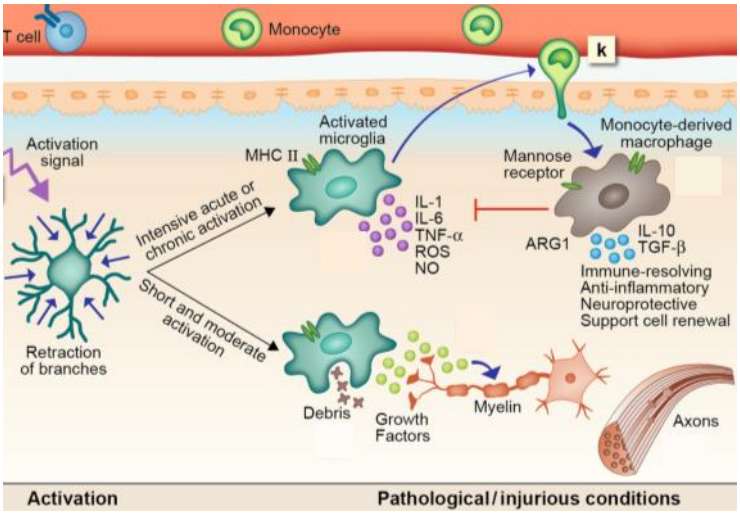


Fig. 7: Microglial activation.

Upon recognition of a danger signal, microglia switch to an activated mode, adopt an amoeboid form and migrate to the injury site. There, they phagocyte damaged cells or debris, secrete a large variety of factors, recruit circulating immune cells and support regeneration. All these events aim to restore the homeostasis in the brain parenchyma. Adapted from (London *et al*, 2013).

2.2. Reactive astrocytes

In case of CNS insults, astrocytes undergo a process of activation called reactive astrogliosis, a hallmark of neuroinflammation. Similarly to microglia, astrocytes can sense endogenous danger signals via the expression of PRRs. These signals are diverse but they mainly result from the microglial response to injury.

The basic process of reactive astrogliosis involves cellular hypertrophy, changes in gene expression and can include astrocyte proliferation after particularly severe insults (Sofroniew & Vinters, 2010). Reactive astrocytes are commonly characterized by an increased synthesis of GFAP and a re-expression of the progenitor markers, vimentin and nestin (Hamby and Sofroniew 2010; Pekny and Nilsson 2005). They also initiate the expression and release of several mediators such as cytokines, chemokines, eicosanoids, ROS, NO and excitatory amino acid that influence the microglial activation and potentiate the recruitment of immune cells to the inflamed sites (Dong & Benveniste, 2001; Lee, 2015).

Accumulating evidence indicates that reactive astrogliosis is not a simple all or none response but could vary in a context-specific manner which corresponds to the level of injury, the astrocyte subtype and the location of the lesion site. Accordingly, the astrocyte phenotypes can be classified into a continuum of progressive activation states from mild

astrogliosis to severe astrogliosis leading to the formation of a glial scar. These different levels are described in figure 8 (Sofroniew and Vinter 2010; Zamanian et al. 2012).

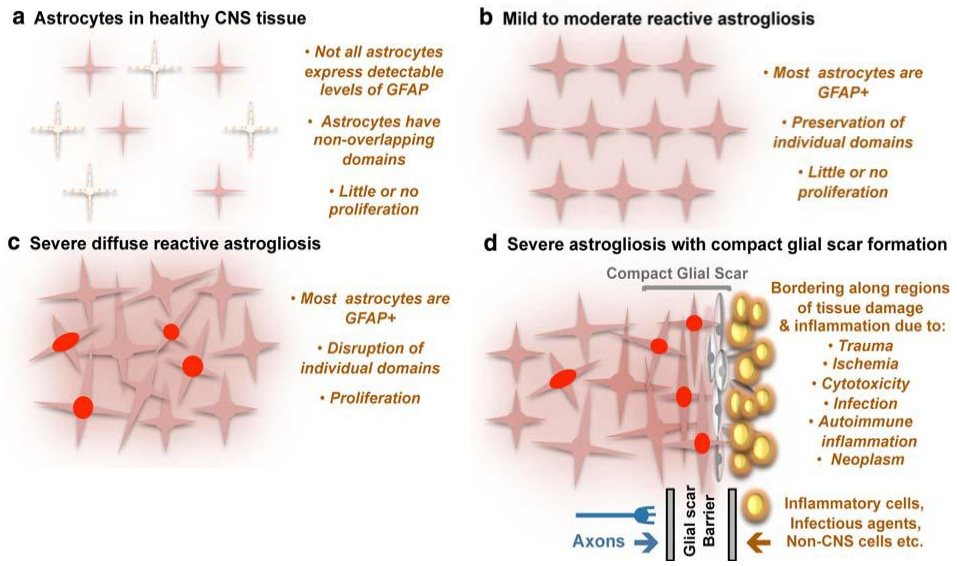


Fig. 8: Reactive astrogliosis.

(a) In healthy CNS tissue, astrocytes occupy a defined domain without overlapping with other cells. (b) In mild to moderate reactive astrogliosis, astrocytes are characterized by anti-inflammatory properties and are found at distal regions from brain injury. There are involved in brain regeneration. (c) In severe diffuse reactive astrogliosis, astrocyte morphology is highly affected. They are located close to the brain injury and release pro-inflammatory cytokines. (d) Further astrocyte activation induces a glial scar formation. This barrier protects the healthy brain region from the spread of inflammation and the damaged tissue. However, it could restrict the regeneration processes (Sofroniew & Vinters, 2010).

2.3. Neuroinflammation: detrimental or not?

Neuroinflammation aims to facilitate the recovery from injury by reducing the size of a lesion, isolating the damaged zone and facilitating wound repair. However, although intended to be beneficial and protective, an excessive inflammation can cause or contribute to tissue damage and disease pathology. The main actors of this neurotoxicity are the pro-inflammatory cytokines and other factors released by both microglia and astrocytes. They can lead to the breakdown of the BBB, the impairment of synaptic activity, the inhibition of the neurogenesis and neuronal regeneration and to neuronal cell death (Lyman *et al*, 2014).

Among the neurotoxic pro-inflammatory factors, the IL-1 family members are well-described. Dysregulated production of these cytokines can give rise to inflammatory diseases and neuronal injury. This is why the production of IL-1 is regulated at multiple levels: transcription, translation, cleavage and cellular release (Allan *et al*, 2005). A key player in this regulation is the inflammasome complex which will be described in the following section.

Part III: The inflammasome

The inflammasome is a large cytosolic multiprotein complex formed upon detection of a large spectrum of infection- or stress-associated stimuli. The assembly of the complex leads to the activation of caspase-1 which is in charge of numerous inflammatory responses, such as the maturation of the cytokines IL-1β and IL-18, the release of alarmins or the initiation of pyroptosis, a characteristic form of caspase-1-dependent cell death (de Zoete *et al*, 2014).

Martinon and collaborators first described the complex in 2002. They reported the identification of a caspase-1-activating complex, which they called “inflammasome” in order to highlight its similarity to the apoptosome complex as well as its particular implication in inflammation (Martinon *et al*, 2002).

1. Inflammasome structure

The inflammasome complex is classically formed of a sensor protein, an adaptor protein and a specific caspase (Fig. 9). Upon activation, the different components assemble through homotypic domain interactions.

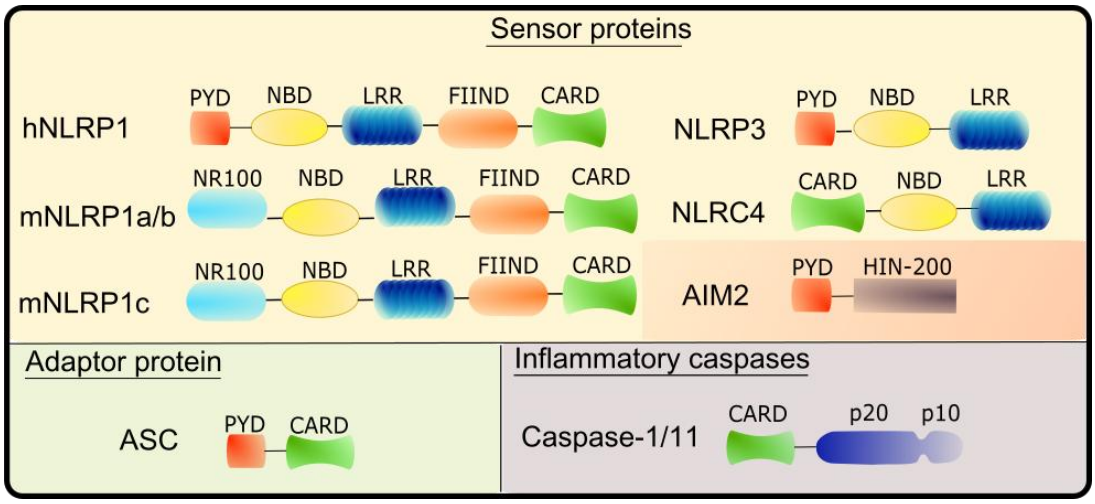


Fig. 9: Principal inflammasome components and their domain structure.

To date, the well-described inflammasomes are the NLRP1, NLRP3, NLRC4 and AIM2 inflammasomes. Their names derived from the sensor protein involved in the complex. Sensor proteins recruit the two other components of the inflammasome, the adaptor protein ASC and the caspase 1/11, by homotypic domain interactions in order to form the inflammasome complex. FIIND = Function to find domain; CARD = Caspase activation and recruitment domain; NBD = Nucleotide-binding domain; PYD = Pyrin domain; NR100 = Amino-terminal domain of rodent NLRP1 of about 100 amino acids; HIN-200 = DNA-binding domain.

1.1 The sensor proteins

Two main protein families act as sensor proteins in inflammasome complexes: the NLR-family (nucleotide-binding domain and leucine-rich repeat containing receptor, previously named NOD-family) and the PYHIN (Pyrin and HIN domain-containing) protein family (Fig.9).

The NLR family consists of 33 genes in mouse and 22 genes in human (Ting *et al*, 2008; Ye & Ting, 2008). All NLRs share a common domain structure: an N-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding domain (NBD) and a C-terminal effector domain in charge of the direct or indirect recruitment of the caspase. This effector domain defines the subfamily classes of NLRs: NLRA (A for acidic transactivating domain), NLRB (B for BIR domain), NLRC (C for CARD), NLRP (P for pyrin domain) and NLRX (X for no significant homology). So far, the capacity to form an inflammasome has been mainly demonstrated for NLRP1, NLRP3, NLRC4, but also AIM2. AIM2 (Absent In Melanoma 2) is a member of the PYHIN protein family and not of the NLR family. Its domain structure is a N-terminal PYD domain coupled to a HIN-200 DNA-binding domain in C-terminus (Fernandes-Alnemri *et al*, 2009).

1.2 The adaptor ASC

The adaptor protein apoptosis-associated speck like protein (ASC) is responsible for the connection between the sensor protein and the caspase in inflammasome complex (Martinon *et al*, 2002; Srinivasula *et al*, 2002). ASC is encoded by the *Pycard* gene and contains two different death-fold domains (Fig. 9): a PYD domain that engages in homotypic interactions with the PYD domain of the sensor protein (NLR or AIM2 ; Fernandes-Alnemri *et al*, 2009; Vajjhala *et al*, 2012) and a CARD domain that is required for the recruitment of caspase-1, again through homotypic protein-protein domain interactions (Proell *et al*, 2013).

Importantly, ASC is dispensable in certain inflammasome complexes such as in NLRP1 and NLRC4 inflammasomes that can directly bind the caspase (Faustin *et al*, 2007; Broz *et al*, 2010). However, some studies propose that ASC might still be required for optimal activation of these complexes (Faustin *et al*, 2007; Case, 2011).

1.3 The pro-inflammatory caspases

Caspases (cysteine-aspartic proteases) are proteolytic enzymes largely known as regulators of cell death and inflammation. This protease family is composed of 12 different members that can be grouped in two distinct classes: apoptotic caspases (casp-2, -3, -7, -8, -9 and -10) and inflammatory caspases (casp-1, -4, -5 / -11) (Shi et al. 2014). More recently, caspases-12 and -14 have been identified but their respective roles are not yet totally understood.

In 1989, a protease responsible for the cleavage of IL-1β into its active form was described for the first time. This protease has been named Interleukin-β Convertase Enzyme (ICE ; Kostura *et al*, 1989). It was rapidly identified as a homolog of the already known caspase-1 (Yuan *et al*, 1993). Caspase-1 is the protease classically involved in the inflammasome complexes. However, recent publications show that caspase-11 can be implicated in a non-canonical inflammasome pathway (Kayagaki *et al*, 2011). While caspase-1 is constitutively expressed in many cell types, the caspase-11 (murine otholog of caspase-4/-5) is inducible. However, both caspases are produced as zymogens, thus existing under an inactive pro-form that needs to be cleaved to become active.

Caspase proenzymes contain three domains: an amino-terminal CARD domain, a large subunit that contains the active-site cysteine (p20) and a carboxy-terminal small subunit (p10 ; Fig. 10). The cleavage of the zymogen generates two subunits, p20 and p10, which associate as a hetero-tetramer to form the active caspase-1. This maturation process occurs via a close proximity model of auto-activation. Indeed, the pro-peptide is cleaved in case of the apposition of more than two monomers of pro-caspase as is the case when inflammasome is assembled (Bauernfeind et al, 2011). However, we have to note that caspase-1 seems to exist in two activation states, unprocessed and fully processed, depending on the composition of the inflammasome (Broz *et al*, 2010).

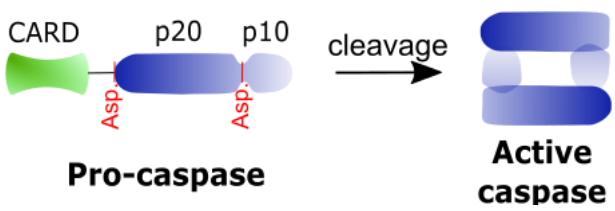


Fig. 10: Caspase 1/11 activation.

Caspase proenzymes contain an amino-terminal CARD and two carboxy-terminal subunits: p10 and p20. Two cleavage events at aspartate (Asp.) residues could occur. The first divides it into large and small caspase subunits, and the second removes the N-terminal prodomain. The resulting functional caspase is a tetramer of two large (p20) and two small (p10) subunits.

2. NLRP3 inflammasome

2.1 Two-step mechanism of inflammasome activation

The NLRP3 inflammasome is the best-characterized inflammasome. It is composed of NLRP3, the adaptor protein ASC and caspase-1. NLRP3 inflammasome activation is generally described as a two-step mechanism requiring two different signals (Fig. 11).

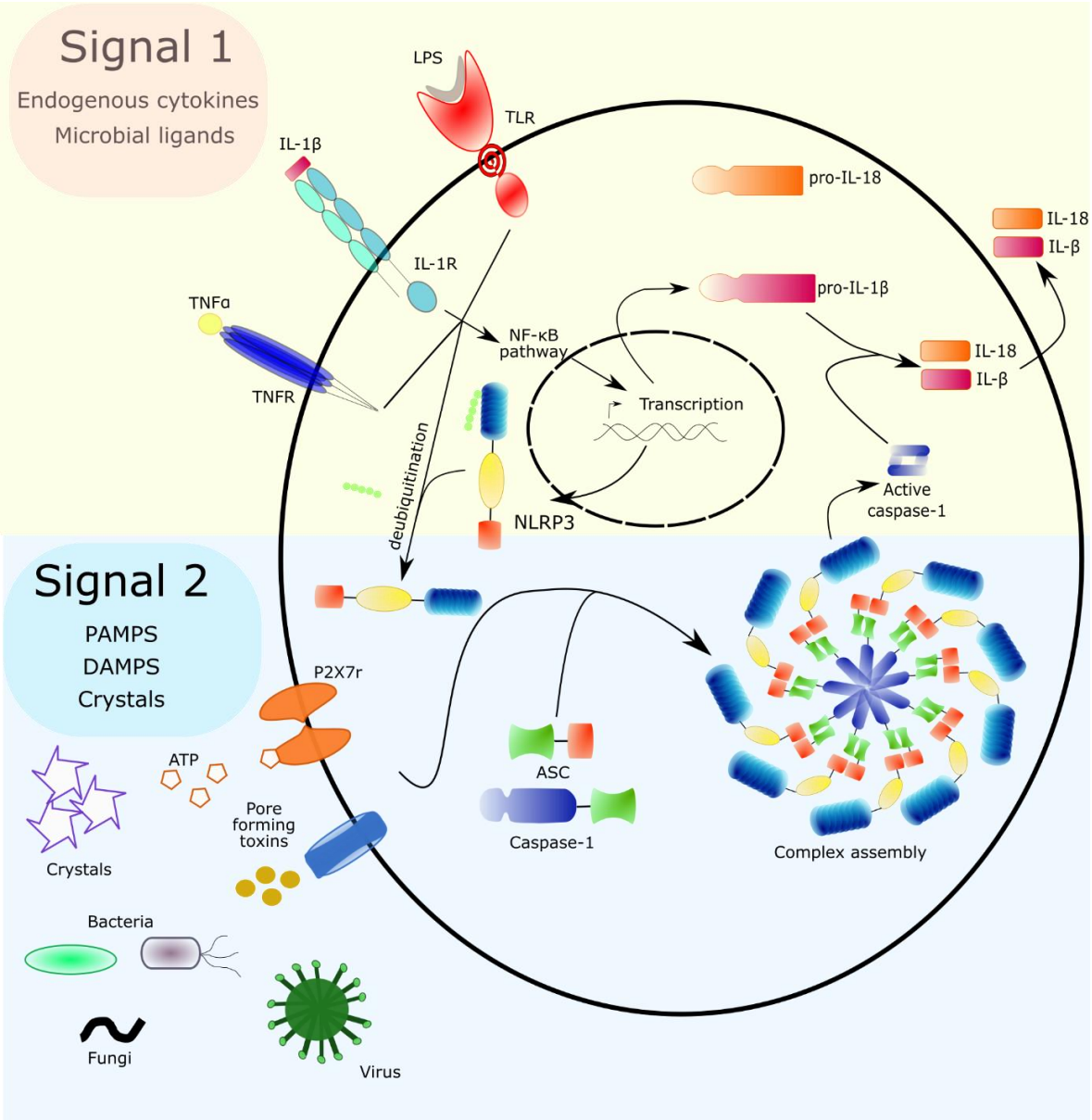


Fig. 11: Activation of the NLRP3 inflammasome requires two signals.

Signal 1 (or priming) is represented by microbial molecules or pro-inflammatory cytokines. They lead to the activation of the NF-κB pathway and to the regulation of the synthesis of NLRP3 and pro-IL-1β. Priming also licenses NLRP3 by inducing its deubiquitination. Signal 2 is responsible for the activation of NLRP3. It can be trigger by a large array of factors (DAMPs, PAMPs, crystals). Once signal 1 and 2 are engaged, the NLRP3 inflammasome is formed which leads to caspase-1 activation and, among other events, to IL-1β/IL-18 maturation and secretion. Inspired from (Franchi *et al*, 2012).

"Signal 1" (or priming) is a sine qua non condition for inflammasome assembly. It aims to upregulate the protein expression level of NLRP3. Priming is triggered by different pro-inflammatory molecules, such as bacterial lipopolysaccharide (LPS) or endogenous cytokines. These factors lead to the activation of the NF-κB pathway activation and to the subsequent upregulation of NLRP3 and pro-IL-1β (Franchi *et al*, 2009; Bauernfeind *et al*, 2010). Additionally, the signal 1 can non-transcriptionally prime NLRP3 by stimulating its deubiquitination (Juliana *et al*, 2012; Py *et al*, 2013).

"Signal 2" consists in an NLRP3 agonist responsible for complex formation (Fig. 11). NLRP3 is a broadly activated sensor protein since it can detect a large array of stimuli. The table 2 presents few examples of the NLRP3 activators described. It is not fully understood how these structurally diverse molecules activate NLRP3. However, we know that their presence induces a change in NLRP3 conformation that allows the recruitment of ASC and caspase-1 and ultimately the cleavage of its substrates (Mayor *et al*, 2007; Compan *et al*, 2012).

Table 2: Diversity of NLRP3 activators.

NLRP3 activators	References
PAMPs	
Bacterial components	(Mariathasan *et al*, 2006; Duncan *et al*, 2009; He *et al*, 2010; Toma *et al*, 2010; Shimada *et al*, 2011)
Fungal components	(Gross *et al*, 2009; Joly *et al*, 2009; Hise *et al*, 2010)
Viral components	(Allen *et al*, 2009; Thomas *et al*, 2009; Ichinohe *et al*, 2010; Rajan *et al*, 2011)
DAMPs	
ATP	(Mariathasan *et al*, 2006)
Uric acid crystals (MSU)	(Martinon *et al*, 2006)
Aβ peptides	(Halle *et al*, 2008)
Environmental factors	
Silica	(Dostert *et al*, 2008)
Abestesos	(Eisenbarth *et al*, 2008)

2.2 Different NLRP3 activation models

Regarding the huge diversity of stimuli, it is unlikely that NLRP3 senses each PAMP or DAMP directly. Instead, a common molecular event is probably leading to its activation. Three main models have been proposed: the ion flux model, the ROS and mitochondrial

impairment model and the lysosomal damage model (Fig. 12). However, so far, none of these models totally accounts for inflammasome activation, probably because all these events are strongly interrelated (Guicciardi *et al*, 2004; Muñoz-Planillo *et al*, 2013).

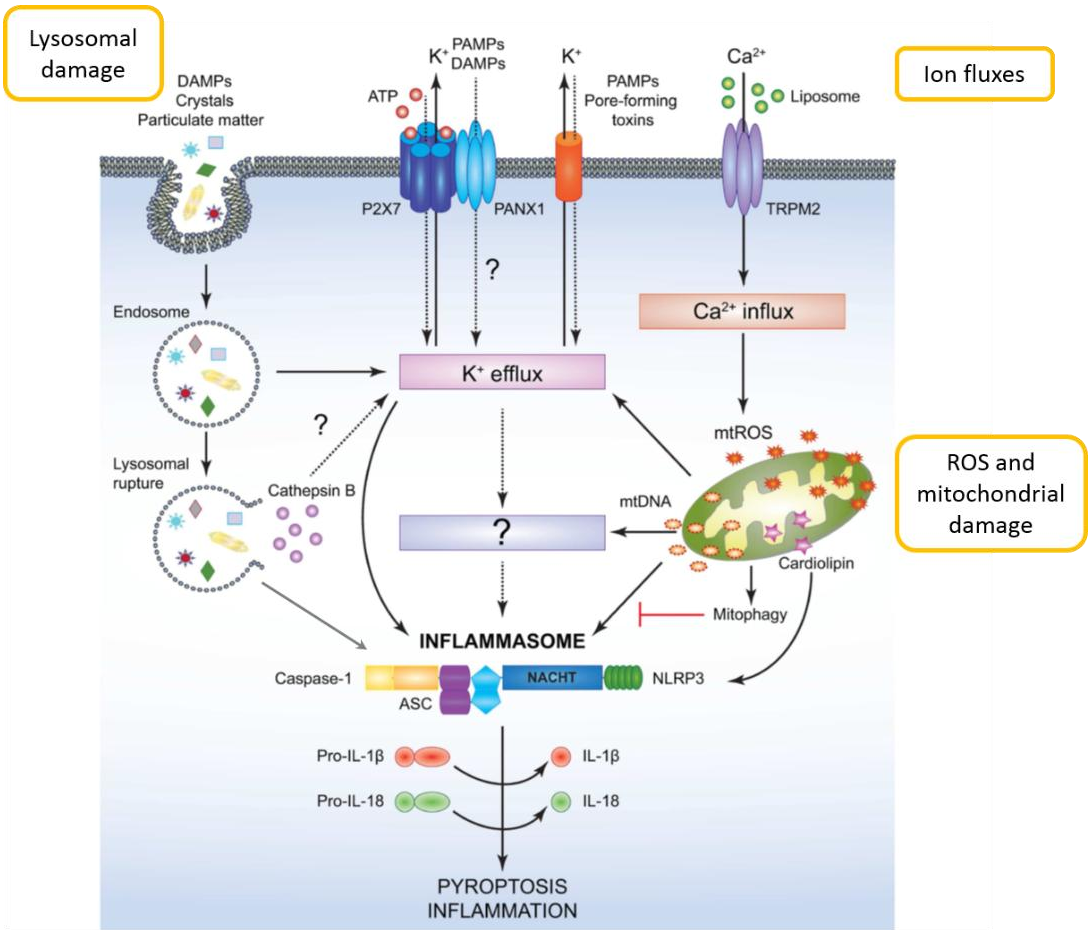


Fig. 12: Models for NLRP3 activation.

Various PAMPs and DAMPs provide the signal 2 required to assemble and activate the NLRP3 inflammasome. Although the precise mechanism leading to NLRP3 activation is still controversial, three models have been proposed: the lysosomal damage, the ions fluxes and the mitochondrial damage and associated ROS. However, these cellular events are strongly correlated and probably act together. Adapted from (Saxena & Yeretssian, 2014).

2.2.1 Ion flux model

Cellular ion fluxes play an important role in NLRP3 activation, in particular potassium (K^+) efflux and Calcium (Ca^{2+}) mobilization from intracellular stores.

Several known inflammasome activators are pore-forming toxins (*i.e.* Nigericin*)* and are described as cause of K^+ efflux. Similarly, ATP activates the ionotropic P2X7 receptor which results in the opening of potassium channels and the release of K^+ in the extracellular space. Incubating cells in KCl-enriched medium inhibits the K^+ efflux and results in reduced IL-1β and IL-18 release (Pétrilli *et al*, 2007), demonstrating the importance of the potassium flux for inflammasome activation.

In addition to K^+, Ca^{2+} fluxes have also been studied in the context of NLRP3 activation. Several recent reports demonstrate that an increase of intracellular calcium is indispensable for NLRP3 activation by ATP, Nigericin, but also lysosomotropic peptides (T. Murakami et al. 2012; Rossol et al. 2012; Zhong et al. 2013). However, even if calcium mobilization is required for NLRP3 activation, it cannot be sufficient, given that calcium signalling is triggered in many settings without coincident inflammasome activation. Moreover, a recent study showed that extracellular calcium activates NLRP3 only when a K^+ efflux is permitted (Muñoz-Planillo *et al*, 2013). This publication also shows that the activation of NLRP3 by Ca^{2+}-enriched media was attributed to the formation of insoluble calcium phosphate salts, which was previously linked to K^+ efflux and NLRP3 activation (Jin *et al*, 2011). Therefore, the current view is that a drop in cytosolic K^+ plays a central role in the NLRP3 activation. However, it remains to be clarified how K^+ efflux are sensed by NLRP3.

2.2.2 Mitochondrial damage and ROS

ROS are chemically reactive compounds (free radicals and molecules) derived from the oxygen metabolism. While ROS are a by-product of physiological cell function, excessive amounts can cause deleterious effects.

Most inflammasome activators, such as ATP, Nigericin, Alum or uric acid induce a ROS production (Tschopp & Schroder, 2010). Experiments using ROS inhibitors have shown that this oxidative stress is important for NLRP3 activation. ROS generated by the NADPH oxidase 2 were first considered to be direct inflammasome activator (Cruz *et al*, 2007; Dostert *et al*, 2008). However, later studies have shown that NOX2-deficient cells exhibit normal NLRP3 activation upon stimulation (Bruggen *et al*, 2010; Latz, 2010; Muñoz-Planillo *et al*, 2013). Moreover, a deficiency in superoxide dismutase (SOD-1), increasing ROS levels in cells, leads to an unexpected inhibition of caspase-1 activity (Meissner *et al*, 2010). Therefore, the discrepant results about the role of ROS and antioxidants on inflammasome activation are still matters of discussion (Rubartelli et al, 2011).

Mitochondria, important cellular organelles, are very sensitive to cellular stress. Its damage triggers molecular events such as the production of ROS or modifications in Ca^{2+} signalling, two processes already relied to NLRP3 activation. Zhou and colleagues have shown that blocking mitophagy induces NLRP3 activation (Zhou *et al*, 2010). Furthermore, mitochondrial DNA, released in case of injury, can directly bind to and activate NLRP3 (Nakahira *et al*, 2011; Shimada *et al*, 2012) similarly to cardiolipin, a phospholipid enclosed in the inner mitochondria membrane (Iyer *et al*, 2013). On the other hand, the

protection of mitochondrial integrity also prevents inflammasome activation (Chang *et al*, 2014). In addition to these results, many reports have demonstrated that a large variety of NLRP3 activators drive mitochondrial damage and subsequent events (Gurung *et al*, 2014). All these findings support the mitochondrial dysfunction as another important cellular event leading to NLRP3 inflammasome activation.

2.2.3 Lysosomal damage

Several particles and crystalline molecules, such as Alum, MSU, Silica or Aβ, have been shown to activate the NLRP3 inflammasome upon “frustrated” phagocytosis and disruption of lysosomal membrane. Proteases, such as cathepsin B that are normally sequestered in this organelle, can then be released. Treatment with cathepsin B inhibitors induce a reduction of NLRP3 activation (Halle *et al*, 2008; Hornung *et al*, 2008; Chu *et al*, 2009; Duncan *et al*, 2009; Codolo *et al*, 2013). However, cathepsin B-deficient mice show no reduction in IL-1β release following particle exposure (Dostert *et al*, 2009; Orlowski *et al*, 2015). Moreover, a recent publication demonstrates that NLRP3 activation by MSU or silica could occur in the absence of phagocytosis but was dependent on K^+ efflux (Muñoz-Planillo *et al*, 2013; Hari *et al*, 2014). These results indicate once again the complexity to construct a unique NLRP3 activation model.

In conclusion, the mechanisms of NLRP3 activation supported by the most studies include potassium efflux, the generation of ROS, mitochondrial damage and the lysosomal destabilization. However, not all of these events are induced by all NLRP3 agonists and some conflicting results on this topic are published. Therefore, the precise mechanism of NLRP3 activation is still debated and need clarification.

3. Other inflammasomes

In addition to NLRP3, other inflammasome complexes have been described. Even if each of them is specifically activated by particular stimuli (Fig. 13), their assembly all triggers caspase-1 activation and the related cellular events.

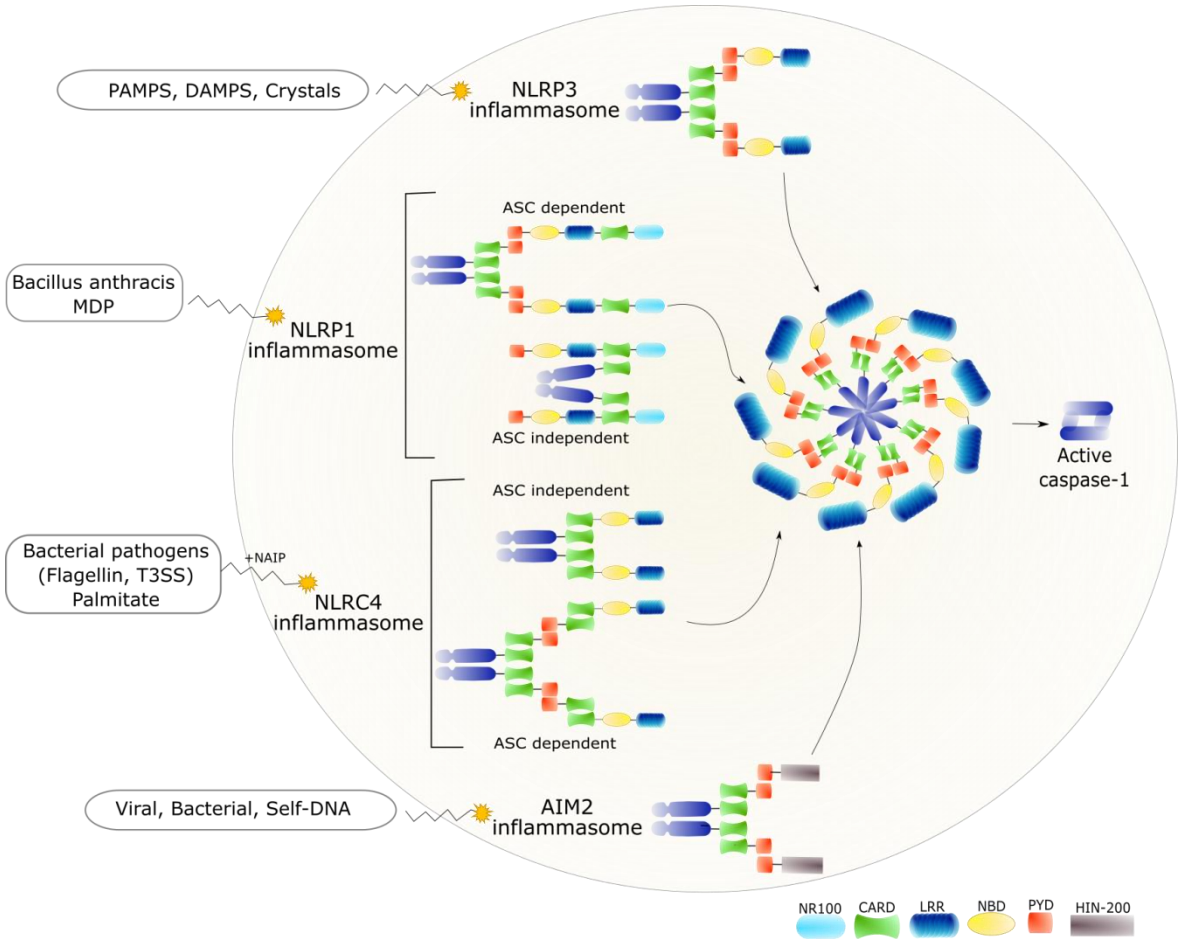


Fig. 13: Distinct stimuli activate different inflammasomes.

A large variety of stimuli, associated with infection or cellular stress, are able to activate the inflammasomes and subsequently the caspase-1. Each of these different triggers preferentially activates one the inflammasome sensor proteins. For example, NLRP1 is activated by Anthrax, *Bacilllus anthracis* or Muramyl dipeptide (MDP) whereas NLRC4 is sensitive to fatty acids (palmitate) or different bacterial PAMPs. Moreover, the nature of the stimuli determines the ASC-dependency of both NLRP1 and NLRC4 complexes assembly. AIM2 can detect the presence of DNA, either viral, bacterial or self-DNA. Inspired from (de Zoete *et al*, 2014)

3.1. NLRP1 inflammasome

NLRP1 was the first described inflammasome complex. It was defined by the spontaneous assembly of NLRP1 protein, ASC and caspase-1 in a cell free system (Martinon *et al*, 2002). Today, we know that ASC is dispensable for NLRP1 inflammasome formation even if its presence enhances the complex's activity (Faustin *et al*, 2007; Van Opdenbosch *et al*, 2014). Although NLRP1 has been described in both humans and mouse, they are encoded by paralog genes. In human, a single genes codes for NLRP1whereas in mice three different genes code for NLRP1a, NLRP1b and NLRP1c. Accordingly, human and mouse NLRP1 display different structures which might explain that they are not sensitive to the same stimuli (Fig. 9). For example, Muramyl dipeptide

(MDP), a bacterial proteoglycan fragment, is a natural ligand of human NLRP1 but not of murine NLRP1 (Faustin *et al*, 2007; Kovarova *et al*, 2012). On the other hand, murine NLRP1 seems sensitive to the *Bacillus anthracis* lethal toxin whereas human NLRP1 is not (Fink *et al*, 2008). More precisely, this sensitivity to anthrax toxin is restricted to specific mouse genetic backgrounds such as in the mice strains BALB/c and 129s1 (Boyden & Dietrich, 2006).

3.2. NLRC4 inflammasome

NLRC4 (formerly called IPAF) is specialized in the detection of bacterial pathogens, such as *Shigella flexneri* (Suzuki & Núñez, 2007), *Legionella pneumophila* (Amer *et al*, 2006; Lamkanfi *et al*, 2007) or *Salmonella typhimurium* (Amer *et al*, 2006; Miao *et al*, 2006). NLRC4 does not directly recognize these pathogens but indirectly interacts with different NLR-family apoptosis-inhibiting proteins (NAIPs) in the cytosol (Kofoed & Vance, 2011). In mice, NAIP1 and 2 bind the bacterial type III secretory system (T3SS) whereas both NAIP5 and 6 bind bacterial flagellin (Kofoed & Vance, 2011; Rayamajhi *et al*, 2013). Once NAIP proteins bind their ligands, they can oligomerize with NLRC4 and induce the recruitment of other NLRC4 inflammasome components. Similar to NLRP1, NLRC4 could directly interact with the CARD domain of caspase-1 (Poyet *et al*, 2001) or indirectly by the recruitment of ASC (Broz *et al*, 2010; Proell *et al*, 2013).

3.3. AIM2 inflammasome

AIM2 is a member of the PYHIN family and is able to recognize cytosolic viral or bacterial double-stranded DNA (Broz *et al*, 2010; Fernandes-Alnemri *et al*, 2010; Rathinam *et al*, 2010; Sauer *et al*, 2010) but also the DNA released by apoptotic cells via its HIN200 domain (W. Zhang et al. 2013). The binding to DNA induces a conformational change of AIM2 and the oligomerization of AIM2 occurs around this DNA molecule allowing the subsequent recruitment of ASC and caspase-1 (Jin *et al*, 2012, 2013).

3.4. Non-canonical inflammasome pathway

Recently, it was shown that caspase-1-deficient mice are also deficient for caspase-11, due to the close proximity of both genes in the mouse genome and the consequent difficulty to segregate these genes during homolog recombination (Kuida *et al*, 1995;

Kayagaki *et al*, 2011). To study the precise role of each caspase, new transgenic mice have been designed expressing caspase-11 from a C57BL/6 bacterial artificial chromosome transgene. The experiments carried out with these new strains show that *Casp11*$^{-/-}$ cells are still able to process IL-1β and IL-18 in response to ATP or MSU but not when infected by some Gram-negative bacteria (Kayagaki *et al*, 2011). Indeed, further investigations have demonstrated that caspase-11 is directly activated after macrophage stimulation with Gram-negative bacteria, such as *E. coli*, *Citrobacter rodentium* or *Vibrio cholerae* (Fig. 14 ; Kayagaki *et al*, 2011; Rathinam *et al*, 2012; Broz & Monack, 2013).

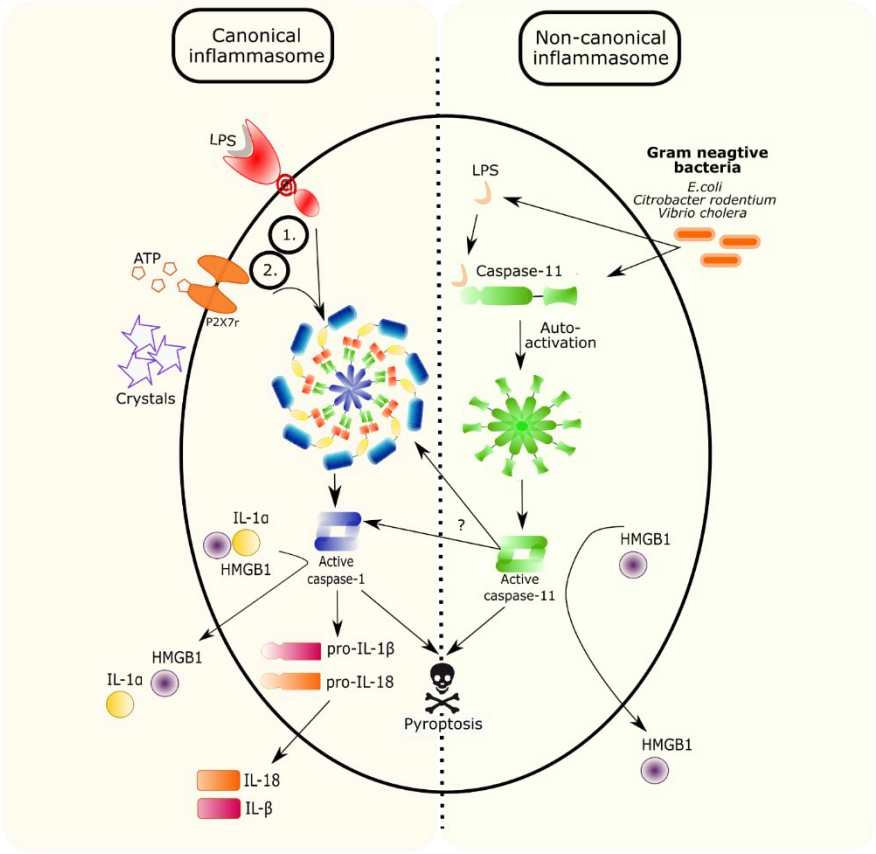


Fig. 14. Non-canonical inflammasome pathway.

Intracellular LPS recognition by caspase-11 triggers its auto-activation. Once activated, caspase-11 triggers pyroptosis and HMGB1 secretion, in a caspase-1-independent way. During this process, IL-1β and IL-18 releases are also observed but they are dependent on the NLRP3 inflammasome and on the caspase-1 activity. This observation suggests a cross-talk between the canonical and non-canonical inflammasome pathways which must be further investigated.

In fact, caspase-11 can be activated by direct binding to intracellular LPS (Hagar *et al*, 2013; Kayagaki *et al*, 2013; Shi *et al*, 2014). Once activated, caspase-11 can trigger pyroptosis and HMGB1 release in a caspase-1-independent way (Kayagaki *et al*, 2011) but also induce IL-1β and IL-18 releases in a caspase-1-, ASC- and NLRP3-dependent way (Kayagaki *et al*, 2011, 2013). These findings and further investigations have shown

that caspase-11 activation is a new way to activate caspase-1 and is therefore called the “non-canonical inflammasome”.

3.5. NLRP2, NLRP6, NLRP12, IFI16 and RIG-I inflammasomes

Other sensor proteins have been described to form functional inflammasomes.

An early study shows that the co-expression of NLRP6 and ASC in fibroblastic cell lines triggers caspase-1 activation and IL-1β release (Grenier *et al*, 2002). Additionally, IL-18-deficient mice present an NLRP6-associated alteration of their microbiota, proposing a role for NLRP6 in IL-18 release (Elinav *et al*, 2011).

NLRP12 closely resembles NLRP6. Its implication in intestinal homeostasis is well described (Zaki *et al*, 2011; Allen *et al*, 2012) and its co-expression with ASC leads to caspase-1 activation and subsequent IL-1β release (Wang *et al*, 2002). Another study showed that *Yersinia pestis* infection triggers IL-1β and IL-18 release in a NLRP12-dependent manner (Vladimer *et al*, 2012).

NLRP2, another member of the NLR subfamily, was already known as a potent inhibitor of NF-κB pathway via the sequestration of the IKK complexes (Bruey *et al*, 2004). More recently, it has been demonstrated that NLRP2 is able to form a functional inflammasome in human astrocytes via assembly with ASC and caspase-1 (Minkiewicz *et al*, 2013).

Finally, some members of the ALR (AIM2 Like Receptor) family were found to colocalize with ASC and generate IL-1β secretion (Brunette *et al*, 2012). Human Interferon-inducible protein 16 (IFI16) has been described to form a functional inflammasome in endothelial cells following herpesvirus infection (Singh *et al*, 2013; Monroe *et al*, 2014). Last but not least, retinoic acid-inducible gene I (RIG-I) seems also able to recruit ASC and caspase-1 via the CARDs to form an inflammasome complex (Poeck *et al*, 2010).

Further studies are needed to validate and complete our knowledge about these inflammasome complexes, notably about the assembly-triggering stimuli specific for each inflammasome.

4. Inflammasome-mediated cellular events

The main outcome of the different inflammasomes is the activation of caspase-1 upon assembly of the multimeric complexes. Once activated, this protease will mature the

pro-inflammatory cytokines IL-1β and IL-18 and induce of pyroptotic cell death. In addition, the inflammasome activation allows the release of alarmins such as IL-1α and HMGB1.

4.1. IL-1β and IL-18 maturation and release

IL-1β and IL-18 are cytokines of the IL-1 family and are expressed as immature pro-peptides that have to be cleaved in order to become biologically active.

4.1.1. IL-1β

IL-1β is a powerful pro-inflammatory cytokine primarily produced by cells of the myeloid lineage, such as monocytes, macrophages and dendritic cells. It is encoded by the *Il1b* gene and is not expressed under resting conditions. Its transcription must be triggered by activation of the NF-κB pathway. Once translated, IL-1β is present in the cytoplasm as a 31 kDa inactive pro-form that is subsequently proteolytically processed into a 17 kDa active cytokine. This maturation step is mainly performed by caspase-1 following inflammasome activation. Nevertheless, in 1997, Fantuzzi et al. published the first evidence of caspase-1-independent IL-1β maturation (Fantuzzi *et al*, 1997). Subsequent studies have validated that neutrophil- and macrophage-derived proteases such as proteinase 3 (Coeshott *et al*, 1999; Joosten *et al*, 2009), elastase, chymase (Guma *et al*, 2009), and the two metalloproteinases, Merpin a and Merpin b (Banerjee & Bond, 2008; Herzog *et al*, 2009; Banerjee *et al*, 2011) are also IL-1β-processing enzymes. More recently, caspase-8 has been identified as another enzyme responsible for the IL-1β maturation (Bossaller et al. 2012).

Regardless of the enzymes in charge of the cleavage, the active 17 kDa IL-1β must be released from the cell to trigger a strong inflammatory response in surrounding cells. Its actual route of secretion is still not entirely clear. The lack of a signal peptide indicates however that secretion does not occur via the classical ER-Golgi route (Rubartelli *et al*, 1990). Different alternatives have been proposed, from the direct externalization at plasma membrane to the implication of vesicles (Piccioli & Rubartelli, 2013).

Once released, IL-1β acts on other cells by binding to the Interleukin-1 Receptor (IL-1R). There are two forms of IL-1R: IL-1R1, which mediates the cellular activation by IL-1β, and IL-1R2, which regulates the activity of IL-1β. When IL-1β binds IL-1R1, a second chain called the IL-1RAcp joins the receptor to form a complex, triggering the IL-1β-dependent cellular events: induction of antimicrobial resistance, activation of NF-κB and MAPK/JNK pathways or cell polarization into TH17 state (Garlanda *et al*, 2013).

IL-1R2 is a biologically inert receptor that competes IL-1β binding to IL-1R1 to negatively regulate cell activation (Hannum *et al*, 1990). A soluble molecule, called IL-1Ra, also acts as specific inhibitor of IL-1β pathway. It is structurally very closed to IL-1β but a mutation renders it incapable to bind IL-1RAcp, preventing the complex assembly of IL-1R1 (Dinarello, 2011).

The release and activity of IL-1β must be tightly controlled in order to prevent an excessive activation of the immune response.

4.1.2. IL-18

IL-18, also called IFNγ-inducing factor, is another cytokine belonging to the IL-1 family. In opposition to IL-1β, IL-18 is constitutively expressed in myeloid cells, keratinocytes and epithelial cells (Puren *et al*, 1999). It is synthetized as an inactive pro-peptide of 24 kDa which is further cleaved into 18 kDa and 6 kDa subunits to become biologically active.

Similar to IL-1β, IL-18 is mainly processed by caspase-1 via the inflammasome complex. Nevertheless, two other caspases are also able to cleave IL-18: 1) Caspase-3 but the resulting product seems to be biologically inactive (Akita *et al*, 1997). 2) caspase-8 which is able to process IL-1β and IL-18 in macrophages (Bossaller *et al*, 2012). In addition to caspases, other proteases are also competent to cleave IL-18 such as the neutrophillic proteinase-3 (Sugawara *et al*, 2001) , Granzyme b (Omoto *et al*, 2010) or Merpin-b (Banerjee & Bond, 2008).

The receptor of IL-18 — a heterocomplex of IL-18Ra and b — is formed upon bonding of mature IL-18 to IL-18Ra. While most cells express IL-18Ra, IL-18Rb seems restricted to T-cells and dendritic cells (Dinarello & Fantuzzi, 2003; Felderhoff-Mueser *et al*, 2005). The IL-18 receptor activation leads to the polarization of T helper cells towards a TH1 state and to the induction of IFNγ (Dinarello, 1999).

As for IL-1β, the downstream effects of IL-18 are strongly controlled by two different mechanisms: 1) The IL-18 binding protein (IL18bp) which binds to IL-18 with higher affinity than IL-18Ra (Novick *et al*, 1999; Kim *et al*, 2000), 2) a truncated form of IL-18Ra that prevents the formation of the complete receptor after IL-18 binding to IL-18-Rb (Andre *et al*, 2003; Fiszer *et al*, 2007; Booker & Grattan, 2014).

4.2. Active IL-1α and HMGB1 alarmin release

Alarmins are endogenous molecules that, in normal condition, remain intracellular to perform their “daily job”. However, they can also be released outside the cell upon injury to alert immune cells. This release can occur passively following cell death or actively, generally by using specialized secretion systems. This active secretion is only described in immune cells.

The alarmin family includes a large variety of structurally different endogenous molecules. Among them are IL-1α and HMGB1, two molecules linked to inflammasome which will be further described below.

4.2.1. IL-1α

IL-1α and IL-1β are encoded by different genes, but share the same receptor (IL-1R) and exhibit similar biological effects. In addition, both proteins are synthetized as precursors and lack a signal peptide suggesting a non-canonical secretion pathway of secretion.

On the other hand, IL-1α and IL-1β are differently regulated: 1) While IL-1β is always induced, IL-1α can be constitutively expressed in some cell types such as keratinocytes or epithelial cells (Hachiya *et al*, 2001; Garlanda *et al*, 2013). 2) Even if IL-1α is also synthetized as a precursor, this latter has been shown to be already active (Mosley *et al*, 1987). 3) The cleavage of IL-1α is not mediated by caspase-1 but by calpaïn-like proteases (Howard et al. 1991; Gross 2012), which is thought to enhance the IL-1α activity (Miller *et al*, 1994).

The secretion mechanism of IL-1α is still not well established. Until recently, it was broadly admitted that it is only passively released after necrotic cell rupture (Chen *et al*, 2007; Eigenbrod *et al*, 2008). Nevertheless, recent publications have demonstrated that IL-1α is also actively secreted by myeloid cells and keratinocytes after exposure to NLRP3, NLRP1 and AIM2 inflammasome-activating stimuli (Guarda et al. 2011; Keller et al. 2008; Yazdi et al. 2010; Gross et al. 2012; Fettelschoss et al. 2011). Initially, it was hypothesised that IL-1β is necessary for the secretion of IL-1α. Accordingly, a model was proposed in which IL-1α directly binds to IL-1β using it as a shuttle for co-secretion (Yazdi & Drexler, 2013). However, some arguments are not in line with this idea. First, the requirement of an inflammasome for IL-1α release seems to be stimuli dependent, at least in case of the NLRP3 inflammasome. Indeed, IL-1α secretion triggered by particles occurs independently of NLRP3, whereas nigericin or ATP lead to IL-1α release in an NLRP3-, ASC- and Caspase-1-dependent manner (Gross *et al*, 2012). Remarkably, such a stimuli-dependency has not been described for IL-1β. Secondly, another refuting argument is that

inhibiting caspase-1 activity does not affect nigericin-induced release of IL-1α, while totally abolishing IL-1β secretion (Gross *et al*, 2012). Finally, and determinately, the active release of IL-1α after inflammasome activation is unchanged in IL-1β knockout mice (Yazdi & Drexler, 2013).

In summary, the inflammasome might participate in the active IL-1α secretion but more investigation is needed to understand by which exact mechanism.

4.2.2. HMGB1

HMGB1 is a non-histone chromatin-binding protein that is highly conserved and ubiquitously expressed. HMGB1 is located in the nucleus where it stabilizes the chromatin structure and controls the expression of genes important for mitochondrial quality control and autophagy (Kashayar *et al*, 1978; Tang *et al*, 2011).

In addition, HMGB1 plays a second role once it is translocated out of the cell, for example upon cell injury. Once outside of the cell, HMGB1 turns into a powerful pro-inflammatory alarmin cytokine (Wang *et al*, 1999). HMGB1 acts on surrounding cells via a different receptors, for example TLRs and RAGE (receptor for advanced glycation end-products ; Bianchi & Manfredi, 2009; Yang *et al*, 2011). According which receptor it binds to, HMGB1 will trigger distinct immune responses such as activation of pro-inflammatory pathways, migration or proliferation (Venereau *et al*, 2012; Keyel, 2014).

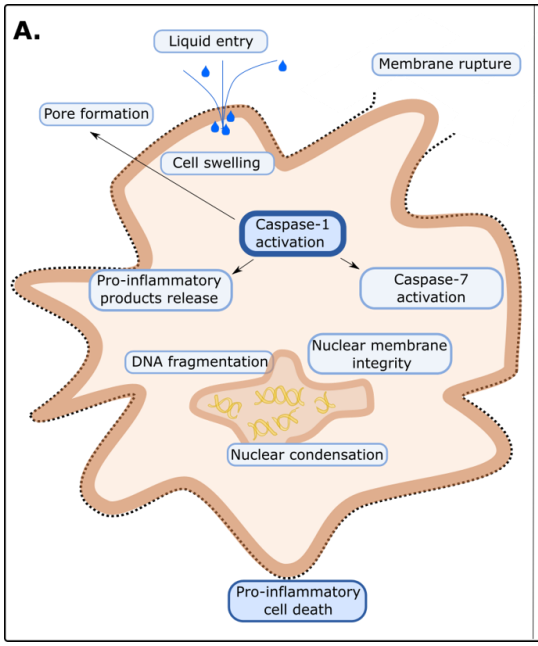
In addition to its “alarmin” function, HMGB1 shares other common characteristics with IL-1α. First, even if HMGB1 can be released passively – for example during necrosis – some cell types, such as macrophages, monocytes, hepatocytes, natural killer cells, and dendritic cells, are also able to secrete HMGB1 actively (Tsung *et al*, 2014). Second, this active HMGB1 secretion mainly occurs in an inflammatory context (Lamkanfi *et al*, 2010; Andersson & Tracey, 2011). Finally, HMGB1 also lacks a classical signal peptide required for the transport through the classical secretory pathway, indicating that an alternative mechanism is responsible for its active release (Gardella *et al*, 2002).

Interestingly, several reports suggest a role for the inflammasome in HMGB1 release. For example, Willingham and colleagues showed that macrophages exposed to bacteria release HMGB1 in an NLRP3 and ASC-dependent manner (Willingham *et al*, 2009). These results were rapidly supported by another publication demonstrating that NLRP3, NLRC4 and ASC are required for HMGB1 release (Lamkanfi *et al*, 2010). However, it is still unclear, how the inflammasome regulates the HMGB1 release.

4.3. Pyroptosis

Pyroptosis is a rapid lytic form of cell death, classically defined as the terminal response of cells with active pro-inflammatory caspase-1 or -11. Currently, it remains to be clarified whether pyroptosis truly constitutes a cell death pathway on its own or whether it represents a particular case of caspase-dependent apoptosis (Galluzzi *et al*, 2012).

An argument to liken pyroptosis to apoptosis is the proteolytic cleavage of caspase-7 by caspase-1, one of the classical apoptotic caspases (Lamkanfi 2008). However, caspase-7 activation, as well as caspase-3 activation, are dispensable for pyroptosis (Lamkanfi 2008), indicating that death by pyroptosis occurs independently of the apoptosis program. Moreover, pyroptosis is characterized by some specific hallmarks digressing to classical apoptotic definition (Fig. 15).



B.

Pyroptosis	Apoptosis
Caspases-1/11 and -7 activation = Inflammatory caspases	Caspases-3 and- 7 activation = Apoptotic caspases
Nuclear condensation and random DNA fragmentation	Nuclear condensation and DNA fragmentation into 180pb pieces
Membrane swelling and rupture	Preservation of membrane integrity
IL-1β, IL-18 and HMGB1 secretion	Apoptotic body formation
Pro-inflammatory cell death	Immunologically silent cell death

Fig. 15: Pyroptosis: main features and differences from apoptosis.

(A) Caspase-1/11 activation is the defining feature of pyroptosis and mediates this form of cell death. Pyroptosis is characterized by the formation of membranous pores, water entry, cell swelling and following membrane rupture. The secretion of pro-inflammatory factors is also an important pyroptosis feature and contributes to its inflammatory nature. **(B)** Pyroptosis differs in several aspects from apoptosis. Adapted from (Lamkanfi, 2011)

A proximal event in pyroptosis is the caspase-1-dependent formation of small cationic pores in the plasma membrane. They appear following cell exposure to numerous inflammasome activators, such as *Salmonella* or anthrax lethal toxin (Fink, Bergsbaken, and Cookson 2008). The subsequent osmotic variations lead to water entry, cell swelling

and finally cell lysis (Fink & Cookson, 2006). Interestingly, the release of pro-inflammatory molecules after caspase-1 activation, such as IL-1β, IL-18, IL-1α and HMGB1 does not directly lead to cell death but contributes to the inflammatory nature of pyroptosis.

During this lytic process, caspase-1 also induces nuclear condensation and DNA fragmentation (via currently unknown DNAses ; Fink & Cookson, 2006) with maintenance of nuclear membrane integrity (Molofsky *et al*, 2006).

5. Inflammasome and diseases

The importance of inflammasome research was underlined by the discovery of its implication in autoinflammatory hereditary syndromes called Cryopyrin-Associated Periodic Syndromes (CAPS). In 2001, Hoffman and colleagues have shown that the hereditary diseases Muckle-Wells syndrome (MWS) and the Familial Cold Autoinflammatory syndrome (FCAS) are both caused by mutations in the *Nlrp3* gene (Hoffman *et al*, 2001). In addition to CAPS, a large variety of diseases are linked to inflammasome perturbation: gout (Martinon *et al*, 2006), osteoarthritis (Denoble *et al*, 2011), atherosclerosis (Duewell *et al*, 2010), Alzheimer's diseases (Heneka *et al*, 2013; Tan *et al*, 2014), multiple sclerosis (Jha et al. 2010) and Type 2 diabetes (Larsen *et al*, 2007; Donath & Shoelson, 2011). Some of these inflammasome-associated diseases are treated with endogenous recombinant proteins targeting IL-1. Among them, the most widely used is Anakinra, a recombinant IL-1R antagonist. However, some antibody-based treatments, such as Canakinumab, a neutralizing IL-1β antibody or the soluble decoy IL-1 receptor Rilonacept, are also used. These molecules have been tested in the treatment of CAPS and other IL-1β-associated diseases, such as type II diabetes (Dinarello *et al*, 2012; Dinarello & van der Meer, 2013).

In the meantime, therapeutic researches were focused on finding a molecule that would directly target the inflammasome complex, in order to gain in specificity. Different pharmacologic inhibitors of inflammasome function have already been described, such as glyburide (Lamkanfi *et al*, 2009), CRID3 (Coll & O'Neill, 2011), Bay 11–7082 (Juliana *et al*, 2010) parthenolide (Juliana *et al*, 2010), 3,4-Methylenedioxy-β-nitrostyrene (He *et al*, 2014) or dimethyl sulfoxide (DMSO, Ahn et al. 2013). However, while all these products have been tested *in vitro*, some *in vivo* reports are missing. Recently, a new NLRP3 inhibitor called MCC950 has been described and tested *in vivo*, in an experimental autoimmune encephalomyelitis (EAE) model and in a CAPS model. In EAE, MCC950 limits the disease severity and in CAPS the treatment protects mice from neonatal lethality

(Coll *et al*, 2015). Further investigations are awaited to confirm this inflammasome inhibitor as a treatment for other inflammatory diseases (de Rivero Vaccari *et al*, 2015).

In conclusion, the inflammasome plays a pivotal role in numerous inflammatory diseases and it seems to be a potential therapeutic target to limit the development of these pathologies. In regard to these observations, studying the inflammasome represents a promising avenue to understand and cure neuroinflammatory diseases.

Part IV: The Inflammasome in CNS

1. Inflammasome function in CNS diseases

IL-1β, IL-18, IL1α and HMGB1 are known for the many functions they play in healthy or injured brain. In the CNS, IL-1 cytokines induce fever, increase slow wave sleep, amplify hypothalamic-pituitary-adrenal activation but also promote the neuroinflammation (Rothwell & Luheshi, 2000; Simi *et al*, 2007). For its part, extracellular HMGB1 is depicted as facilitator of neurite outgrowth and cell migration during early brain development and as neuroinflammatory compound after injury (Fang *et al*, 2012).

All these inflammasome-related factors are also associated to the pathogenesis of several neurological diseases including AD, PD, traumatic brain injury, epilepsy and stroke (Alboni *et al*, 2010; Fang *et al*, 2012; Walsh *et al*, 2014). Both neuroprotective and detrimental effects are described, depending on the timing, the context and the disease.

As IL-1β, IL-18, IL1α and HMGB1 secretions are all linked to the inflammasome activation, one expectation could that, depending on the context, they play similar beneficial or deleterious roles on the brain parenchyma. The reality is far more complex. For example, *Il18* knockout mice are not protected from CNS ischaemic injury (Wheeler *et al*, 2003) while mice lacking IL-1α, IL-1β or HMGB1 present a resistance to the pathogenic effects of ischemia (Boutin *et al*, 2001; Kim *et al*, 2006). Another illustration is the divergent effects of IL-1β and IL-18 on remyelination after cuprizone treatment, where IL-1β is protective (Mason *et al*, 2001) and IL-18 detrimental (Jha *et al*, 2010).

In light of these differences, it seems judicious to investigate how their production is regulated and, therefore explore the inflammasome implication in CNS diseases. Indeed, this field of research is currently deeply studied (for review see, Singhal *et al*, 2014; Walsh *et al*, 2014).

These last years, a lot of publications have depicted the inflammasome as an important player in different neurological disorders such as CNS infections, acute sterile injuries or chronic neurodegenerative diseases. An overview of these findings is summarised in table 3.

Table 3: Inflammasome in neurological diseases.

	Disease context	Inflammasome involvement	References
Infection	Turberculous meningitis	*M. tuberculosis* induces NLRP3- and ASC-dependent IL-1β secretion from microglial cultures.	(Lee *et al*, 2013)
	Pneumococcal meningitis	ASC- and NLRP3-deficiency decrease the neuroinflammation and the scores of disease severity *via* reduction of IL-1β and IL-18 secretion.	(Hoegen *et al*, 2011; Mitchell *et al*, 2012)
	Brain abcesses	*S. aureus* infection activates microglial NLRP3 and IL-1β secretion *in vitro*.	(Hanamsagar *et al*, 2011)
	Viral encephalitis	West Nile virus activates NLRP3 inflammasome *in vivo*. NLRP3- and ASC-deficiency reduce the survival after mice were infected with the virus. Murine Encephalitis virus activates caspase-1 and subsequent IL-1β and IL-18 secretion, *in vitro* and *in vivo*.	(Kaushik *et al*, 2012; Ramos *et al*, 2012; Jha *et al*, 2013)
Acute and chronic sterile inflammation	Traumatic brain injury (TBI)	TBI induces NLRP1 inflammasome assembly, caspase-1 activation and IL-1β release in rat neurons. The antibodies against ASC or NLRP1 are neuroprotective in this model. Patients with cranial trauma exhibit higher CSF levels of NLRP1 and ASC.	(de Rivero Vaccari *et al*, 2009; Adamczak *et al*, 2012)
	Spinal cord injury (SCI)	Processing of IL-1β and IL-18 by active caspase-1 is observed in SCI rat model. Anti-ASC neutralizing antibodies promote tissue integrity and functional improvement.	(de Rivero Vaccari *et al*, 2008)
	Thromboembolic stroke	NLRP1 inflammasome, IL-1β and IL-18 are implicated in a mouse model of thromboembolic stroke. Neutralising antibodies against NLRP1 act as anti-inflammatory compound after ischemia.	(Abulafia *et al*, 2009)
	Ischemic stroke	NLRP3 deficiency improves cerebral injury after ischemic stroke.	(Yang *et al*, 2014)
	Demyelinating autoimmune inflammatory disease	The experimental autoimmune encephalomyelitis (EAE) and the cuprizone models, two model of multiple sclerosis are linked to inflammasome. The ASC- and NLRP3-deficiency reduce the disease progression in both models, at least to a certain extent.	(Gris *et al*, 2010; Jha *et al*, 2010)
	Prion disease	*In vitro* exposure of microglia to prion protein peptides induces NLRP3 inflammasome-depend release of IL-1β. However, mice lacking NLRP3 or ASC do not survive better in an *in vivo* prion pathogenesis model.	(Hafner-Bratkovič *et al*, 2012; Shi *et al*, 2012; Nuvolone *et al*, 2015)
	Amyotrophic Lateral Sclerosis	Mutant SOD-1 triggers IL-1β secretion in an ASC-dependent manner. Caspase-1- and IL-1β-deficient mice expressing the mutant form of SOD-1 show better survival compared to the wild-type mice.	(Meissner *et al*, 2010; Johann *et al*, 2015)
	Alzheimer's disease	Different forms of Aβ are implicated in the formation of the NLRP3 and NLRP1 inflammasomes. NLRP3- and caspase-1-deficiency are protective in an APP/PS1 transgenic AD model.	(Halle *et al*, 2008; Heneka *et al*, 2013; Tan *et al*, 2014)

Adapted from Walsh *et al*, 2014

All these studies suggest an important role of inflammasomes in CNS diseases and lead to the emergence of new research avenues. However, most of these studies were descriptive and based on tissue analysis and did not identify the respective role of each cell type in the inflammasome signalling. In the next section, we will review the actual knowledge of the expression and regulation of inflammasomes in the different brain cells.

2. Inflammasome expression in the brain

2.1. Inflammasome in neurons

Studies addressing the inflammasome function within neurons have particularly focused on the NLRP1 complex. They have been performed in different neuronal cell types (cortical and spinal motor neurons), as well as in different models of CNS disorders (traumatic brain injury, spinal cord injury, age-related cognitive impairment or stroke), in both rat and human (de Rivero Vaccari *et al*, 2008, 2009; Abulafia *et al*, 2009; Mawhinney *et al*, 2011; Adamczak *et al*, 2012). More recently, new *in vitro* and *in vivo* reports have shown the importance of the neuronal NLRP1 inflammasome in AD, ischemic stroke, Rasmussen's encephalitis and neuropathic pain (Fann *et al*, 2013; Li *et al*, 2013; Ramaswamy *et al*, 2013; Tan *et al*, 2013).

It has been shown for instance that the activation of neuronal NLRP1 inflammasome depends on Pannexin-1 (Silverman *et al*, 2009). In summary, high extracellular K^+ lead to the opening of pannexin-1 channels, allowing a strong ATP efflux, which in turn stimulates the P2X7 receptor and finally activates the NLRP1 inflammasome (de Rivero Vaccari *et al*, 2015).

In addition to NLRP1, neurons express AIM2 inflammasome components, which could be assembled in presence of aberrant DNA (Adamczak *et al*, 2012, 2014; Kaushal *et al*, 2015). Similarly for NLRP1, the activation of AIM2 seems to be pannexin-1-dependent and leads to the secretion of IL-1β as well as to pyroptotic cell death (Adamczak *et al*, 2014).

The ability of neurons to express other inflammasome components, and in particular NLRP3, is less clear. It has been for example reported that mouse cortical neurons, placed in ischemia-like conditions, overexpress NLRP1 and NLRP3 and increase their secretion of IL-1β and IL-18 (Fann *et al*, 2013). However, other studies suggest that NLRP3 is expressed neither in mice nor in human neurons (Kummer *et al*, 2007; Ramaswamy *et al*, 2013; Yang *et al*, 2014; Kaushal *et al*, 2015). Similarly, the presence of

an active NLRC4 inflammasome in neurons is also controversial. One report suggests that neurons exposed to flagellin express and form a NLRC4 inflammasome (Kaushal *et al*, 2015) whereas another study failed to detect any NLRC4 mRNA in these cells (Ramaswamy *et al*, 2013).

2.2. Inflammasome in astrocytes

The presence of activated caspase-1 in astrocytes growing in K^+-enriched medium raised the question if an inflammasome complex could be present in these cells (Silverman *et al*, 2009).

Several reports analysed the expression patterns of NLR and other inflammasome components in astrocytes. They commonly showed that human and murine astrocytes express NLRP1, NLRC4, AIM2, ASC and caspase-1 proteins, albeit at lower levels than in other cell types (Ramaswamy *et al*, 2013; Alfonso-Loeches *et al*, 2014; Kaushal *et al*, 2015; Zeis *et al*, 2015).

Regarding the expression of NLRP3, the reports diverge. On the one hand, some authors claim that NLRP3 protein is upregulated in cortical astrocyte cultures after exposure to LPS, ethanol or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ; Zou & Crews, 2012; Alfonso-Loeches *et al*, 2014; Lu *et al*, 2014; Zeis *et al*, 2015). This has been strengthened by *in vivo* observations showing that, in different neuropathological conditions, NLRP3 could be upregulated in astrocytes (Tezel *et al*, 2012; Kawana *et al*, 2013). On the other hand, other recent publications fail to detect any NLRP3 mRNA in human and murine astrocytes (Ramaswamy *et al*, 2013; Kaushal *et al*, 2015; Nuvolone *et al*, 2015). Thus, additional studies are still required to determine if astrocytes express NLRP3 and whether they can form a functional NLRP3 inflammasome.

More interestingly, the existence of a functional inflammasome has only been described in human astrocytes. This inflammasome assembles after treatment with ATP and is composed of NLRP2, ASC and caspase-1 (Minkiewicz *et al*, 2013). However, this finding needs confirmation and in particular with regard to the existence of such an inflammasome in mice.

2.3. Inflammasome in microglia

It is well known that microglia express different inflammasome-related proteins such as NLRP1, NLRP3, NLRC4, AIM2, ASC and Caspase-1 (Halle *et al*, 2008; Hanamsagar

et al, 2011; Kaushik *et al*, 2012; Jamilloux *et al*, 2013; Cox *et al*, 2015). At first glance, their expression profile seems equivalent to the one of macrophages. However, a recent publication shows that there are differences between microglia and macrophages, especially in regard to the expression level of inflammasome components in primates (Burm et al. 2015).

To date, the NLRP3 inflammasome remains the most studied inflammasome complex in microglia. A great number of reports demonstrate that activated microglia can form a functional NLRP3 inflammasome in sterile or infectious conditons. ATP, MSU and pathogens, representing different classical NLRP3 inflammasome activators, are able to trigger the release of IL-1β and IL-18 in microglia (Halle *et al*, 2008; Hanamsagar *et al*, 2011; Kaushik *et al*, 2012; Savage *et al*, 2012; Burm *et al*, 2015). In addition, the microglial NLRP3 inflammasome can be activated after *in vitro* exposure to different neuropathology-related proteins, such as prion protein (Shi *et al*, 2012) or the AD-related proteins Aβ and Chromogranin A (Halle et al. 2008a; Parajuli et al. 2013; Terada et al. 2010; Wu et al. 2013). The mechanisms leading to NLRP3 inflammasome activation in microglia and in macrophages have been described to be very similar (Halle et al. 2008a; Wu et al. 2013; Parajuli et al. 2013; Murphy et al. 2014).

All these studies confirm that microglia express a functional NLRP3 inflammasome. However, it has been poorly investigated if other inflammasomes need to be implicated in the secretion of IL-1β by microglia. Indeed, a single report described a NLRC4 inflammasome-dependent caspase-1 activation after brain infiltration by *Legionella pneumophila* (Jamilloux *et al*, 2013).

In conclusion, microglia, astrocytes and neurons have all been reported to express functional inflammasomes. However, some of the cited publications have to be confirmed by others. Moreover, little is known about the conditions under which inflammasome activation occurs in these cells as well as how these cell-specific inflammasome participate to CNS pathologies.

II.
Scope & Aims of the thesis

Since 2008, several research groups started to investigate whether the inflammasome is active in the CNS. The first published results were very encouraging since they demonstrated an important implication of the inflammasome signalling in diverse neuroinflammatory conditions. For example, recent evidences highlighted a role of the inflammasome in the development of two neurodegenerative diseases: Amyotrophic Lateral Sclerosis and Alzheimer's disease (Heneka 2013, Meissner 2010).

Parkinson's disease (PD) is another well described neurodegenerative disease, which also presents an important inflammatory component but, surprisingly, little is known about the implication of the inflammasome in this pathology. However, many PD reports suggest such a link: i) the detection of IL-1β in the CSF of patients, ii) ROS production and mitochondrial damage implicated in the neuronal death, or iii) the presence of misfolded proteins in Lewy bodies and their capacity to activate microglia. According to these considerations, **we aim to investigate the possible involvement of inflammasome in Parkinson's pathology.**

In order to correctly study the link between the inflammasome and PD, we first decided to clarify the expression profile and activation of the inflammasome in microglia and astrocytes. While different publications already addressed this topic, some critical questions remains. For example, it is admitted that microglia express a functional NLRP3 inflammasome but there is no evidence of such an expression in astrocytes. It is also unknown whether microglia and astrocytes are able to actively secrete IL-1α and HMGB1 or to trigger the pyroptosis after inflammasome activation. In addition, the expression of alternative inflammasome complexes by both cell types is, until now, largely under investigated. In respect with these observations, the first part of this work will be **to characterize the detailed *in vitro* capacities of murine microglia and astrocytes in term of inflammasome expression, activation and regulation**.

In the second part of the project, we will focus our attention on **the implication of the inflammasome in Parkinson's disease**. For this purpose, we will first test the reactivity of the glial inflammasome to products-related to neurodegenerative diseases *in vitro*. Then, we will study the effect of the NLRP3 deficiency on the neurodegenerative process occurring during PD, via an *in vivo* 6-hydroxydopamine (6-OHDA) PD model.

A summarized view of these objectives is depicted in the figure 16.

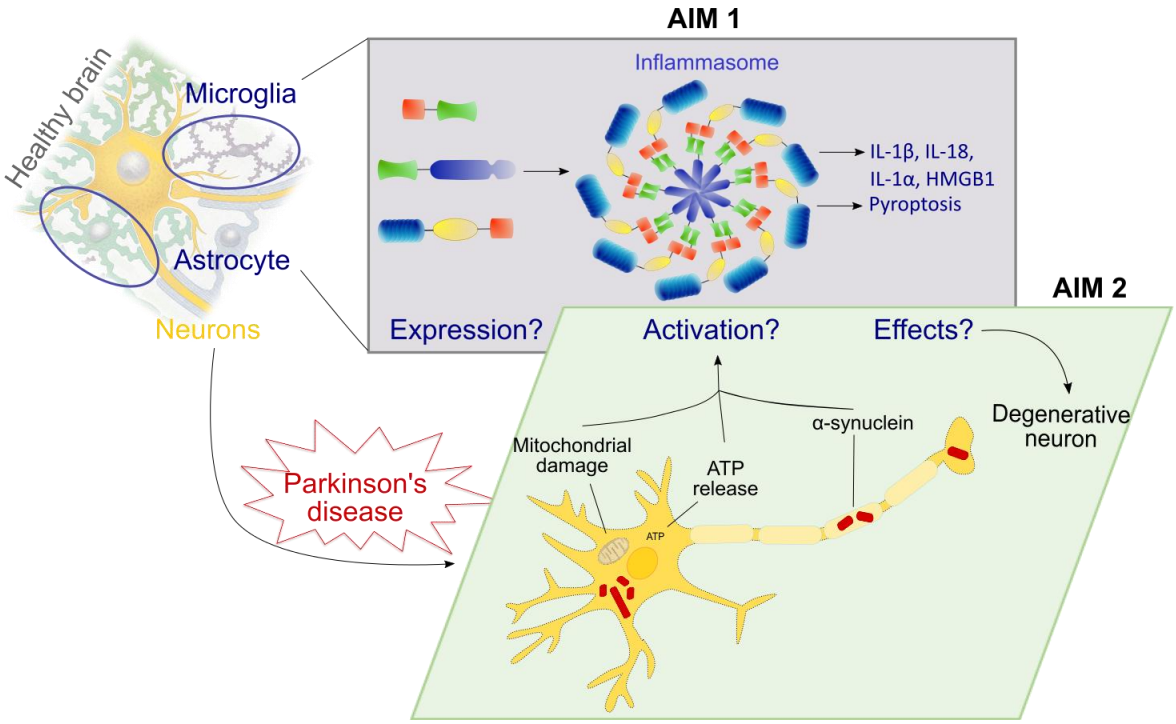


Fig. 16: Aims of the work.

Aim 1: Characterization of inflammasome components expression and regulation in mouse astrocyte and microglia. Aim 2: Study of the implication of inflammasome in Parkinson's disease.

III.
Materials & Methods

1. Mice

1.1 Mice strains

C57BL/6JOlaHsd mice (WT) were from Harlan (The Netherlands). *Nlrp3*$^{-/-}$ mice were provided by the University of Lausanne (Martinon, 2002). *Casp1*$^{-/-}$/*Casp11*$^{-/-}$ (hereafter referred to as Casp1$^{-/-}$) and *P2rx7*$^{-/-}$ mice were obtained from The Jackson Laboratory (Bar Harbor, USA) (see Table 4). All mouse strains were housed and bred in our animal house facility approved by the National Veterinary Inspection. The cages were maintained around 22°C with a 12:12 hour light:dark cycle and food and water were available *ad libitum*. All animal stabulation/procedures were conducted in accordance with European regulations.

Table 4. Knockout mice information.

Common name	Strain name	Background	Origin
Nlrp3$^{-/-}$	/	C57BL/6JOla	University of Lausanne
Casp1$^{-/-}$/*11*$^{-/-}$	B6.129S2*Casp1tm1Flv*J	C57BL/6NJ	Jackson Laboratory (# 016621)
P2rx7$^{-/-}$	B6.129P2P2rx7tm1Gab/J	C57BL/6J	Jackson Laboratory (# 005576)

1.2 Genotyping

DNA was extracted from mouse tail biopsies digested with 20 mg/mL proteinase K (Invitrogen) in Direct PCR buffer (Viagen Biotech, USA). After a 5 h incubation at 55°C under orbital shaking (550 rpm), the proteinase K was inactivated by heating the sample at 85°C during 45 min. The presence of each gene of interest was detected by performing a polymerase chain reaction (PCR). The primer sequences used for each strain are summarized in Table 5.

Table 5. Primer sequences for genotyping.

Mice	Primer sequences
Nlrp3$^{-/-}$	Forward: 5' -TCAAGCTAAGAGAACTTTCTG-3' Reverse: 5'-ACACTCGTCATCTTCAGCA-3' Reverse (EGFP cassette): 5'-AAGTCGTGCTGCTTCATGT-3'
Casp1$^{-/-}$/*11*$^{-/-}$	Reverse: 5'-GAGACATATAAGGGAGAAGGG–3' Forward (WT): 5'-GAGACATATAAGGGAGAAGGG-3' Forward (KO): 5'-TGCTAAAGCGCATGCTCCAGACTG-3'

P2rx7$^{-/-}$	Reverse (WT): 5'-TGGACTTCTCCGACCTGTCT-3' Forward (WT): 5'-TGGCATAGCACCTGTAAGCA-3' Reverse (KO): 5'-CTTGGGTGGAGAGGCTATTC-3' Forward (KO): 5'-AGGTGAGATGACAGGAGATC-3'

Subsequently, the PCR samples were migrated on a 1.2% agarose gel (E-gel double comb 1.2% agarose, Invitrogen). The presence of amplicon bands were revealed on a ChemiDoc MP system and its ImageLab software (BioRad, Belgium), as shown in the example presented in figure 17.

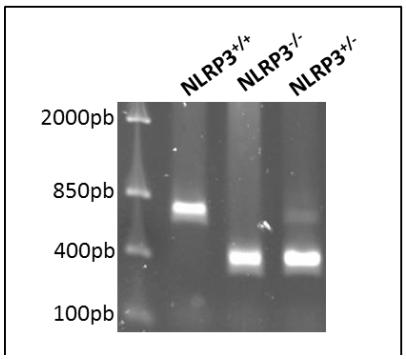


Fig. 17: Genotyping of *Nlrp3*$^{+/+}$, *Nlrp3*$^{-/-}$, *Nlrp3*$^{-/+}$ mice.

2. Cell cultures

2.1 Mouse primary cultures

2.1.1 Mixed glial cell cultures

Mixed glial cell cultures (MGC P0) were prepared from newborn C57BL/6 mice brains (Fig. 18 ; Losciuto *et al*, 2012). Briefly, after removing meninges, large blood vessels and the diencephalon, the brains were pooled and minced in cold phosphate-buffered saline (PBS, Sigma, Diegem, Belgium) solution by mechanical dissociation. Cell suspensions were centrifuged (10 min, 1100 rpm, 4°C), washed and plated in Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 100 U/mL penicillin and 100 U/mL streptomycin (Lonza, Verviers, Belgium) at 37°C, in a humidified atmosphere containing 5% CO_2. The culture medium was changed after three days and new fresh medium was added after 10 days. Mixed glial cultures had reached confluence after 2 weeks and were ready for passage or purification.

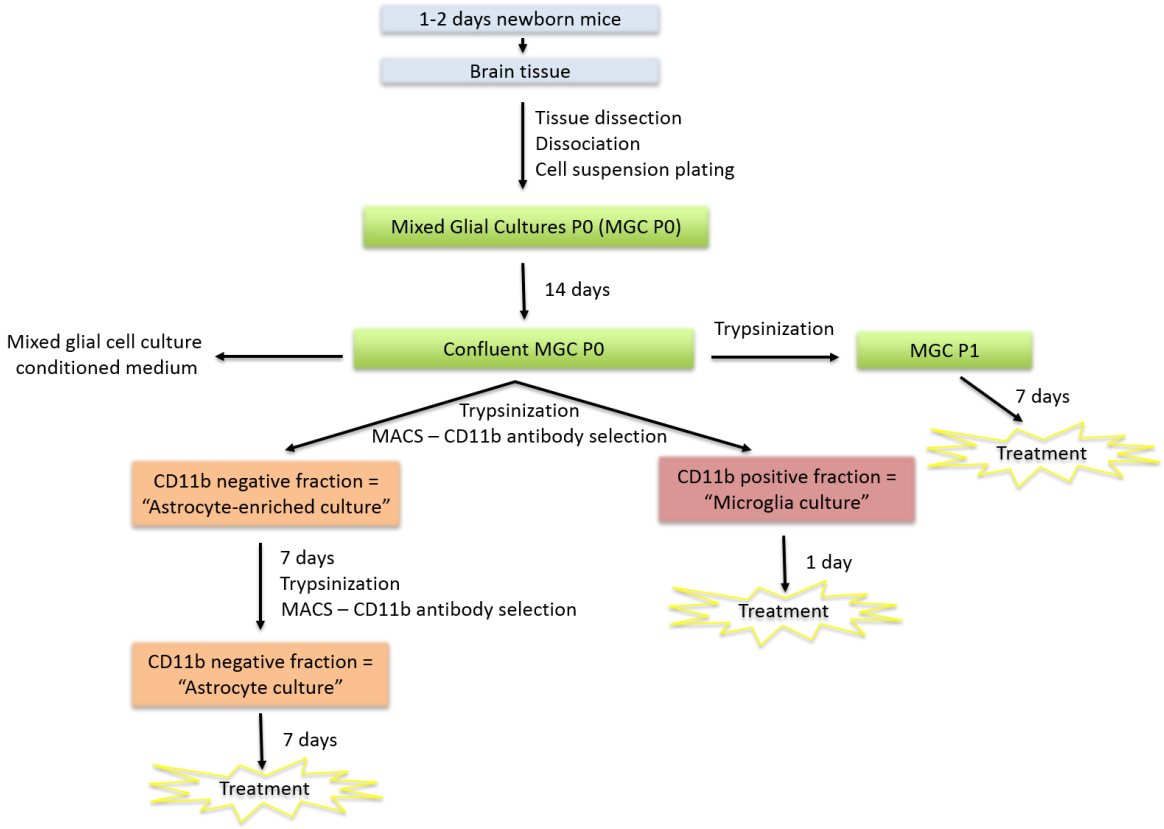


Fig. 18. Workflow for glial primary cultures achievement.

2.1.2 Microglial cultures

Microglia were positively sorted out from MGC P0 by the MACS (Magnetic Cell Sorting) technique (see Fig. 18). Before to start, the supernatant of MGC P0 cultures was collected to be further used as "mixed glial cell culture conditioned medium". Then, cells were washed with PBS and subsequently incubated with Trypsin/EDTA (Ethylenediaminetetraacetic acid) for 5 min at 37°C. After trypsin inactivation by FBS, cells were collected and centrifuged at 1100 rpm during 10 min. Then, the cells were washed by adding 10 mL of MACS buffer (PBS, 2 mM EDTA, 0.5% BSA ; all from Sigma) and counted. After an additional centrifugation step, the cells were resuspended in 90 µL of MACS buffer per 10^7 cells and complemented with anti-CD11b antibody coated magnetic nanoparticules, following the manufacturer's instructions (10 µl /10^7 cells, Miltenyi Biotec). CD11b is widely used as marker for microglial identification. Following 20 minutes of incubation at 4°C, the cells were washed again with 2 mL of buffer by 10^7 cells. After a final centrifugation step, the cells were resuspended in 500 µl of buffer and transferred on a LS column (Miltenyi Biotec) placed in a strong magnetic field (MidiMACS magnetic separation unit, Miltenyi Biotech). CD11b expressing microglia were trapped in the column due to their binding to magnetic nanoparticules. The remaining cells flowed through the

column and were harvested to constitute astrocyte-enriched cultures (see section 2.1.3.1). Afterwards, microglia were harvested by removing the column from the magnetic field and flushing it with 5 mL of MACS buffer. The cell suspension was centrifuged at 1100 rpm during 10 min and resuspended in DMEM supplemented with 20% of "mixed glial culture conditioned medium" and plated at a density of 2000 cells/mm² in MW96 plates and 1300 cells/mm² in MW6 plates. Microglia were treated twenty-four hours later.

2.1.3 Astrocyte cultures

2.1.3.1. Primary astrocytes

Astrocytes were negatively sorted during the MACS procedure dedicated to the microglia isolation and described above (Losciuto *et al*, 2012). The obtained "astrocyte-enriched cultures" (AEC-M1) were plated in complete medium (DMEM containing 10% FBS and 1% antibiotics) in 75 cm² flasks. After 3 days, medium was renewed and after seven days cultures reached confluence. At this time, the MACS procedure was repeated in order to reduce the microglial contamination of the astrocyte population. Cells were plated at a density of 800 cells/mm² in MW6 plates and were ready to use 7 days later.

2.1.3.2. Neurosphere-derived astrocytes

Neurospheres were obtained from embryonic murine NSCs derived from the ventricular zone of C57BL/6 mouse embryos at E14, as described previously (Grandbarbe *et al*, 2003; Crocker *et al*, 2008).

Neurospheres were cultured in Neurobasal medium supplemented with 2mM glutamine, penicillin (100 U/mL), streptomycin (100 U/mL), mouse recombinant Epidermal Growth Factor (EGF, 20 ng/mL) and 2% B27 without Vitamin A (All from Invitrogen, Belgium). Neurospheres were plated in 75 cm² flasks. After 4-7 days of proliferation, cells were dissociated with an enzyme-free cell dissociation buffer (Invitrogen), and reseeded as a single cell suspension after a 1:2 dilution.

Neurospheres were differentiated into astrocytes as previously described (Crocker *et al*, 2008). Briefly, non-dissociated neurospheres were plated on poly-L-ornithine (Sigma) coated multiwell plates. The cells were grown in DMEM containing 10% FBS with 1% Penicillin-Streptomycin (Invitrogen) until they reached confluence. The medium was renewed after 1, 2 and 7 days of differentiation and then, once a week. Mature astrocytes were submitted to treatment after 8 weeks of differentiation.

2.1.5. Bone marrow-derived macrophages (BMDM)

Bone marrow-derived macrophages (BMDM) were generated as previously described (Guarda *et al*, 2009). Bone marrow cells were isolated from tibia and femur of mice. To differentiate the cells into macrophages, primary bone marrow cells were cultured for 6 days in DMEM supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 10% FBS and 30% of L929 cells conditioned medium providing M-CSF (Macrophages colony-stimulating factor) among other factors. Cells were plated at a density of 2000 cells/mm² in MW96 plates and 1300 cells/mm² in MW6 plates.

2.2 Cell lines

2.2.1. MMGT12

MMGT12 are a murine cell line and are a subclone of the MMGT1, a murine cell line established after transfection of primary microglial cell cultures with a v-myc-containing plasmid. MMGT12 were provided by Dr. Vanmechelen (Innogenetics, Belgium; Briers *et al*, 1994). Cells were cultured in DMEM/F12 (Life Technologies) supplemented with 2% FBS, 1% Insulin-Transferrin-Selenium (ITS, Life Technologies) and 15% filtered WEHI conditioned medium (WEHI 3, WEHI3B and WEHI 3D, producers of interleukin-3 and granulocyte-macrophage-colony stimulating factor). Cells were passaged twice a week by mechanical dissociation (scrapping). For the experiments, MMGT12 cells were seeded at a concentration 1000 cells/mm² in MW6 plates and cultured in DMEM/F12 supplemented with 10% FBS and 1% ITS.

2.2.2. BV2

BV-2 cells were derived from raf/myc-immortalised murine neonatal microglia and were provided by Prof. Blasi (University Medical School, Perugia, Italy ; Blasi *et al*, 1990). Cells were maintained in DMEM with 10% FBS and passaged twice per week after mechanical dissociation (scrapping). For the experiments, cells were plated on poly-L-lysine coated-plate, at a density of 1200 cells/mm² in MW96 and grown in DMEM with 5% FBS. Any antibiotic was added to the medium.

2.3 Reagents

2.3.1 Pro-inflammatory agents

Cells were primed for 6 h or 24 h with ultrapure liposaccharide (LPS, 10 ng/mL), Pam3CSK4 (10 ng/mL) (both from Invivogen, Toulouse, France) or cytokines IL-1β (10 ng/mL), TNFα (10 ng/mL) or IFNγ (20 ng/mL) (all from R&D, Abingdon, UK), alone or in combination in case of the Complete Cytokine Mix (CCM: 10 ng/mL IL-1β, 10 ng/mL TNFα and 20 ng/mL IFNγ).

2.3.2 Inflammasome activators

Classical inflammasome activation was performed by Adenosine 5'-triphosphate (ATP, 1 mM, 30 min, Sigma). Other inflammasome activators used were Nigericin (1.34 μM, 2 h, Sigma), Aluminium hydroxide (100 μg/mL, 5 h, Pierce) or Ultra-pure flagellin (2.5 μg/mL, 5 h, Invivogen). Poly(dA:dT) (Invivogen) and highly concentrated ultrapure LPS (Invivogen) were delivered to the cytoplasm by transfection with Lipofectamine 2000 (Life technologies). Briefly, activators were mixed with lipofectamine in Opti-MEM medium during 5 min at room temperature. After the incubation, the mix was added to the wells at a final concentration of 2.5 μg/mL for poly(dA:dT), 1 μg/mL for LPS and 10 μg/mL for Lipofectamine. The cells were then incubated during 5 h poly(dA:dT) and 16 h for LPS.

2.3.3 Others activators

The amyloid beta peptide 25-35 ($A\beta_{25-35}$), its reverse form $A\beta_{35-25}$ and rotenone (all from Sigma) were used as indicated in the figure legends.

$A\beta_{1-42}$ peptide (from Sigma or Anaspec, Fremont, Canada or Bachem, Weil am Rhein, Germany) was resuspended following manufacturer's instructions, aliquoted and stored at -20°C. The fibrillar form was obtained by heating a 1:10 dilution (DMEM) of the peptides at 37°C during 7 days.

WT and A53T mutant α-synuclein were purchased from rPeptide (Bogart, USA). Aliquots were resuspended in H_2O to obtain a 100 μM solution. For an activation by oligomeric α-synuclein, the preparation was used directly. To obtain a fibrillar α-synuclein preparation, oligomeric form was diluted twice with Tris-HCl buffer and then incubated for 4 days at 57°C with shaking.

The fibrillar Aβ and α-synuclein preparations were characterized by western blotting (see section 3.3) using 6E10 anti-Aβ antibody (Eurogentec SIG-39320, Belgium) and 4D6 anti-α-synuclein antibody (Abcam, ab1903).

Nucleotides (ATP, ADP and Adenosine) were purchased from Sigma and used at 1 mM during 30 min to 6 h. The ATP contamination was controlled by the ATP Determination Kit (Life Technologies).

All these products have been tested for endotoxin contamination using the LAL PYROGENT™ Plus Single Test Vials (Lonza).

2.3.4. Inhibitors

The following molecules have been tested to inhibit the inflammasome activation: Potassium Chloride (KCl; 130, 75 or 25 mM), N-acetyl-L-cysteine (NAC, 5mM, antioxidant) and Cytochalasin D (cytoD, 2µM, inhibitor of actin polymerization) (all from Sigma) but also (L-3-trans-(Propylcarbamoyl)oxirane-2- Carbonyl)-L-Isoleucyl-L-Proline Methyl Ester (CA074-Me, 10 µM, inhibitor of cathepsin B, PeptANOVA). All inhibitors were added 30 min before the inflammasome activation except KCl which, was added in the same time than LPS priming.

Z-YVAD-FMK, a specific caspase-1 inhibitor (SantaCruz, Heidelberg, Germany) had also been used at a concentration of 20 µM and was added 15 min prior priming step.

3. Analytical techniques

3.1 Cell death/viability measurement

3.1.1 MTT assay

Cell viability was assessed after treatment by the mitochondria-dependent reduction of Thiazolyl Blue Tetrazolium Bromide (MTT) assay (Mosmann, 1983). MTT assay is based on the MTT (Sigma) reduction into the water-insoluble formazan by mitochondrial succinate dehydrogenase. Since the reduction of MTT can only occur in metabolically active cells, its measure reflects the viability of the cells. After treatment, cells were incubated with a 5 mg/mL MTT solution diluted 1:10 in DMEM during 30 min to 4 h, at 37°C. Then, medium was removed and the formazan was solubilized by 10 min incubation in DMSO at 37°C. The absorbance was read at 540 nm using a microplate reader

(TECAN, Austria). The viability was estimated by comparison to untreated cells (100% viability).

3.1.2 Lactate dehydrogenase release assay

Cell death and cell lysis were quantified by the colorimetric assay based on measurement of lactate dehydrogenase (LDH) released into the cell supernatant. LDH is a stable cytoplasmic enzyme released in case of membrane damage. Quantity of LDH in supernatant was determined following the assay protocol of Cytotoxicity Assay kit (Roche). Briefly, cells were submitted to treatment of interest or to medium containing 2% Triton 100x, which induced 100% of cell lysis. The cell-free culture supernatant were collected and incubated with the commercial reaction mixture (freshly prepared; 25 µL of solution 1 + 1.125mL of solution 2), during 30 min. The absorbance was read at 490 nm with a microplate reader. The determination of the cytotoxicity was calculated by comparison to untreated cells (= low control) and lysed cells (high control).

$$\text{Cytotoxicity (\%)} = \frac{\text{exp. value} - \textit{low control}}{\text{high control} - \text{low control}} x\ 100$$

3.2 Real-Time PCR gene expression analysis

3.2.1 Total RNA extraction and quantification

Total RNA was isolated using the Invisorb™ Spin Cell RNA mini Kit (Invitek) or InnuPREP RNA mini Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's protocol. Briefly, cell lysates were loaded on a first column to remove DNA. The filtrate was mixed with a 70% ethanol solution and loaded on a second column. After two washing steps with provided buffer, RNA was eluted with H_2O. RNA samples were then stored at -80°C.

Total RNA concentration was quantified by reading the absorbance at 260 nm of each sample using a Nanodrop 2000 spectrophotometer (Thermo Scientific). The sample contamination by protein, phenol or other contaminants was estimated by the absorbance ratio 260/280 that should be greater than 2.

3.2.2 Reverse transcription

Complementary DNA ($_c$DNA) was synthetized from RNA samples using the ImProm-II Reverse Transcription System (Promega, Leiden, The Netherlands). Two mixes

were prepared (see Table 6): the primer-sample mix and the reverse transcription mix. The secondary structures of RNAs were denaturated by heating the primer-sample mix at 70°C for 5 min followed by a flash cooling step on ice. Then, the two mixes were pooled together to obtain a final volume of 40 µL. Samples were then incubated for 5 min at 25°C (annealing), 1 h at 42°C (extension) and 15 min at 70°C (reverse transcriptase inactivation) and subsequently stored at -20°C until use.

Table 6. Reverse transcription mixes.

Primers sample mix	
Oligo(dT)	1µl
Total RNA	0,1µg to 2µg
Nuclease free water	qs 10µl
Final volume	10µl

Reverse transcription mix	
ImProm-II 5x Reaction buffer	8µl
MgCl2 (25mM)	6µl
dNTP mix (10mM)	2µl
Recombinant RNAsin Ribonuclease inhibitor	1µl
ImProm-II reverse transcriptase	2µl
Nuclease free H2O	11µl
Final volume	30µl

3.2.3 Real-time polymerase chain reaction

Gene expression was analysed using Bio-Rad Thermal Cyclers (iQ5 and CFX Real-Time PCR Detection System, Bio-Rad Laboratories) with SYBR Green Supermix (Promega). Expression was normalized to the housekeeping genes (*Rpl27*). Primer sequences were designed using the Beacon Designer Software (Bio-Rad) and are listed in Table 7. Analysis of gene expression was performed using the comparative 2^{-dCt} method:

$$dCt = (C_{t,\ target} - C_{t,\ Rpl27}) \qquad 2^{-dCt} = \text{Relative expression}$$

Table 7. Mouse primers sequences.

Gene	Accession number	Sequences
Aif1	NM_019467	F: 5'-TTCCCAAGACCCACCTAG-3' R: 5'-TCCTCATACATCAGAATCATTC-3'
Aim2	NM_001013779	F: 5'-ATAGGAGGAACAACAACAT-3' R: 5'-GCCATCTTCTGCTACATA-3'
Casp1	NM_009807	F: 5'-AGGAATTCTGGAGCTTCAATCAG-3' R: 5'-TGGAAATGTGCCATCTTCTTT-3'

Casp4	NM_007609	F: 5'-GCTCTTACTTCATCACTA-3' R: 5'-AATATCTCGTCAAGGTTG-3'
Cxcl10	NM_021274	F: 5'-TTCTGCCTCATCCTGCTG-3' R: 5'-AGACATCTCTGCTCATCATTC-3'
Gfap	NM_010277	F: 5'-GGTTGAATCGCTGGAGGAG-3' R: 5'-CTGTGAGGTCTGGCTTGG
Hmgb1	NM_010439	F: 5'-TGGCAAAGGCTGACAAGGCTC-3' R: 5'-GGATGCTCGCCTTTGATTTTGG-3'
Il18	NM_008360	F: 5'-ACCAAGTTCTCTTCGTTGAC-3' R: 5'-TCACAGCCAGTCCTCTTAC-3'
Il1a	NM_10554	F: 5'-GTATCAACTCTAAGAACTACT-3' R: 5'-ATATCTGGAAGTCTGTCATA -3'
Il6	NM_031168	F: 5'-ACCGCTATGAAGTTCCTCTC-3' R: 5'-CTCTGTGAAGTCTCCTCTCC-3'
Itgam	NM_008401	F: 5'-TGGACGCTGATGGCAATACC-3' R: 5'-GGCAAGGGACACACTGACAC-3'
Nlrc4	NM_001033367	F: 5'-GTCAAGTGTTATCCAAGTTA-3' R: 5'-CGCTAATATCATAGTCATCAA-3'
Nlrp1	NM_001004142	F: 5'-GGTGTGCTGGTTGGTCTGC-3' R: 5'-GTGCTGTGGTGGTCTGTGAG-3'
Nlrp12	NM_001033431	F: 5'-AAGAGATGAGATGCTACCTTGAGAG-3' R: 5'-ATGCCAACACTTCCTCCTTCAC-3'
Nlrp2	NM_177690	F: 5'-AAGCCTGTAGAGGTCTTACTG-3' R: 5'-ACTGTGTCCGTGTGGTTAC-3'
Nlrp3	NM_145827	F: 5'-GCTCCAACCATTCTCTGACC-3' R: 5'-AAGTAAGGCCGGAATTCACC-3'
Nlrp6	NM_001081389	F: 5'-GGACGAGAGGAAGGCAGAG-3' R: 5'-GCACACGAAGGGCACAAAG-3'
Nos2	NM_010927	F: 5'-AGCCCTCACCTACTTCCTG-3' R: 5'CAATCTCTGCCTATCCGTCTC-3'
Ptgs2	NM_011198	F: 5'-GCCTGGTCTGATGATGTATGC-3' R: 5'-GAGTATGAGTCTGCTGGTTTGG-3'
Pycard	NM_023258	F: 5'-AGGAGTGGAGGGGAAAGC-3' R: 5'-AGAAGACGCAGGAAGATGG-3'
Rpl27	NM_011289	F: 5'-ACATTGACGATGGCACCTC-3' R: 5'-GCTTGGCGATCTTCTTCTTG-3'
Tlr4	NM_021297	F: 5'-TTCACCTCTGCCTTCACTAC-3' R: 5'-CACTACCACAATAACCTTCCG-3'
Tnf	NM_013693	F: 5'-GGTTCTGTCCCTTTCACTCAC-3' R: 5'-TGCCTCTTCTGCCAGTTCC-3'

3.3 Immunoblotting

3.3.1 Protein extraction

Cell extracts were harvested after direct cell lysis by 100µL of SB3x buffer (187.5 mM Tris-HCl, 6% w/v SDS, 0,012% w/v bromophenol blue, 30% w/v glycerol and 50 mM DTT (dithiothreitol, added freshly), pH 6.8).

Proteins from supernatant were precipitated following a Chloroform-Methanol precipitation protocol (Wessel, 1984). Briefly, supernatants were collected, centrifuged and mixed with 1:1 methanol and 1:5 chloroform. After centrifugation (13 000 rpm, 5 min), the upper aqueous phase was removed and 1:1 methanol was added. Samples were newly centrifuged (13 000 rpm, 5 min), and the liquid phase discarded. The pellets were resuspended in 60 µL of SB3x buffer.

All protein samples were denaturated by a heating step (95°C for 5 min) and then stored at -20°C.

3.3.2 Western Blot

Cell lysates were separated by a denaturing SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis). The samples were migrated at 100 V during 120 min (47 mA). The proteins were transferred onto a nitrocellulose membrane at 400 mA during 50 min (5-7 V). To avoid aspecific protein interactions, the membranes were blocked with a 3% milk PBS-0.1% Tween solution during 1 hour, at room temperature. The blots were then incubated overnight (4°C) with a specific primary antibody diluted in blocking buffer (see Table 8). The next day, the membranes were washed with PBS-0.1% Tween solution and subsequently incubated with Horseradish Peroxidase (HRP)-linked secondary antibody for 2 hours at room temperature. After washing, the presence of labelled protein bands was revealed with the SuperSignal West Femto Chemiluminescence Substrate (Life Technologies) and the pictures were acquired on the Bio-Rad ChemiDoc XRS Imager and its Image Lab Software (Bio-Rad Laboratories).

Table 8. Immunoblotting antibodies.

Primary antibody	Host	Dilution	Origin
Anti-ASC	Rabbit	1:1000	AdipoGen (AG_25B-0006)
Anti-Caspase-1	Mouse	1:1000	AdipoGen (AG-20B-0042)
Anti-HMGB1	Rabbit	1:1000	Abcam (Ab18256)
Anti-IL-1α	Goat	1:500	R&D systems (AF-400-NA)
Anti-IL-1β	Goat	1:500	R&D systems (AF-401-NA)
Anti-NLRP3	Mouse	1:1000	AdipoGen (AG-20B-0014)
Anti-α-tubulin	Mouse	1:1000	Abcam (Ab7291)

Secondary antibody	Host	Dilution	Origin
HRP-conjugated anti-rabbit	Donkey	1:5000	Amersham (NA934)
HRP-conjugated anti-mouse	Sheep	1:2000	Amersham (NA931)
HRP-conjugated anti-goat	Bovine	1:1000	Jackson ImmunoResearch (205-035-108)

3.4 Immunocytochemistry

Cells dedicated to an immunocytochemistry analysis were cultured on poly-L-lysine coated coverslips, stimulated and then fixed with paraformaldehyde (4% in PBS) for 20 min. After 3 washing steps with PBS (5 min), the cells were permeabilized during 5 min in PBS containing 0.3% Triton X-100 (Sigma). The blocking step was done during 30 min using a PBS solution containing 3% BSA (Bovine Serum Albumin, buffer is named BSA buffer) (Sigma). A double immunostaining was performed by simultaneously incubating the coverslips with primary antibodies of interest overnight at 4°C. The used primary antibodies are listed in Table 9. After 3 additional washing steps in PBS (5 min each), the cells were incubated with the secondary antibodies during 2 h at room temperature (for dilutions in BSA buffer, see Table 9). Cells were then washed and mounted with DAPI-Fluoromount G (SouthernBiotech, USA). The picture acquisition was performed with a LSM 510 META inverted confocal microscope (Carl Zeiss Micro Imaging, Göttingen, Germany).

Table 9. Immunocytochemistry antibodies.

Primary antibody	Host	Dilution	Origin
Anti-Iba1	Rabbit	1:200	Biocare medical (CP290)
Cy3-labelled anti-GFAP	Mouse	1:800	Sigma (C9205)

Secondary antibody	Host	Dilution	Origin
Alexa-Fluor 555 anti-rabbit	Donkey	1:1000	Life Technologies (A-31572)

3.5 Protein release quantification

3.5.1 IL-1α, IL-1β, IL-18, TNFα, HMGB1 and CXCL10 quantification.

After treatment, cell culture supernatants were harvested and assayed for the presence of IL-1α, IL-1β, TNFα and CXCL10 by Enzyme Immunoassay's according to the manufacturer's instructions. Briefly, 96-well microplates (NUNC immuno plate Maxisorb, Thermo Scientific) were coated overnight with the appropriate "*Capture antibody*". On the next day, the plates were washed and incubated with a blocking buffer (specific to each ELISA). After 1 h and additional washing steps, the samples and standards were incubated in the capture antibody-coated plates for 2 h. Then, after additional washing steps, a biotinylated "*Detection antibody*" recognizing the protein of interest was incubated for 2 h. Exceeding antibodies were removed by several washing steps and peroxidase-conjugated streptavidin was added in each wells for 20 min. The incubation of the peroxidase with its substrate produced a colorimetric reaction. After 20 min of incubation, the reaction was stopped by adding sulfuric acid. The absorbance was measured at 450 nm with a reference wavelength of 540 nm using an absorbance microplate reader. A standard curve was generated by plotting the optical density of standards. On this base, the sample optical density values were converted into concentrations.

The IL-18 and HMGB1 ELISA kits used the same principle than exposed before. However, IL-18 ELISA kit contains a 96-wells microplate already coated with two monoclonal antibodies against two different epitopes of IL-18. In the case of HMGB1 quantification, the wells of the provided microtiter strips were already coated with purified anti-HMGB1 antibody and the detection antibody was directly bind to peroxidase, suppressing one step of the previous protocol.

Table 10. Enzyme linked Immunobsorbent assay's list.

Target	Kit name	Reference	Origin
IL-1β	Mouse IL-1 beta/IL-1F2 DuoSet	DY401	R&D systems
IL-1α	Mouse IL-1 alpha/IL-1F1 DuoSet	DY400	R&D systems
IL-18	Mouse IL-18 ELISA Kit	7625	MBL
CXCL10	Mouse CXCL10/IP-10/CRG-2 DuoSet	DY466	R&D systems
TNFα	Mouse TNF-alpha DuoSet	DY410	R&D systems
HMGB1	Mouse HMGB1 ELISA	ST51011	IBL international

3.5.2 PGE2 quantification

The PGE2 EIA kit (ADI-900-001, Enzo Life Sciences) is a competitive immunoassay.

In a MW96 plate, 50 µL of cell supernatant were added to 50 µL of "PGE enzyme conjugate". The plate was then shaking at 500 rpm during 2 h. After washing, 150 µL of substrate solution were added for 30 min. Then, the reaction was stopped by adding of 50 µL of trisodium phosphate solution to each well. The absorbance was immediately measured at 405 nm using a microplate reader (TECAN). Sample concentrations were determined with a PGE2 standard curve.

4. 6-OHDA Parkinson's disease mouse model

The 6-hydroxydopamine (6-OHDA) is an organic compound derived from dopamine by addition of a 6-hydroxyl group (Fig. 19). It is known to be neurotoxic as it selectively causes the death of the dopaminergic and noradrenergic neurons. 6-OHDA enters into neurons via the dopamine reuptake transporter. Once in the cytosol, 6-OHDA (or its metabolite) induces an oxidative stress resulting in dopaminergic neuron cell death. The administration of 6-OHDA into the striatum cause a retrograde degeneration of dopaminergic neurons within the substantia nigra (Schober, 2004; Dooley *et al*, 2012).

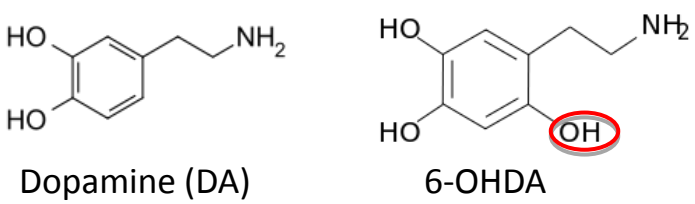


Fig. 19. Dopamine and 6-hydroxydopamine structures.

4.1 Reagents and Solutions

The 6-OHDA solution was prepared under sterile conditions at a final concentration of 2 µg/µL. To this end, an ascorbic acid solution was prepared by dissolving 2 mg of ascorbic acid (Sigma) in 10 mL of 0.9% saline solution. 10 mg of 6-OHDA hydrochloride (Sigma) was dissolved in 4 mL of the ascorbic acid solution to obtain a 2 µg/µL solution. The 6-OHDA and vehicle (ascorbic acid) solutions were sterilized through a 0.22 µm filter. As 6-OHDA oxidized rapidly, the solutions were aliquoted into 100 µl tubes, stored on ice, protected from light and were always freshly prepared.

4.2 Surgery

12 weeks (for transcripts analysis) or 18 weeks old (for immunohistochemistry) wild-type and *Nlrp3*$^{-/-}$ mice were used. Mouse anaesthesia was induced under 3% isoflurane. Mice were then transferred into the stereotaxic frame (Kopf) and the skin was incised with a scalpel. After revelation of sutures by a 3% H_2O_2 solution, the skull was pierced with a 0.7 mm drill at following coordinates: ML: +2.3 mm; AP: +0.5 mm. The needle of the Hamilton syringe, previously loaded with either the vehicle or the 6-OHDA solution, was introduced down into the striatum, to the +3.3 mm dorso-ventral (DV) coordinates for 10 seconds and then withdrawn back to +3.2 mm DV (Fig. 20).

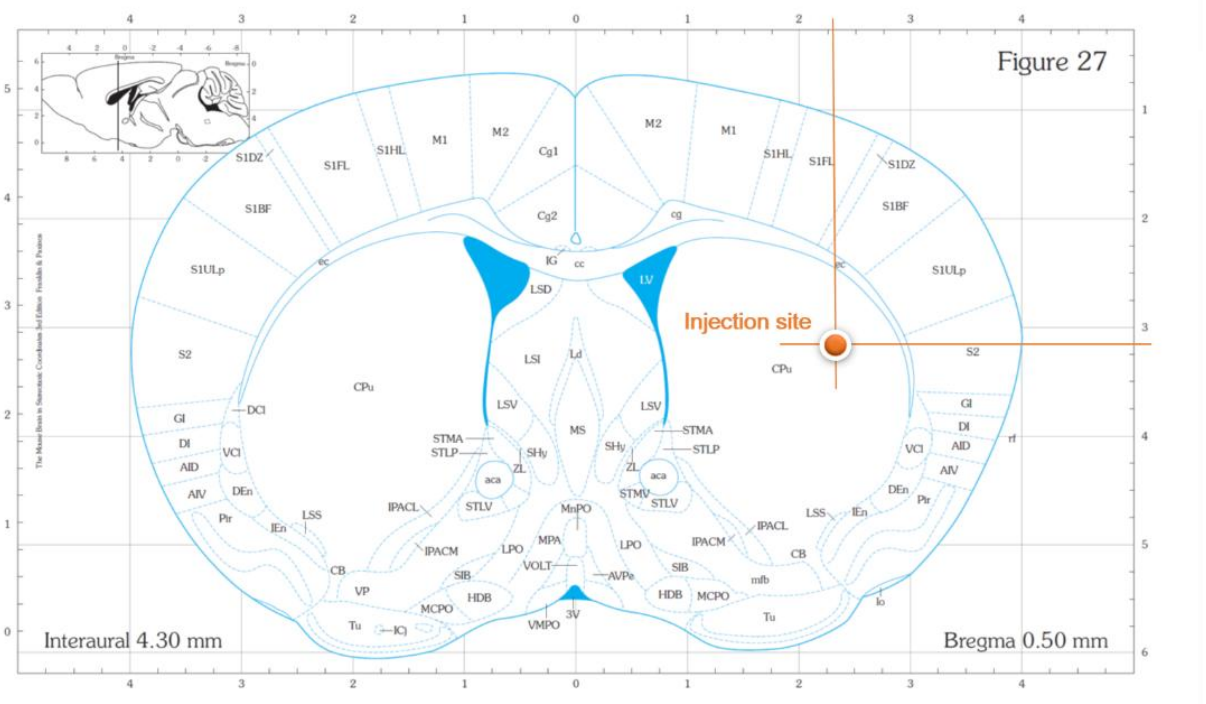


Fig. 20. Theoretical coordinates for the injection site. From Franklin and Paxinos, 2007.

After 2 min, 6-OHDA or vehicle was injected at a rate of 2 µL/5 min. After 2 additional minutes, the needle was withdrawn, the skull hole sealed with bone wax and the wound clipped. In order to avoid any post-operative pain we locally administered 2% lidocaine gel onto the wound (Astra Zeneca 2% Xylocaine gel). After the surgical procedure, the mice were housed at 2 animals per cage and monitored during the following days.

4.3 Sacrifice and brain sampling

Prior perfusion, the anesthetic solution was prepared by mixing 550 µL of ketamine solution (Nimatek 100 mg/mL) together with 323.4 µL of Dorbene Vet (1mg/mL) and 2.2 mL of PBS. The mice were anesthetized with a 200 µL intra-peritoneal injection. Once sedated, the skin was incised below the sternum and after cutting, the heart and liver were exposed. A 25 gauge needle was introduced into the left ventricle and after the PBS flow was turned on, the right atrium was incised. After 7 minutes, the brain was extracted and processed according to the following analyses.

4.4 Immunohistochemistry

4.4.1 Brain fixation

After extraction, the brain was placed into a 50 mL falcon tube containing 7.5 mL of a 4% formaldehyde solution and cooled at +4°C. After 2 days of fixation, the brains were washed 3 times in PBS and stored in a 0.02% PBS–azide solution at +4°C.

4.4.2 Brain sections

Thick sections (40µm) were realized on a Leica VT1000S vibratome. The brain was cut into two hemispheres, which were glued onto the object holder with cyanoacrylate adhesive. The dorsal parts of both hemispheres (cortices) were oriented toward the razor blade. The buffer tray was filled with PBS and the cutting speed as well as the frequency set to 5. The first 1 mm of the brain were cut into 100 µm thick slices and discarded. After discarding two additional 40 µm thick sections, the further sections (40 µm) were serially collected into eight 2 mL tubes containing 1.8 mL of cryoprotective medium (PBS – Ethylene glycol V/V supplemented with 1% PVP40). The tubes were stored at -20°C until use for immunohistochemical analysis.

4.4.3 Floating sections immunohistochemistry

The free-floating sections were first removed from the cryoprotective medium and washed twice with PBS containing 0.1% Triton X-100. Next, the permeabilization was performed by a 20 min incubation in PBS with 3% H_20_2 and 1% Triton X-100. Once again, sections were washed twice and incubated during 1 h in PBS with 0.1% triton X-100 and 5% BSA. After new washing steps, slices were incubated overnight with primary antibodies (see Table 11) diluted in PBS with 0.1% triton X-100 and 0.5% BSA. On the next day, slices were newly washed and then incubated for 90 min, at room temperature and under shaking, with the secondary antibodies (see Table 11) diluted in PBS containing 0.1% Triton X-100 and 0.5% BSA. Then, sections were washed 10 min and stained with DAPI (Molecular probes, The Netherlands) diluted 1:5000 in PBS containing 0.1% Triton X-100 to label the nuclear DNA. Finally, after twice washing steps, slices were transferred into TBS (pH 7.4) before being mounted onto slides with fluoromount-G (Southern Biotech).

Table 11. Immunohistochemistry antibodies.

Primary antibody	Host	Dilution	Origin
Anti-Iba1	Rabbit	1:2000	Wako (019-19741)
Anti-CD68	Rat	1:2500	AbDserotec (MCA1957)
Anti-GFAP	Mouse	1:2000	Cell Signalling (3670S)
Anti-TH	Rabbit	1:2000	Millipore (AB152)

Secondary antibody	Host	Dilution	Origin
Alexa-Fluor 488 anti-rat	Goat	1:1000	Life Technologies (A11006)
Alexa-Fluor 647 anti-rabbit	Donkey	1:1000	Life Technologies (A31573)
Cy3 anti-rat	Donkey	1:2000	Jackson ImmunoResearch (712-165-153)
Cy3 anti-mouse	Donkey	1:2000	Jackson ImmunoResearch (715-165-151)

4.4.4 Picture acquisition and analysis

Pictures were acquired by using a LSM 510 META inverted confocal microscope (Carl Zeiss Micro Imaging, Göttingen, Germany). Striatum and midbrain pictures were acquired with 20x and 10x objectives respectively. For each animal, the striatum was analysed at 4 different medio-lateral levels (3, 2.7, 2.4 and 2.1 mm from midline) and the

midbrain at 5 different medio-lateral levels (1.8, 1.64, 1.48, 1.32, and 1.16). After acquisition, the pictures were imported in the ImageJ software and binarized. The regions of interest were delimited and the stained surface measured. For each hemisphere the integrated value were used for the subsequent analysis (sum of 4 values for the striatum or 5 values for the midbrain).

4.5 Transcript analysis

4.5.1 Sampling

Once removed, the brain was dissected in order to recover the midbrain and striatum regions. These biopsies were immediately dry frozen at -80°C until the RNA extraction.

4.5.2 RNA extraction

Total RNA were extracted following a phenol-chloroform protocol using RNA NOW reagent (Biogentex, Seabrook, USA), according to the manufacturer's protocol. Briefly, tissues were mechanically lysed and homogenized in the RNA NOW solution. After addition of chloroform, samples were frozen overnight at -80°C. Following defrosting and centrifugation, the homogenate layers were into two phases. The upper phase, containing RNA, was carefully collected and transferred to a new tube. Then, isopropanol was added to the samples, which were subsequently placed overnight at -80°C. The next step consisted in a centrifugation in order to obtain RNA precipitated pellets. After 2 ethanol washing steps, pellets were air dried and finally dissolved in water.

4.5.3 Quantification, Reverse Transcription and RT-PCR

Please refer to the section 3.2.

5. Statistical analysis

The significance of multiple treatments was analysed by a non-parametric analysis of variance Kruskal–Wallis test followed by Dunn's multiple comparisons test or by a parametric One-way analysis of variance (ANOVA) followed by Dunnett's multiple

comparisons tests.

ANOVA on gene expression was performed on the delta-Ct values ($Ct_{gene\ of\ interest}$ - $Ct_{housekeeping\ gene}$) and the analysis of protein quantification (ELISA) was performed on log-transformed concentrations (if present, zero values were removed by adding an offset to the dataset equal to half the smallest non-zero concentration).

All statistical analyses were performed using GraphPad Prism 6 software and differences with p-values less than 0.05 were considered significant. All experiments have been performed at least three times.

IV. Results

Part I: Characterization of inflammasome expression in glial cells.

Until now, the inflammasome signalling in glial cells has only been partially addressed. This is why the main focus of this thesis was to characterize the expression and regulation of inflammasome components in glial cells. We turned our attention to all inflammasome complexes expressed in both microglia and astrocytes but with a special regard to the NLRP3 inflammasome.

1. Inflammasome expression and regulation in microglia.

1.1 The NLRP3 inflammasome in microglia.

1.1.1 Primary culture is the best model to study NLRP3 inflammasome in microglial cells.

In order to investigate the expression and regulation of the NLRP3 inflammasome in microglia, we first tried to identify the best-suited model. We tested three different microglial models: the MMGT12 and BV2 cell lines as well as the mouse primary microglia (hereafter simply referred as microglia). In addition, we used bone marrow-derived macrophages (BMDM) as a positive control for NLRP3 inflammasome expression and activation. All cells were submitted to the classical protocol of NLRP3 inflammasome activation, consisting in a priming step of 6 h with LPS (signal 1) followed by a 30 min ATP stimulation (signal 2 ; Fig. 21A).

First, we analysed, in all cell types and by RT-PCR, the gene expression of the NLRP3 inflammasome components NLRP3, ASC and Caspase-1. We observed that the constitutive expression levels (Ctrl) of all these genes were, on average, 30-fold lower in both microglial cell lines compared to primary microglia and BMDM (Fig. 21B). However, an exposure to LPS could upregulated the *Nlrp3* and *Casp1* genes in all cells (Fig. 21B). In addition to the inflammasome components, we also studied the expression level of the *Il1b* gene. Its expression was upregulated in all cell types (161-fold for MMGT12, 70-fold for BV2, 49-fold for microglia and 43-fold for BMDM ; Fig. 21C). This result was confirmed at the protein level by Western blot (WB) analysis. All studied microglial cells were able to upregulate the expression of the pro-IL-1β protein after LPS priming (Fig. 21D).

As IL-1β secretion is commonly used as a read out for inflammasome activation, we analysed the ability of the different microglial cells to release IL-1β upon inflammasome

stimulation. To this end, we performed WB assays (Fig. 21D) and ELISAs (Fig. 21E). The WB analysis showed that, while all microglia models produced pro-IL-1β after LPS priming (Fig. 21D), the primary microglia were the sole to secrete detectable mature IL-1β into the supernatant (Fig. 21D). Moreover, this microglial IL-1β secretion, which we quantified by ELISA (2172±359 pg/mL), was in the same order of magnitude as for BMDM (4636±1163 pg/mL, Fig. 21E). In line with these observations, we selected primary microglia to further investigate NLRP3 activation and regulation in microglial cells.

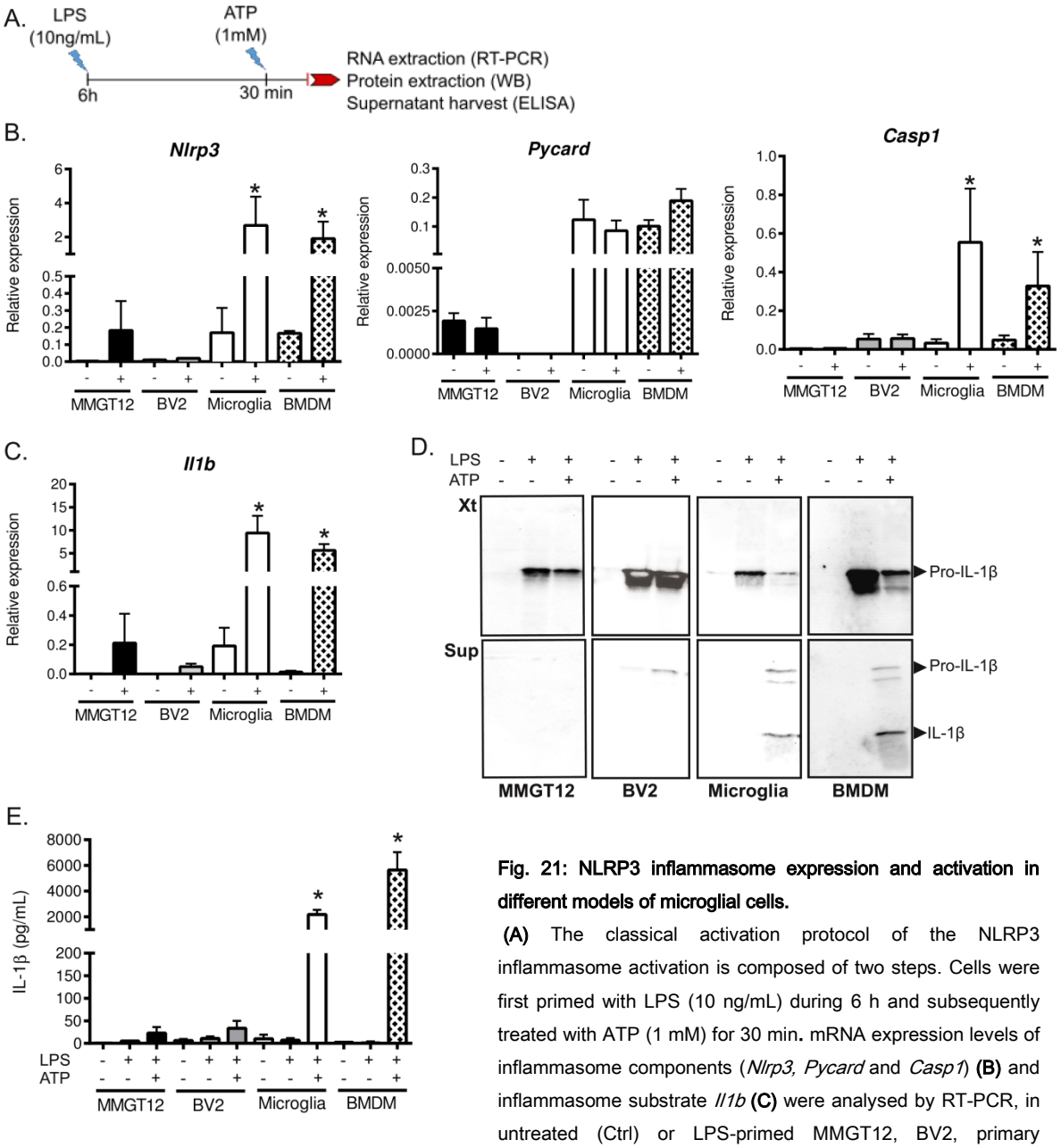


Fig. 21: NLRP3 inflammasome expression and activation in different models of microglial cells.

(A) The classical activation protocol of the NLRP3 inflammasome activation is composed of two steps. Cells were first primed with LPS (10 ng/mL) during 6 h and subsequently treated with ATP (1 mM) for 30 min. mRNA expression levels of inflammasome components (*Nlrp3, Pycard* and *Casp1*) **(B)** and inflammasome substrate *Il1b* **(C)** were analysed by RT-PCR, in untreated (Ctrl) or LPS-primed MMGT12, BV2, primary and bone marrow-derived macrophages (BMDM). Data are normalized to *Rpl27*. **(D, E)** Cells were activated following the classical activation protocol by LPS and/or ATP. **(D)** Cell-free culture supernatant (Sup) and cell lysates (Xt) were analysed by WB for the expression of IL-1β after activation with LPS and/or ATP. **(E)** IL-1β released in culture supernatant was quantified by ELISA. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test

1.1.2 Microglia secrete IL-1β in an NLRP3 inflammasome-dependent way.

In macrophages, NLRP3 inflammasome is known to be activated by a large variety of factors including PAMPs and DAMPs (see Introduction, Part II. 2.1). Here, we wanted to investigate if the microglial NLRP3 inflammasome also responds to ATP, Nigericin and Alum. To this end, we treated LPS-primed microglia with these NLRP3 activators. WB assays and ELISA analysis revealed that these treatments induced caspase-1 cleavage as well as IL-1β secretion in microglia (Fig. 22A and Fig. 22B). It is noteworthy that the priming step is prerequisite for these events, as we were not able to observe any IL-1β secretion without LPS treatment (Fig. 22B). Moreover, we noted that ATP and Nigericin treatments induced a stronger IL-1β release (respectively 2945±638 pg/mL and 4260±994 pg/mL) than particulate stimuli (Alum, 457±112 pg/mL ; Fig. 22B). In addition to IL-1β production, a trend in an increase of LDH release was observed in the supernatant after inflammasome activation (Fig. 22C).

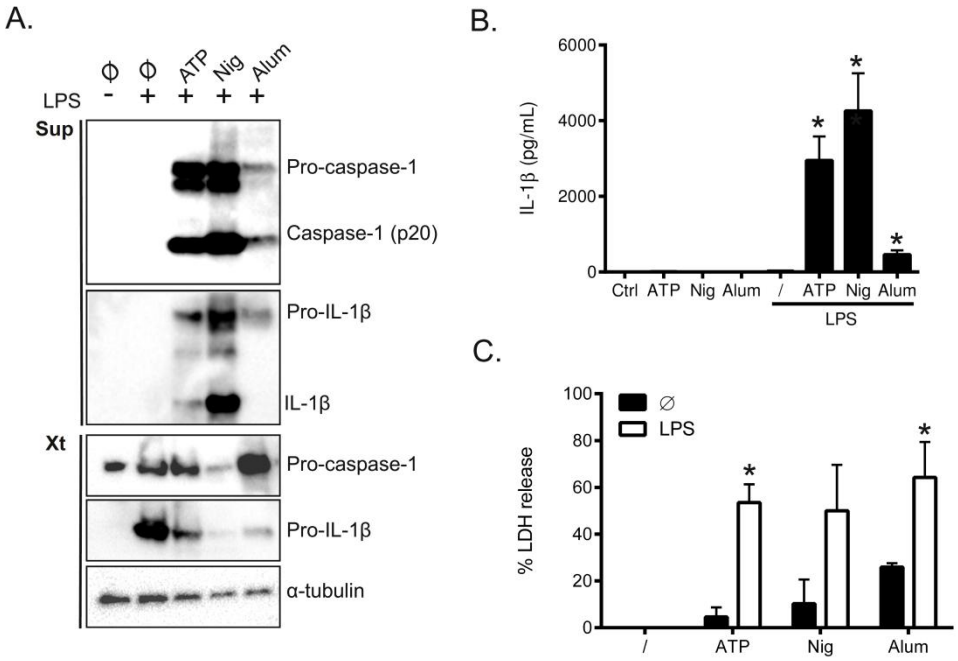


Fig. 22: ATP, Nigericin and Alum induce caspase-1 cleavage and IL-1β release by LPS-primed microglia.

Untreated or LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). **(A)** Cell-free culture supernatants (Sup) and cell lysates (Xt) were analysed by WB for IL-1β and caspase-1 expressions. **(B)**. IL-1β secretion in the supernatant was analysed by ELISA. **(C)** LDH release in the supernatant was analysed by LDH cytotoxicity test. LDH results are shown as percentage of LDH release by cells treated with Triton-X100 (100%). Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *=p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

In order to analyse whether this IL-1β production depends on the NLRP3 inflammasome, we submitted *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia to the same activation protocol. We observed a dramatic decrease of IL-1β secretion in both deficient cell types and in response to all tested stimuli (ATP, Nigericin or Alum ; Fig. 23A). Similarly, the treatment with z-YVAD, a caspase-1 inhibitor, reduced the release of IL-1β subsequent to the inflammasome activation (Fig. 23B). In contrast, the NLRP3- or Caspase1-deficiency did not affect LPS-mediated TNFα secretion (Fig. 23C).

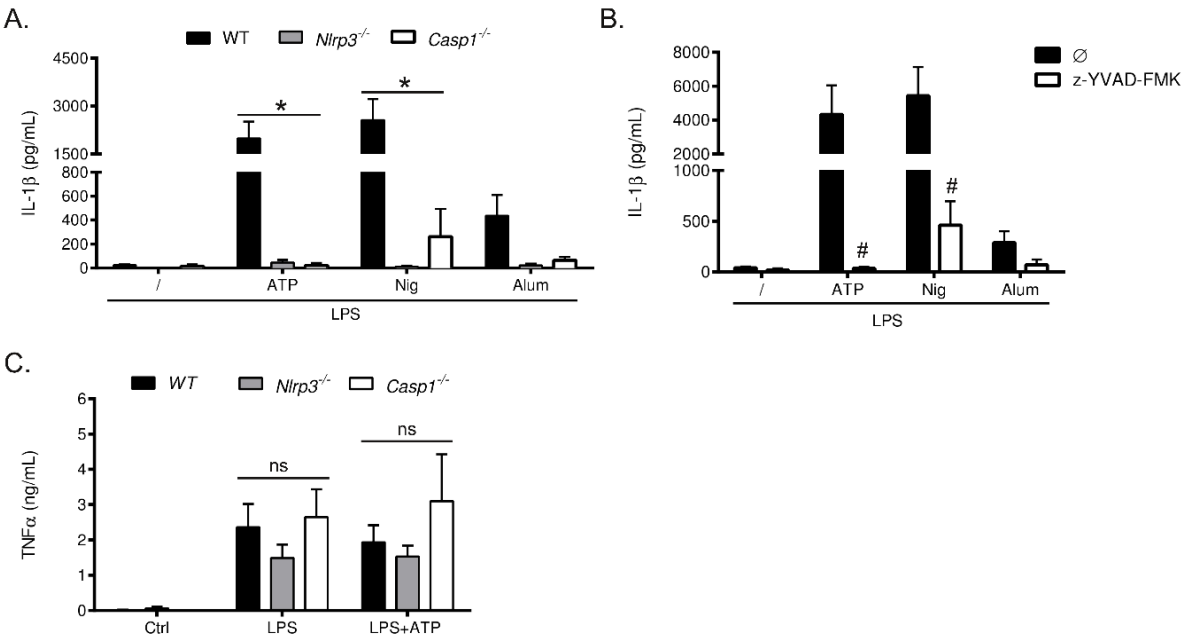


Fig. 23: Microglia secrete IL-1β in an NLRP3 inflammasome-dependent way.

LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). **(A)** Secretion of IL-1β in the supernatant of wild-type (WT), *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia was quantified by ELISA. **(B)** IL-1β secretion in the supernatant of microglia exposed to z-YVAD-FMK, a specific caspase-1 inhibitor, was quantified by ELISA. The inhibitor was added 15 min before the LPS-priming. **(C)** Secretion of TNFα into the supernatant of microglia was quantified by ELISA. The bars represent the mean ± SEM of at least three independent experiments, *=p<0.05 KO compared to WT, #=p<0.05 compared to ø, ns= not significant. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

To determine if Nigericin and Alum directly trigger the inflammasome assembly or if they act indirectly by inducing the intermediate release of ATP, we quantified the ATP concentration in the supernatant following Nigericin or Alum treatment. In these conditions, no intermediate ATP release was detected (0.08 mM ATP for Nigericin treatment ; Fig. 24A), suggesting that Nigericin and Alum directly activate IL-1β production. Accordingly, we observed no statistical difference in IL-1β secretion upon Nigericin or Alum treatment between wild-type and *P2xr7*$^{-/-}$ cells (Fig. 24B). The P2X7

receptor was only required for IL-1β release in response to the stimulation with ATP (1969±502 pg/mL in wild-type *vs* 39±22 pg/mL in *P2rx7*$^{-/-}$, Fig. 24B).

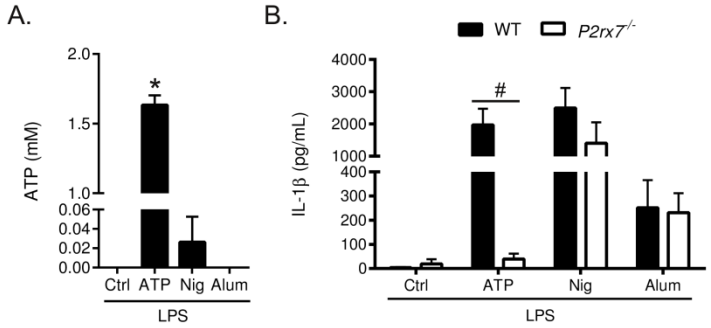


Fig. 24: Microglia secrete IL-1β in a P2X7R-independent-way.

LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). **(A)** ATP release was quantified in cell supernatant upon treatment using a luminescent assay **(B)** IL-1β secretion was assessed by ELISA in wild-type (WT) and *P2rx7*$^{-/-}$ microglia. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, #=p<0.05 KO compared to WT. One-way ANOVA followed by Dunnet's multiple comparisons test.

1.1.3 Microglia secrete IL-18, IL-1α and HMGB1 after NLRP3 inflammasome activation.

IL-1β is not the sole molecule to be released after inflammasome activation. IL-18 or alarmins, such as IL-1α and HMGB1, are also secreted following inflammasome assembly in macrophages. We investigated the expression and secretion profile of these factors in microglia.

First, primary microglia constitutively expressed the *Il18* gene (Ct_{Il18}=25.1, Ct_{L27}=22.3). However, LPS priming significantly increased by 10.4-fold its expression (Fig. 25A). In order to investigate the release of IL-18 in microglia, we activated LPS-primed cells with either ATP, Nigericin or Alum. We quantified the IL-18 released in the culture supernatant by ELISA. Similar to IL-1β, the priming of cells by LPS was required to detect any release of IL-18 after ATP, Nigericin or Alum exposure (187±71 pg/mL, 297±87 pg/mL and 47±18 pg/mL respectively, Fig. 25B). In addition, the production of IL-18 occurred in an NLRP3 inflammasome-dependent manner, as its secretion was significantly decreased in supernatants of NLRP3- and Caspase-1-deficient microglia (Fig. 25C). Similarly, the caspase inhibitor z-YVAD-FMK almost completely abrogated the IL-18 secretion (Fig. 25D).

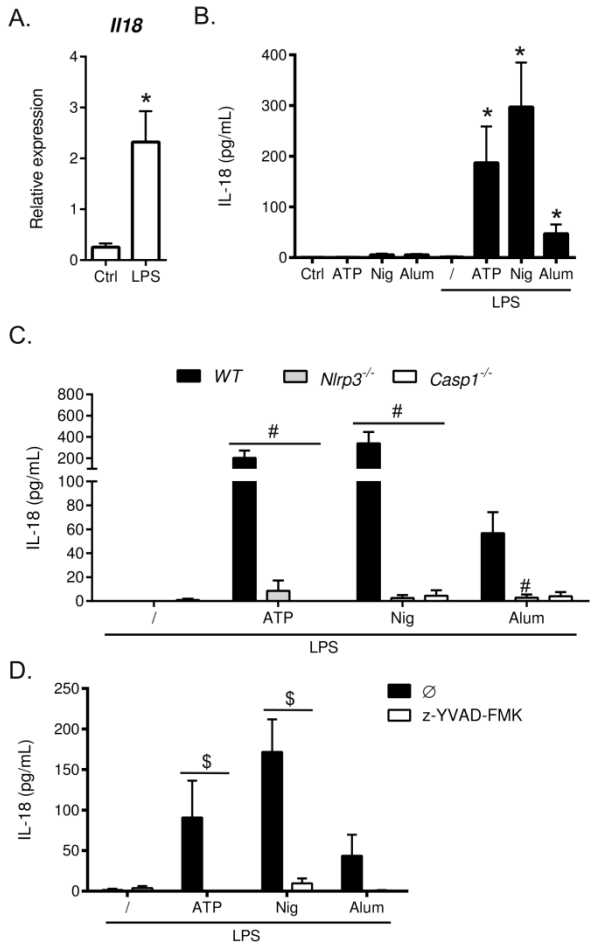


Fig. 25: IL-18 is expressed in microglia and released after NLRP3 inflammasome activation.

(A) Expression level of *Il18* was analysed by RT-PCR in untreated or LPS-primed microglia. Data are normalized to *Rpl27*. *=p<0.05 compared to Ctrl. Mann and Whitney test **(B, C, D)** Untreated or LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). IL-18 secretion was assayed by ELISA in supernatant of wild-type (WT) **(B)**, *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia **(C)** and in supernatant of WT microglia exposed to z-YVAD-FMK, a specific caspase-1 inhibitor. The inhibitor was added 15 min before the LPS-priming **(D)**. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, #=p<0.05 KO compared to WT, $=p<0.05 compared to ø. One-way ANOVA followed by Dunnet's multiple comparisons test.

We further investigated the expression and secretion of both alarmins IL-1α and HMGB1. We analysed whether IL-1α was constitutively expressed by RT-PCR and WB. Since the basal *Il1a* mRNA level was low (Ct_{Il1a}=30.7, Ct_{L27}=22.1) and that we were not able to detect the protein, we considered that IL-1α was not constitutively expressed in our microglia (Fig. 26A and Fig. 26B). However, stimulating the cells with LPS induced 1744-fold the *Il1a* gene expression (Fig. 26A) and upregulated the IL-1α translation (Fig. 26B). While the treatment with LPS alone was not sufficient to trigger the release of IL-1α into the supernatant, subsequent stimulation with either ATP, Nigericin or Alum led to the release of IL-1α (at respectively 435±259 pg/mL, 622±314 pg/mL and 121±64 pg/mL ; Fig. 26C).

In order to investigate whether the release of IL-1α was dependent on the NLRP3 inflammasome, we compared the IL-1α protein levels in the supernatant of wild-type, *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ stimulated microglia. The results showed that ATP or Nigericin treatment required the presence of the NLRP3 inflammasome to induce IL-1α release, whereas Alum did not (Fig. 26D). Moreover, while the ATP- and Nigericin-mediated IL-1α release required the presence of caspase-1, its activity was dispensable, since the

treatment with the caspase-1 inhibitor z-YVAD-FMK did not significantly alter the levels of IL-1α release (292 *vs* 198 pg/mL for ATP, 582 *vs* 601 pg/mL for Nigericin and 126 *vs* 69 pg/mL for Alum; Fig. 26E).

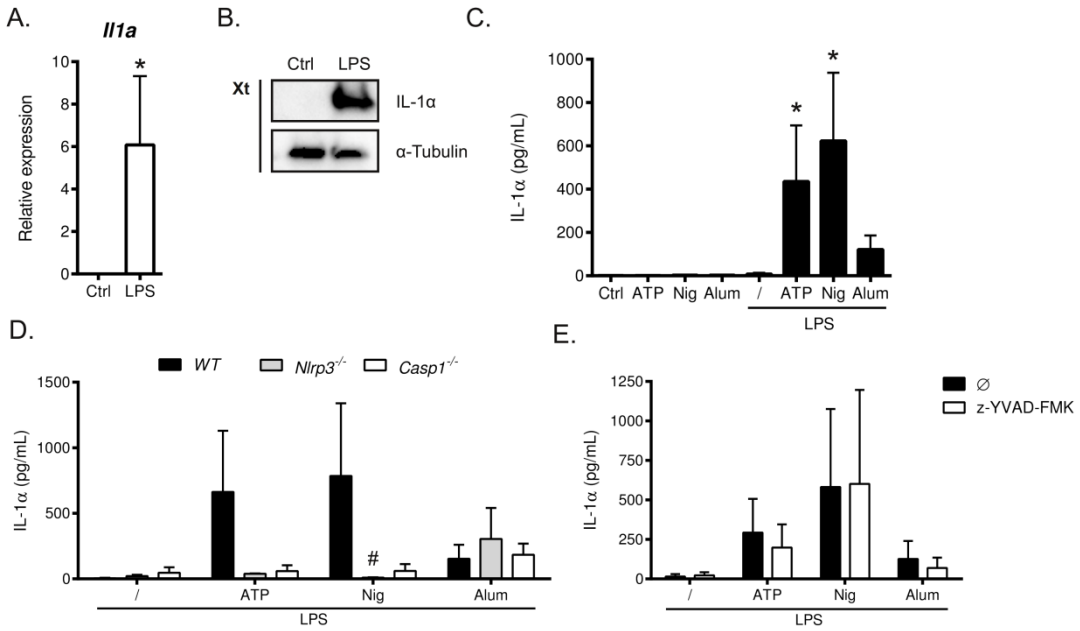


Fig. 26: NLRP3 activators induce IL-1α secretion in LPS-primed microglia, but display differential requirement for the inflammasome components.

(A, B) Microglia were stimulated with 10 ng/mL LPS during 6 h. **(A)** Expression level of *Il1a* was analysed by RT-PCR. Data are normalized to *Rpl27*. *=p<0.05 compared to Ctrl. Mann and Whitney test. **(B)** Cell lysates (Xt) were analysed by WB for the expression of IL-1α in untreated or primed condition. α-Tubulin was used as a loading control. **(C, D, E)** Untreated or LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). IL-1α secretion was assayed by ELISA in the supernatant of wild-type (WT) **(C)**, *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia **(D)** and in the supernatant of WT microglia exposed to z-YVAD-FMK, a specific caspase-1 inhibitor. The inhibitor was added 15 min before LPS-priming **(E)**. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *=p<0.05 compared to Ctrl and #=p<0.05 KO compared to WT. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

A recent report showed that microglia actively secrete HMGB1 in response to acute stress (Weber 2015). However, the authors did not study whether the inflammasome was implicated in this process. Thus, we wanted to study if microglia could be able to secrete HMGB1 following NLRP3 inflammasome activation. As expected, HMGB1 was constitutively expressed in microglia (Ct_{Hmgb1}=22.1, Ct_{L27}=22.3 ; Fig. 27A and Fig. 27B). However, we could not detect its secretion, neither in the control condition nor after stimulation with LPS. In fact, we were only able to detect HMGB1 in the supernatant of LPS primed cells once the cells were treated with either ATP (6.4 ng/mL) or Nigericin (18.8 ng/mL; Fig. 27C). Interestingly, Alum treatment was not able to trigger the release of HMGB1 by LPS-primed microglia (Fig. 27C). Consistently with a role for the NLRP3

inflammasome, ATP- and Nigericin- mediated HMGB1 release were abrogated in NLRP3-deficient (2.4±0.7 ng/mL with ATP and 4.3±1.6 ng/mL with Nigericin) or Caspase-1-deficient microglia (3.7±1.2 ng/mL with ATP and 5.6±1.9 ng/mL with Nigericin) compared to wild-type microglia (13.8±4.9 ng/mL with ATP and 32±9 ng/mL with Nigericin, Fig. 27D). Similar to our observation with IL-1α, we were still able to detect HMGB1 in the supernatant of the cells treated with the caspase-1 inhibitor z-YVAD (Fig. 27E). Yet, in both cases, we were able to confirm the efficacy of the caspase-1 inhibitor by quantifying IL-1β on the same supernatants.

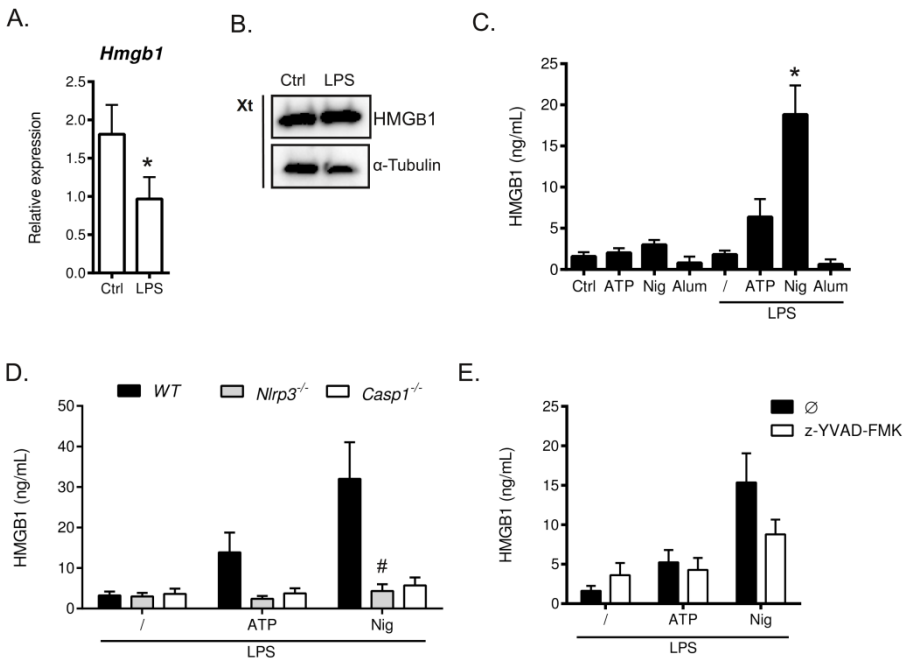


Fig. 27: LPS-primed microglia actively released HMGB1 after inflammasome activation by ATP and Nigericin.

(A, B) Microglia were stimulated with 10 ng/mL LPS during 6 h. **(A)** Expression level of *Hmgb1* was analysed by RT-PCR. Data are normalized to *Rpl27*.*=p<0.05 compared to Ctrl. Mann and Whitney test. **(B)** Cell lysates (Xt) were analysed by WB for the expression of HMGB1 in untreated or primed condition. α-Tubulin was used as a loading control. **(C, D, E)** Untreated or LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h). HMGB1 secretion was assayed by ELISA in supernatant of wild-type (WT) **(C)**, *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia **(D)** and in supernatant of WT microglia exposed to z-YVAD-FMK, a specific caspase-1 inhibitor. The inhibitor was added 15 min before the LPS-priming **(E)**. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *=p<0.05 compared to Ctrl and #=p<0.05 KO compared to WT. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

1.1.4 Inflammasome-dependent IL-1β production in microglia occurred through similar mechanisms than described in macrophages.

We wanted to investigate whether the mechanisms underlying inflammasome activation in microglia are similar to those described in macrophages. To this purpose, we

studied the implication of potassium efflux, ROS and lysosomal damage in microglial NLRP3 inflammasome activation.

First, we stimulated the cells in a potassium-enriched medium (25 to 130 mM KCl) in order to investigate the importance of potassium efflux for inflammasome activation. We tested whether the potassium-enriched conditions affect the viability of microglia by using a MTT assay. We found that KCl seemed not toxic despite a slight decrease in viability when its concentration reached 130 mM (86.4% of viability, Fig. 28A). When we treated microglia with increasing concentrations of KCl (25 mM, 75 mM and 130 mM), we observed a dose-dependent reduction in ATP-mediated IL-1β release (Fig. 28A). The concentration decreased from 1362 pg/mL in the control condition to 114 pg/mL with 25 mM KCl, 12 pg/mL with 75 mM KCl and 1.7 pg/mL with 130 mM KCl (Fig. 28B). Similarly, we observed that the presence of 25 mM KCl also inhibited the release of IL-1β following Nigericin (3674 pg/mL to 1207 pg/mL) or Alum treatment (571 pg/mL to 118 pg/mL, Fig. 28C).

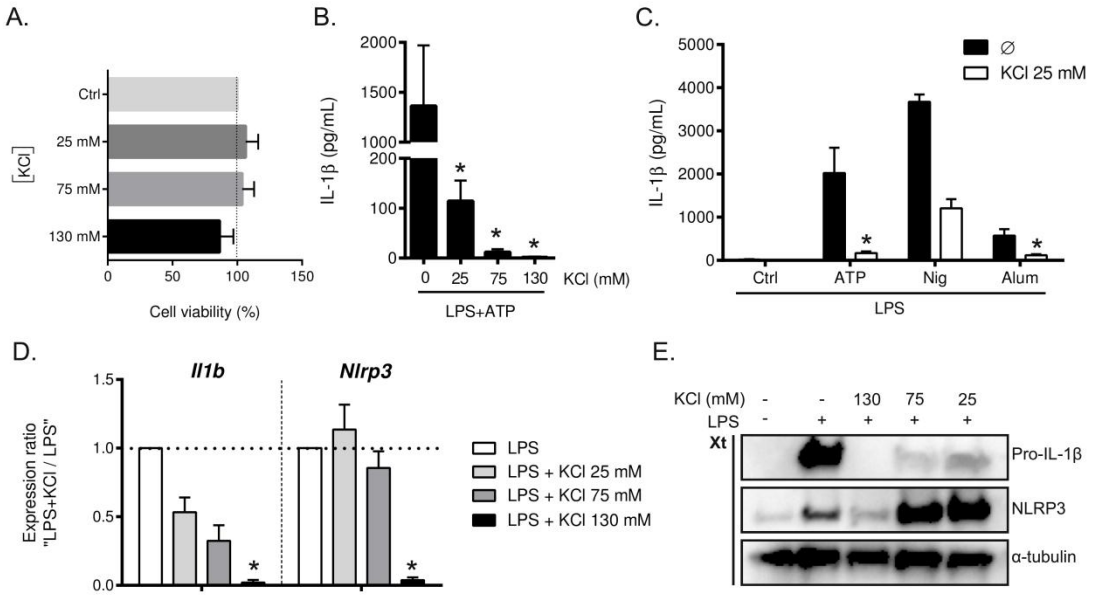


Fig. 28: High extracellular concentration of KCl inhibits *Il1b* and *Nlrp3* expression as well as IL-1β release.

(A) Microglia were cultured in classical medium or in 25 mM, 75 mM, or 130 mM KCl-enriched medium. Cell viability was tested by MTT assay. Data are shown in percentage of the non-treated cells viability (Ctrl). **(B, C)** LPS-primed microglia were stimulated with ATP (1 mM, 30 min), Nigericin (Nig, 1.34 µM, 2 h), or Aluminium hydroxide (Alum, 300 µg/ml, 5 h) in presence of KCl-enriched medium. IL-1β secretion in supernatant was quantified by ELISA after exposure to ATP (B) and Nigericin or Aluminium hydroxide **(C)**. **(D, E).** LPS-primed microglia were cultured in classical medium or in 25 mM, 75 mM, or 130 mM KCl-enriched medium. **(D)** Expression levels of *Il1b* and *Nlrp3* were analysed by RT-PCR and normalized to *Rpl27*. The expression ratio of these genes was calculated for "LPS+KCl" *vs* "LPS" conditions. **(E)** Cell lysates (Xt) were analysed by WB for the expression of IL-1β and NLRP3. α-Tubulin was used as a loading control. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments) *=p<0.05 compared to Ctrl. One-way ANOVA followed by Dunnet's multiple comparisons test.

Surprisingly, we noted that adding KCl not only affected the activation of NLRP3 but also the priming step (Fig. 28D and Fig. 28E). Indeed, when we analysed the transcripts by RT-PCR, we observed that KCl was able to downregulate the LPS-induced *Il1b* expression in a dose-dependent manner (Fig. 28D). *Il1b* expression decreased of 53.2% with 25 mM KCl, 67.6% with 75 mM KCl and 97.9% with 130 mM of KCl. Interestingly, the expression of *Nlrp3* was only affected in presence of the highest concentration of KCl (130 mM) and dropped down by 96.3% in this condition (Fig. 28D). Our WB analysis confirmed these observations at the protein level (Fig. 28E).

ROS have previously been proposed as being crucial for NLRP3 activation. Thus, we analysed their implication in microglial inflammasome activation by testing the effect of the antioxidant molecule N-Acetyl-L-Cystein (NAC) on IL-1β secretion. As a prerequisite, we performed an MTT assay which showed that NAC did not exhibit any adverse effect on the cell viability (Fig. 29A). Our experiments indicated that the exposure to NAC impaired the IL-1β secretion induced by either ATP (from 3933±952 pg/mL to 2091±89 pg/mL) or Alum (from 283±42 pg/mL to 71±7 pg/mL ; respectively Fig. 29B and Fig. 29C). In addition, the effect of NAC on the release of IL-1β was priming-independent, as the RT-PCR analysis showed no variation in the expression of either *Il1b* or *Nlrp3* gene in presence of NAC (Fig. 29D).

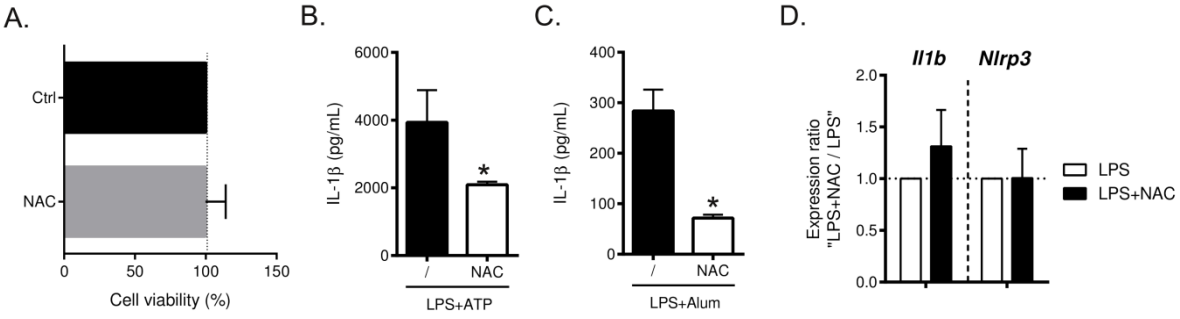


Fig. 29: IL-1β release is decreased in presence of NAC antioxidant.

(A) Microglia were treated for 330 min with the antioxidant N-Acetyl Cysteine (NAC, 5 mM). Cell viability was tested by MTT assay. Data are shown in percentage of non-treated cells viability (Ctrl). **(B and C)**. IL-1β secretion was quantified by ELISA performed on supernatant of LPS-primed microglia stimulated with ATP (1 mM, 30 min) **(B)**, or Aluminium hydroxide (Alum, 300 µg/ml, 5 h) **(C)** and exposed to NAC (added 30 min prior to the inflammasome activator). **(D)** LPS-primed microglia treated for 330 min with NAC were submitted to transcripts analysis. Expression levels of *Il1b* and *Nlrp3* were analysed by RT-PCR and normalized to *Rpl27*. The expression ratio of these genes was calculated for "LPS+NAC" *vs* "LPS" conditions (**D**). Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl. One-way ANOVA followed by Dunnet's multiple comparisons test.

It has also been reported that, in macrophages, particulates could induce lysosomal damage and subsequent release of cathepsin B, which are responsible for the activation of the NLRP3 inflammasome. To study whether particulates act in the same way on microglia, we first inhibited their phagocytosis capacity by using Cytochalasin D (Cytod), an inhibitor of actin polymerization. Then, we also blocked cathepsin B activity by using the inhibitor CA074-Me. These compounds did not give rise to significant cell death (Fig. 30A). As expected, both inhibitors did not affect the IL-1β secretion observed in response to ATP (Fig. 30B), whereas Cytod and CA074-Me induced a decrease of Alum-triggered IL-1β secretion of respectively 98.6% and 86.3% (Fig. 30C). Moreover, this effect was priming-independent, as RT-PCR analysis showed no difference in the expression of either *Il1b* or *Nlrp3* gene in presence of CA074-Me or Cytod (Fig. 30D).

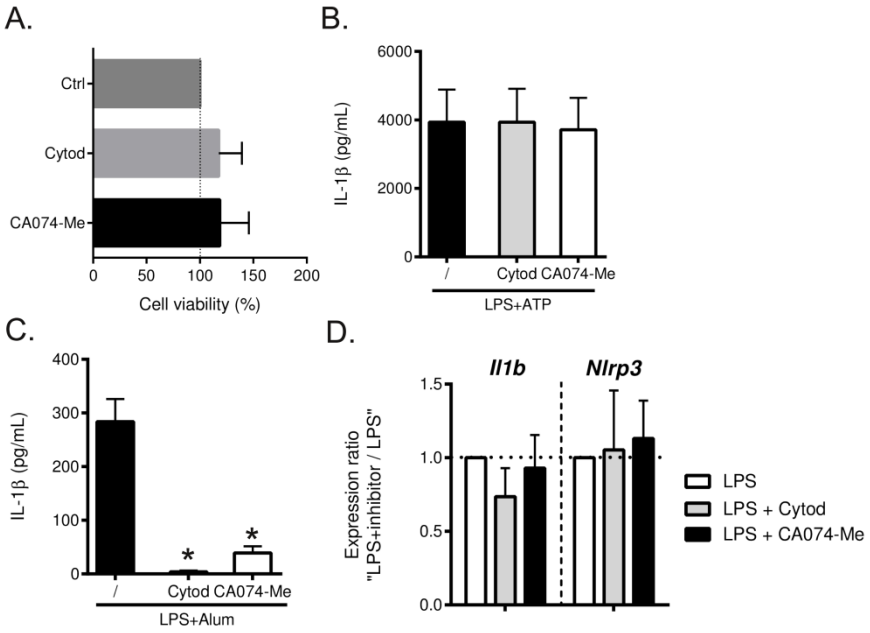


Fig. 30: Phagocytosis and cathepsin B activity are required for IL-1β release following Alum exposure.

(A) Microglia were treated for 330 min with Cytochalasin D (Cytod, 2 µM) or CA074-Me (10 µM). Cell viability was tested by MTT assay after inhibitor exposure. Data are shown in percentage of non-treated cells viability (Ctrl). **(B and C)** The IL-1β secretion in supernatant of LPS-primed microglia stimulated with ATP (1 mM, 30 min) **(B)**, or Aluminium hydroxide (Alum, 300 µg/ml, 5 h) was quantified by ELISA **(C)** in presence of Cytod or CA-074Me. **(D)** LPS-primed microglia treated for 330 min with Cytod or Ca-074Me were submitted to transcripts analysis. Expression levels of *Il1b* and *Nlrp3* were analysed by RT-PCR and normalized to *Rpl27*. The expression ratio of these genes was calculated for "LPS+inhibitor" *vs* "LPS" conditions (**D**). Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl. One-way ANOVA followed by Dunnet's multiple comparisons test.

1.2 Preliminary results: Other functional inflammasomes in microglia.

Even if NLRP3 inflammasome is the best characterized, other inflammasome complexes have been described (see Introduction, Part II, section 3). As some of them have been linked to neuroinflammation, we further investigated the expression of the inflammasome-related proteins in microglia. To this purpose, we performed RT-PCR on $_c$DNA derived from untreated or LPS-primed microglia. The different expression levels are reported in figure 31 as a heat map representing the -dCt ($-Ct_{gene\ of\ interest} - Ct_{Rpl27}$) values. The expression data of all genes are provided in supplemental table 1. We detected the constitutive expression of *Nlrp1* (Ct = 30.2±1.9), *Nlrp6* (Ct = 26.9±1.5), *Aim2* (Ct = 20.5±0.6) and *Nlrc4* (Ct = 27.5±1.5) genes in microglia (Fig. 31). In addition, we observed that *Casp4* (caspase-11) mRNA could be upregulated 240-fold when microglia were primed with LPS (Fig. 31).

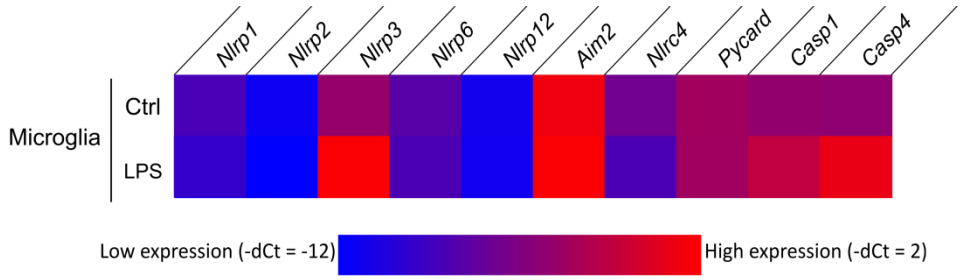


Fig. 31: Expression profile of inflammasome-related genes in microglia.

Microglia were primed with LPS (10 ng/mL) during 6 h. RNA were extracted and analysed for gene expression of *Nlrp1, Nlrp2, Nlrp3, Nlrp6, Nlrp12, Aim2, Nlrc4, Pycard, Casp1* and *Casp4* by RT-PCR. Results were normalized to *Rpl27*. The Heat map represents the normalized expression values (-dCt). Blue represents low level of expression (-dCt = -12) and red represents high level of expression (-dCt = -2). Data are the mean of at least three independent experiments.

Based on these results, we started a functional analysis of AIM2 and NLRC4 inflammasome complexes in microglia, since this question has, until now, been poorly addressed.

As AIM2 is able to sense cytosolic double stranded DNA, we transfected LPS-primed cells with the synthetic double-stranded DNA poly(dA:dT). After the transfection, we were able to observe that microglia released a highly variable but significant amount of IL-1β (729±423 pg/mL ; Fig. 32A). As expected, this release was NLRP3-independent since the level of IL-1β was not significantly altered in NLRP3-deficient microglia (Fig. 32B).

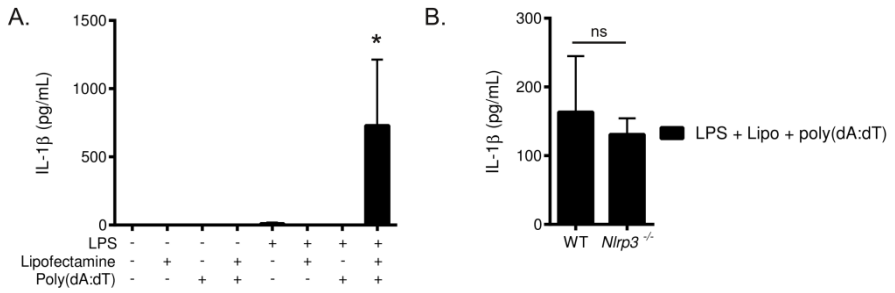


Fig. 32: The AIM2 inflammasome ligand, poly(dA:dT), induces IL-1β release by LPS-primed microglia.

Microglia were primed for with LPS (10 ng/mL, 6 h) and transfected with Poly(dA:dT) (2.5 µg/mL, 5 h) by using Lipofectamine (10 µg/mL). IL-1β production in culture supernatant was assessed by ELISA in wild-type (WT) **(A)** or *Nlrp3*$^{-/-}$ microglia **(B)**. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, ns= not significant. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Next, we investigated the functionality of the NLRC4 inflammasome, which is classically assembled after detection of bacterial flagellin. Thus, we stimulated LPS-primed microglia with flagellin during 5 h. We could indeed observe a significant release of IL-1β (244±161 pg/mL), but only after 24 h of LPS priming (Fig. 33A). Here again, we further used NLRP3-deficient microglia to show that this flagellin-mediated IL-1β release was NLRP3-independent (Fig. 33B).

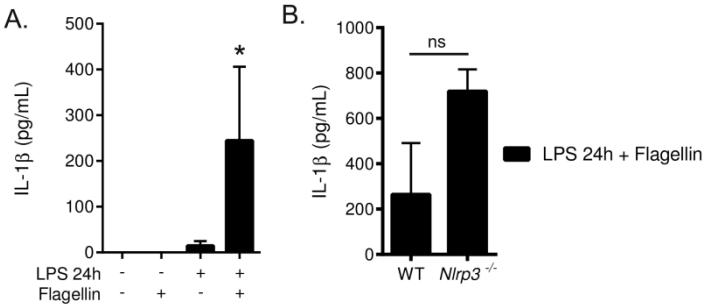


Fig. 33: LPS-primed microglia release IL-1β following flagellin exposure.

Microglia were primed with LPS (10 ng/mL, 24 h) and stimulated with ultra-pure flagellin (2.5 µg/mL, 5 h). IL-1β production in culture supernatant was assessed by ELISA in wild-type (WT) **(A)** or *Nlrp3*$^{-/-}$ microglia **(B)**. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, ns= not significant. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

We have previously mentioned that LPS was able to induce the upregulation of *Casp4* (Fig. 31). In fact, this gene encodes mouse caspase-11, which is implicated in the "non-canonical inflammasome" pathway after binding to intracellular LPS (see the section 3.4 in Part II of the introduction). This observation led us to study the microglial capacity to

trigger this non-canonical inflammasome pathway. To this purpose, we used lipofectamine to transfect a high dose of LPS (1 µg/ml) into microglial cells. This transfection induced the release of IL-1β (461±254 pg/mL), as revealed by ELISA (Fig. 34A), but also led to an increase in LDH release (13.5-fold compared to Ctrl, Fig. 34B). Surprisingly, when we treated the cells with high LPS concentration (1 µg/ml), without transfection agent, we were nevertheless able to detect a significant release of IL-1β into the supernatant (239±82 pg/mL, Fig. 34A) as well as a release of LDH (16%, Fig. 34B). We further noted that the release of IL-1β tended to decrease in *Nlrp3*$^{-/-}$ mice (467±359 pg/mL *vs* 228±183 pg/mL, Fig. 34C) whereas the LDH release remained similar in wild-type and NLRP3-deficient cells (respectively 23% and 28%, Fig. 34D).

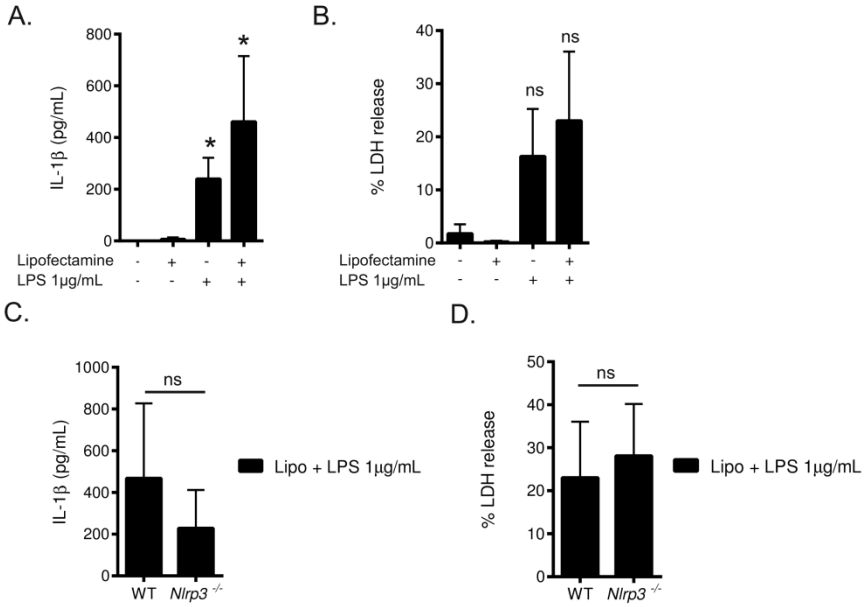


Fig. 34: High dose of LPS triggered IL-1β release by microglia.

Microglia were stimulated for 16 h with a high dose of LPS (1 µg/mL), transfected by using Lipofectamine (10 µg/mL). IL-1β production and LDH release in culture supernatant were assessed respectively by ELISA and LDH cytotoxicity test in wild-type (WT) **(A, B)** or *Nlrp3*$^{-/-}$ microglia **(C, D)**. LDH results are shown as percentage of LDH release by Triton-X100 treated cells. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, ns= not significant. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

2. Inflammasome expression in astrocytes.

Even if astrocytes physiology is currently an important field of research, their direct contributions under inflammatory conditions is still controversial. For example, it is still debated if astrocytes are able to produce IL-1β. Moreover, only few studies tried to characterize inflammasomes in astrocytes. Therefore, we aimed to investigate inflammasome expression and activation in astrocytes.

2.1 Astrocyte culture purity is crucial.

First, we wanted to emphasize the importance to work with astrocyte cultures devoid of microglia in inflammatory studies. Primary mixed glial cultures (MGC P0) contain at least both glial cell types (astrocytes and microglia). As microglia are extremely reactive immune cells, it is important to deplete them as much as possible from astrocyte cultures-derived from MGC P0.

In order to obtain highly astrocyte-enriched cultures *in vitro*, we performed a MACS isolation where microglia were positively selected during the sorting. The resulting astrocyte-enriched cultures were named AEC-M1. To characterize the purity of this type of culture, we realized an immunocytochemistry staining using antibodies against GFAP and Iba1 in order to label astrocytes or microglia respectively (Fig. 35A). Cell counting revealed that AEC-M1 contained less than 2% of Iba1 positive microglial cells. While this level of purity is often described as being acceptable in the literature, our preliminary experiments have demonstrated that the remaining microglial cells were still introducing a bias during inflammatory studies. Indeed, experimental results were not reproducible as the pro-inflammatory gene expression was highly variable, probably due to different amounts of microglial contamination. For example, the Ct value obtained by RT-PCR for *Il1b* varied from 32 to 38. In order to fix this issue, we decided to perform a second MACS sorting to reduce even more the remaining microglial cells. The thereby obtained cultures were called AEC-M2. In these cultures, we were not able to detect Iba1-positive cells anymore (Fig. 35A), indicating a purity close to 100%. To confirm these results by an alternative method, we characterized the gene expression levels of relevant cell type specific markers, *i.e. Aif1* (Allograft inflammatory factor 1), *Itgam* (Integrin alpha M) and *Gfap* in AEC-M1, AEC-M2, microglial cultures and MGC P0. *Itgam* and *Aif1* are the genes respectively coding for CD11b and Iba1 proteins and expressed in microglia. Figure 35B shows that *Aif1* and *Itgam* were expressed at higher levels in microglia cultures than in MGC P0 (respectively 14-fold and 4-fold) and in AEC-M1 (respectively 14-fold and 6-fold).

Moreover, these genes were less expressed in AEC-M2 than in AEC-M1 cultures (3-fold for *Aif1* and 2-fold for *Itgam*).

Based on these observations, we decided to use AEC-M2 to investigate inflammasome expression and regulation in astrocytes *in vitro*.

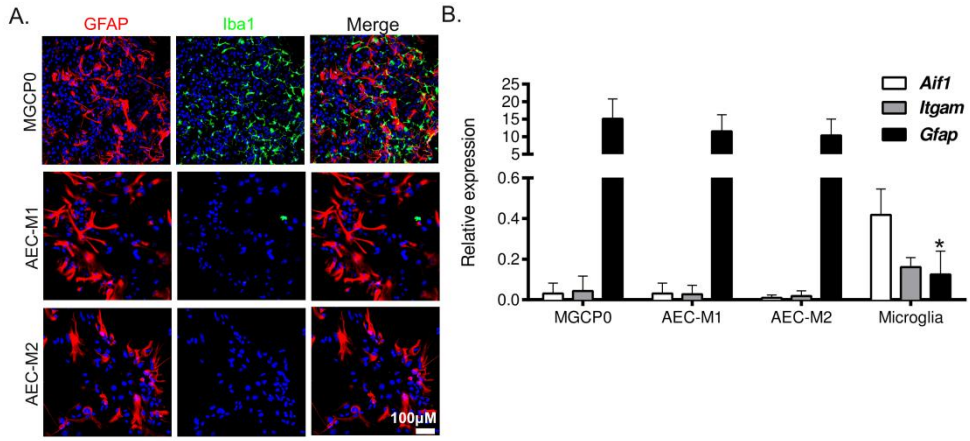


Fig. 35: AEC-M2 contain less contaminant microglia than AEC-M1 or MGC P0.

(A) Immunocytochemistry was performed on non-treated mixed glial cultures (MGC P0) and on astrocyte-enriched cultures (AEC) sorting once (AEC-M1) or twice (AEC-M2) by MACS technique. Astrocytes are detected using anti-GFAP antibody (red). Microglia were detected using anti-Iba1 antibody (green). DAPI staining identifies nucleus (blue). Pictures are one representative experiment of at least three independent experiments. **(B)** Gene expressions of *Aif1*, *Itgam* (microglia markers) and *Gfap* (astrocyte marker) were analysed in untreated MGC P0, AEC-M1, AEC-M2 and microglial cultures by RT-PCR. Results were normalized to *Rpl27*. Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to MGC P0. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

2.2 Astrocytes do not express all NLRP3 inflammasome components.

As astrocytes do not strongly respond to LPS stimulation (Henn 2011), probably due to their weak *Tlr4* expression (Suppl. Fig. 1), we tried to setup an alternative priming method. To this aim, we tested the TLR2 ligand Pam3CSK4 (P3C, 10 ng/mL) or the cytokines IL-1β (10 ng/mL), TNFα (10 ng/mL) and IFNγ (20 ng/mL) separately or in combination (CCM: IL-1β + TNFα + IFNγ). Untreated and LPS-primed microglia were used as positive controls even if P3C and CCM were also able to efficiently prime NLRP3 in these cells (Suppl. Fig. 2).

After 6 h of astrocytes priming by the cited activators, we performed a RT-PCR to monitor the expression levels of selected pro-inflammatory genes (*i.e. Il6, Nos2, Ptgs2 Cxcl10* and *Tnf*) but also of NLRP3 inflammasome-related genes (*i.e. Il1b, Il1a, Il18, Hmgb1, Nlrp3, Pycard* and *Asc* ; Fig. 36A). The obtained data are represented as a heat map representing the -dCt values for each genes. The exhaustive expression values are provided in supplemental Table 2.

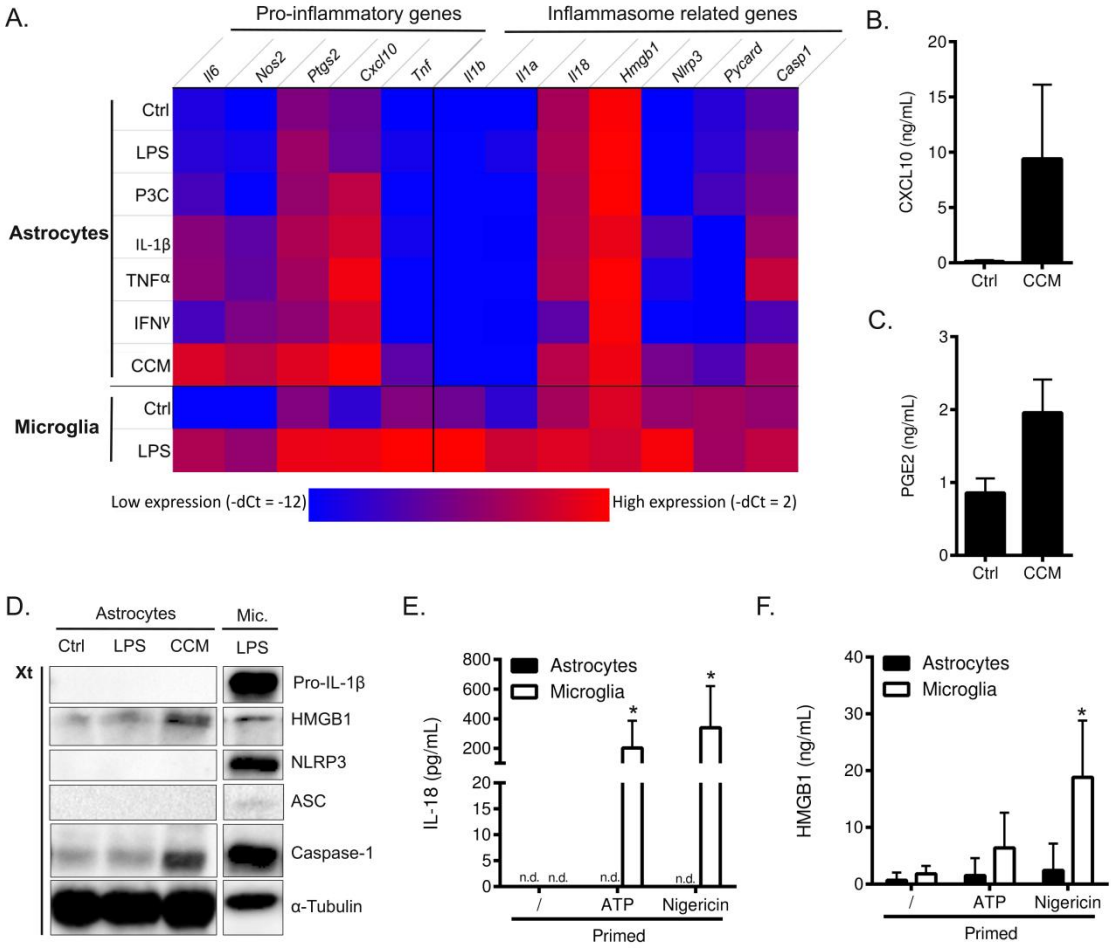


Fig. 36: Astrocytes weakly expressed *Il1b*, *Il1a*, *Nlrp3* and *Pycard* genes and do not respond to inflammasome activation, even after an efficient priming.

(A) Primary astrocytes (AECM2) were primed for 6 h with LPS (10 ng/mL), P3C (10 ng/mL), IL-1β (10 ng/mL), TNF- (10 ng/mL), IFNγ (10 ng/mL) or CCM (10 ng/mL IL-1β + 10 ng/mL TNFα + 20 ng/mL IFNγ). Primary microglia were primed with LPS (10 ng/mL, 6 h) and served as control. RNA were extracted and analysed by RT-PCR for expression of the pro-inflammatory genes *Il6, Nos2, Ptgs2, Cxcl10* and *Tnf*) and expression of NLRP3 inflammasome-related genes (*i.e. Il1b, Il1a, Il18, Hmgb1, Nlrp3, Pycard* and *Asc)*. Results were normalized to *Rpl27*. The Heat map represents these normalized expression values (-dCt). Blue represents low level of expression (-dCt = -12) and red represents high level of expression (-dCt = -2). Data are mean of at least three independent experiments. CXCL10 **(B)** and PGE2 **(C)** secretions were assessed after 6 h of CCM exposure. **(D)** Primary astrocytes (AEC M2) were primed for 6 h with LPS (10 ng/mL) or CCM. LPS-primed microglia served as control. IL-1β, HMGB1, NLRP3, ASC and Caspase-1 protein levels were analysed by WB. α-Tubulin was used as a loading control. IL-18 **(E)** and HMGB1 **(F)** secretions were assayed by ELISA on supernatant of primed astrocytes and microglia treated with ATP (1 mM, 30 min) or Nigericin (Nig, 1.34 µM, 2 h). Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *=p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

By looking at the expression levels of *Il6, Nos2, Ptgs2*, *Cxcl10* and *Tnf* in astrocytes, we observed that, among the tested activators, CCM was the most potent one (108-, 675-, 38.5-, 3777- and 25-fold inductions respectively for *Il6*, *Nos2*, *Ptgs2*, *Cxcl10* and *Tnf ;* Fig. 36A). We further confirmed the capacity of CCM to activate astrocytes by measuring the secretion of CXCL10 or PGE2 by ELISA. We were able to detect a 78-fold increase of CXCL10 in the supernatant after CCM stimulation (0.12±0.01 ng/ml in Ctrl and

9.4±6.7 ng/ml with CCM, Fig. 36B), as well as an upregulation of 2.3-fold of the PGE2 release (0.85±0.19 ng/ml in Ctrl and 1.96±0.45 ng/ml with CCM, Fig. 36C).

We also analysed the expression levels of inflammasome-related genes in untreated or inflammatory conditions. Surprisingly, we failed to detect any *Il1a* and *Il1b* transcripts in astrocytes, in both unprimed and primed conditions. In addition, the *Nlrp3* and *Pycard* genes were poorly expressed in untreated astrocytes, compared to microglia (Fig. 36A). Nevertheless, *Nlrp3* was significantly upregulated after CCM exposure (332-fold, Fig. 36A). The heat map also illustrates that astrocytes constitutively expressed *Il18* (Ct = 24.7±2.3), *Hmgb1* (Ct = 20.5±1.8) or *Casp1* (Ct = 29.3±1.7) and that treating the cells with CCM was able to upregulate *Casp1* expression by 12.8-fold (Fig. 36A). We attempted to confirm these observations at the protein level by WB assays. Interestingly, we failed to detect pro-IL-1β, NLRP3 and ASC proteins in primed or unprimed astrocytes (Fig. 36D). However, we noted that these cells expressed HMGB1 and caspase-1 in both conditions, with an upregulation of caspase-1 expression after priming, consistently with our transcripts results (Fig. 36D).

Although we failed to detect the expression of all NLRP3 inflammasome components in astrocytes, we have tested the effects of the inflammasome activators ATP and Nigericin on CCM-primed astrocytes. As expected, we did not detect any IL-18 (Fig. 36E) or HMGB1 (Fig. 36F) release after treatment, even if they are expressed by astrocytes. IL-1β release was also undetectable in the supernatant (Suppl. Fig. 3).

In order to confirm our results, these experiments have been repeated on another model of astrocytes devoid of microglia: neurosphere-derived astrocyte cultures (Crocker *et al*, 2008). Similar to primary astrocytes, these cells exhibited a very low expression of *Asc* and *Nlrp3* but expressed *Casp1* which was upregulated after CCM-priming (Suppl. Fig. 4). On another hand, *Il1b* and *Il18* genes were not expressed by the neurosphere-derived astrocytes, either in unstimulated or in CCM-primed conditions (Suppl. Fig. 4).

2.3 Astrocytes do not express other functional inflammasomes.

As we failed to detect a functional NLRP3 inflammasome in astrocytes, we further investigated the expression of other inflammasome-related proteins in these cells. To this purpose, we performed RT-PCR on RNA extracted from untreated or primed astrocytes. Figure 37 shows a heat map representation of the normalized expression levels. The expression data of all genes are provided in supplemental table 3. We found that astrocytes and microglia contained similar transcript levels of the intracellular sensors

Nlrp6 (respectively Ct = 29.9±1.9 and 29.6±1.5) and *Aim2* (respectively Ct = 22±0.7 and 20.5±0.6 ; Fig. 37 and Suppl. Table 3). On the contrary, *Nlrp1*, *Nlrp2*, *Nlrp12* and *Nlrc4* genes were poorly expressed in astrocytes (Fig. 37) as demonstrated by their respective Ct values: 32.6, 34.2, 33.2 and 35.3 (Suppl. Table 3). Interestingly, we were able to observe a 27-fold upregulation of *Casp4* (Caspase-11) gene expression after CCM-priming in astrocytes, although the expression levels remain much lower than in LPS-primed microglia (Ct=30.2±3.1 in primed astrocytes *vs* 21.1±0.2 Ct in primed-microglia; Fig. 37 and Suppl. Table 3).

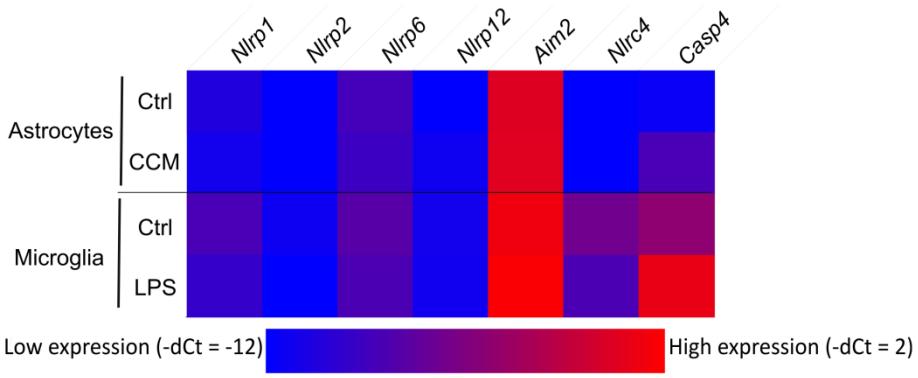


Fig. 37: Expression profile of other inflammasome-related genes in astrocytes.

Primary astrocytes were untreated or primed with CCM (10 ng/mL IL-1β + 10 ng/mL TNFα + 20 ng/mL IFNγ, 6 h). Primary microglia were untreated or primed with LPS (10ng/mL, 6 h) and served as comparison. RNA were extracted and analysed for gene expression of *Nlrp1, Nlrp2, Nlrp6, Nlrp12, Aim2, Nlrc4* and *Casp4* by RT-PCR. Results were normalized to *Rpl27*. The Heat map represents these normalized expression values (-dCt). Blue represents low level of expression (-dCt = -12) and red represents high level of expression (-dCt = -2). Data are the mean of at least three independent experiments.

In view of these results we tried to activate the AIM2 inflammasome complex as well as the non-canonical inflammasome pathway in astrocytes. As for LPS-primed microglia (Results section 1.2), we transfected CCM-primed astrocytes with the AIM2 inflammasome activator poly (dA:dT) or with LPS. Both stimulations failed to induce the release of IL-1β, IL-18 and HMGB1 (data not shown).

Part II: The inflammasome in neurodegenerative diseases.

The development of Parkinson's disease (PD) is accompanied by chronic inflammation in which IL-1β plays an important role. However, a possible implication of the inflammasome in the pathogenesis of PD remains poorly addressed. In order to investigate this relation, we developed *in vitro* assays to study inflammasome activation in microglia, in response to neurodegenerative diseases-associated stimuli. In a second time, the *in vivo* significance of NLRP3 deficiency in Parkinson's disease physiopathology was addressed.

1. *In vitro* NLRP3 inflammasome activation in response to neurodegenerative diseases-related signals.

As our previous results demonstrated the capacity of microglia to form a functional NLRP3 inflammasome, we aimed to explore the reactivity of this complex to three different types of factors that have been associated with the neurodegenerescence: the neurodegenerative diseases-related proteins, the purine metabolites and the pesticide rotenone.

1.1 Differential microglial inflammasome activation in response to the neurodegenerative disease-related peptides Aβ and α-synuclein.

Before the onset of this project, in 2011, a single report suggested that the stimulation of microglia with Amyloid-β (Aβ) was able to induce an NLRP3-dependent release of IL-1β (Halle *et al*, 2008). Thus, we wanted to confirm these observations but also to investigate the inflammasome activation capacity of α-synuclein, the principal constituent of the Lewy bodies. To this end, we exposed microglia to these both peptides and analyzed their potential to prime (signal 1) or activate NLRP3 (signal 2).

We tested two different fragments of the amyloid-β protein: $A\beta_{25-35}$ and $A\beta_{1-42}$ (see Introduction, Part I, section 3).

First, we study the effect of the $A\beta_{25-35}$, as it is endogenously found in elderly people and has been described to be a more toxic form (Millucci *et al*, 2009). To explore the priming capacity of this $A\beta_{25-35}$ peptide, we activated microglia for 6 h with 50 µM of the peptide as well as with its reverse form ($A\beta_{35-25}$, negative control) and LPS (positive

control). The gene expression analysis performed by RT-PCR indicated that the $A\beta_{25-35}$ was unable to prime microglia, as *Nlrp3* and *Il1b* were not upregulated after treatment (Fig. 38A).

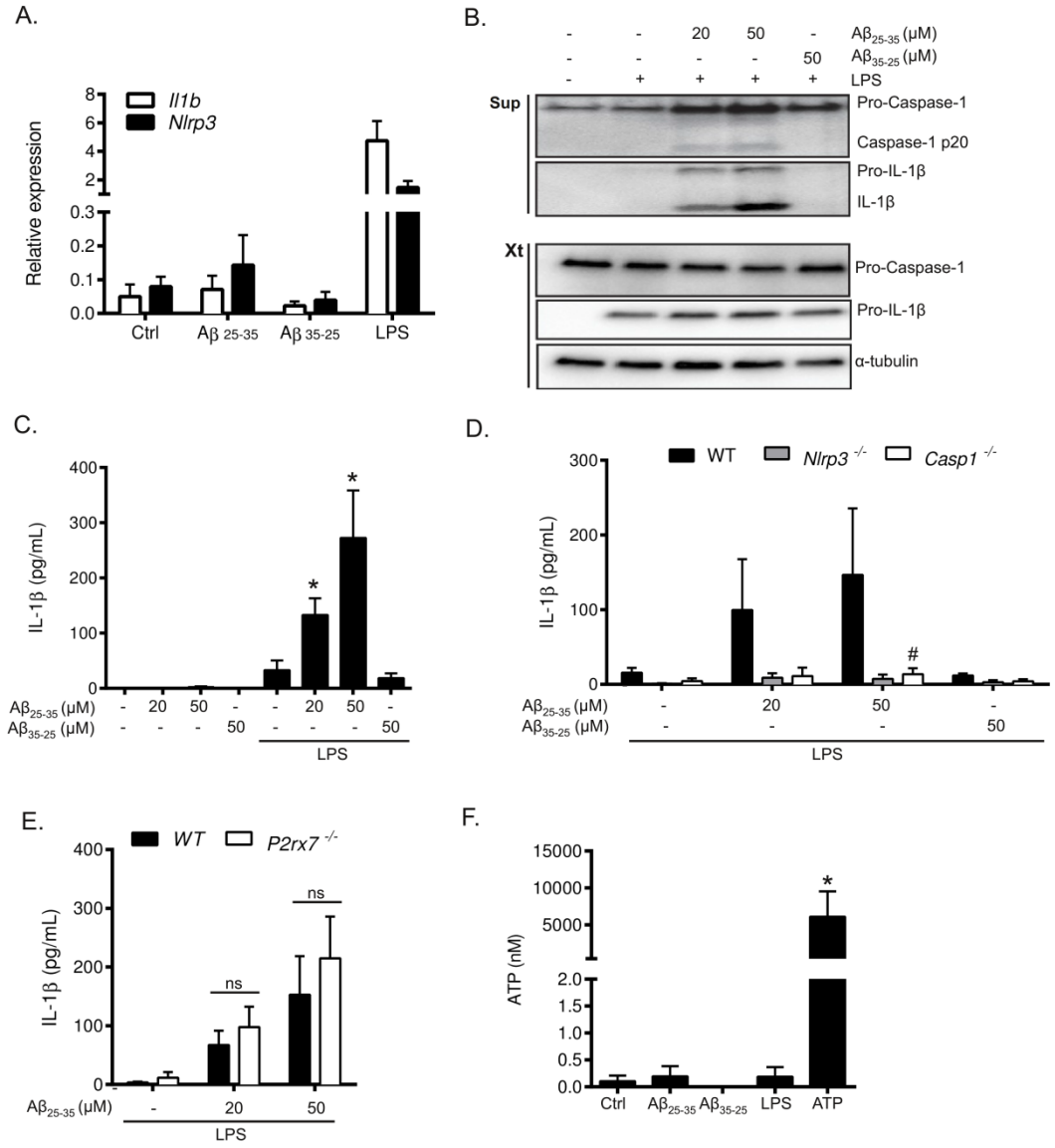


Fig. 38: $A\beta_{25-35}$ induces caspase-1 cleavage and IL-1β release in an NLRP3-dependent and P2X7R-independent way.
(A) Microglia were stimulated with $A\beta_{25-35}$ (20 or 50 μM, 5 h), $A\beta_{35-25}$ (50 μM, 5 h) or LPS (10 ng/mL, 6 h). Transcripts were analysed for expression of *Nlrp3* and *Il1b* by RT-PCR. Data were normalized to *Rpl27*. **(B, C, D)** Untreated or LPS-primed microglia were stimulated with $A\beta_{25-35}$ (20 or 50 μM) or $A\beta_{35-25}$ (50 μM) for 5 h. **(B)** Cell free culture supernatants (SN) and cell lysates (XT) were analysed by WB for the expression of caspase-1 and IL-1β. α-Tubulin was used as a loading control. IL-1β production in culture supernatant was assessed by ELISA in wild-type **(C)**, *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ **(D)**, or *P2rx7*$^{-/-}$ microglia **(E)**. **(F)** ATP release was quantified in microglial supernatant upon treatment with $A\beta_{25-35}$ (50 μM, 5 h) or $A\beta_{35-25}$ (50 μM, 5 h) or LPS (10 ng/mL, 6 h) performed using a luminescent assay. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *p<0.05 compared to Ctrl, #p<0.05, KO compared to WT, ns= not significant. One-way ANOVA followed by Dunnet's multiple comparisons test.

Even if the $A\beta_{25-35}$ peptide was unable to act as a signal 1, it seemed to be able to act as a signal 2. Indeed, WB analysis revealed that, in LPS-primed microglia, $A\beta_{25-35}$ was able to induce caspase-1 cleavage and IL-1β secretion, whereas the control peptide $A\beta_{35-25}$ was not (Fig. 38B). The additional IL-1β quantification performed by ELISA demonstrated that this IL-1β release was dose-dependent since the levels increased from 132±30.5 pg/mL with 20 µM of $A\beta_{25-35}$ to 272±85 pg/mL with 50 µM (Fig. 38C). To confirm the involvement of the NLRP3 inflammasome, we used this peptide and its negative reverse control on *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ microglia. We observed a strong reduction of IL-1β release in the supernatant of the knockout cells compared to the wild-type (95% and 77% of decrease respectively in *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$, Fig. 38D). To determine if this activation could be due to an indirect release of ATP, we also treated P2X7 receptor-deficient microglia. Both wild-type and *P2rx7*$^{-/-}$ cells secreted similar amounts of IL-1β, respectively 152±65 pg/mL and 214±71 pg/mL in response to 50 µM of $A\beta_{25-35}$ (Fig. 38E). This latter result was strengthened by the absence of ATP in the supernatant of microglia treated with $A\beta_{25-35}$ (Fig. 38F).

In addition to the peptide fragment $A\beta_{25-35}$, we wanted to test the capacity of the full length peptide $A\beta_{1-42}$ to induce NLRP3 activation. This peptide is supposed to be physiologically more relevant as it is found in amyloid plaques of AD patients (Shahdat & Hashimoto, 2012). The $A\beta_{1-42}$ peptide was used in its oligomeric and fibrillar forms. The fibrillation protocol (see Materials and Methods, section 2.3.3.) led to the formation of larger oligomers even if monomers were still present (Fig. 39A). Microglia exposed to 10 µM of both oligomeric and fibrillar forms, for 6 h or 24 h, failed to significantly modulate *Il1b* and *Nlrp3* gene expressions, as shown by RT-PCR analysis (Fig. 39B). This observation was confirmed by WB analysis, where we failed to detect pro-IL-1β after $A\beta_{1-42}$ treatment (Fig. 39C). These experiments also demonstrated that, even after LPS-priming step, $A\beta_{1-42}$ was still failing to induce a significant IL-1β release, independently from its conformation or incubation times (Fig. 39C and Fig. 39D). To exclude the possibility that the lack of activity could be linked to the peptide manufacturer (Bachem), we also tested $A\beta_{1-42}$ from other providers (Anaspec and Sigma) and observed similar results (data not shown).

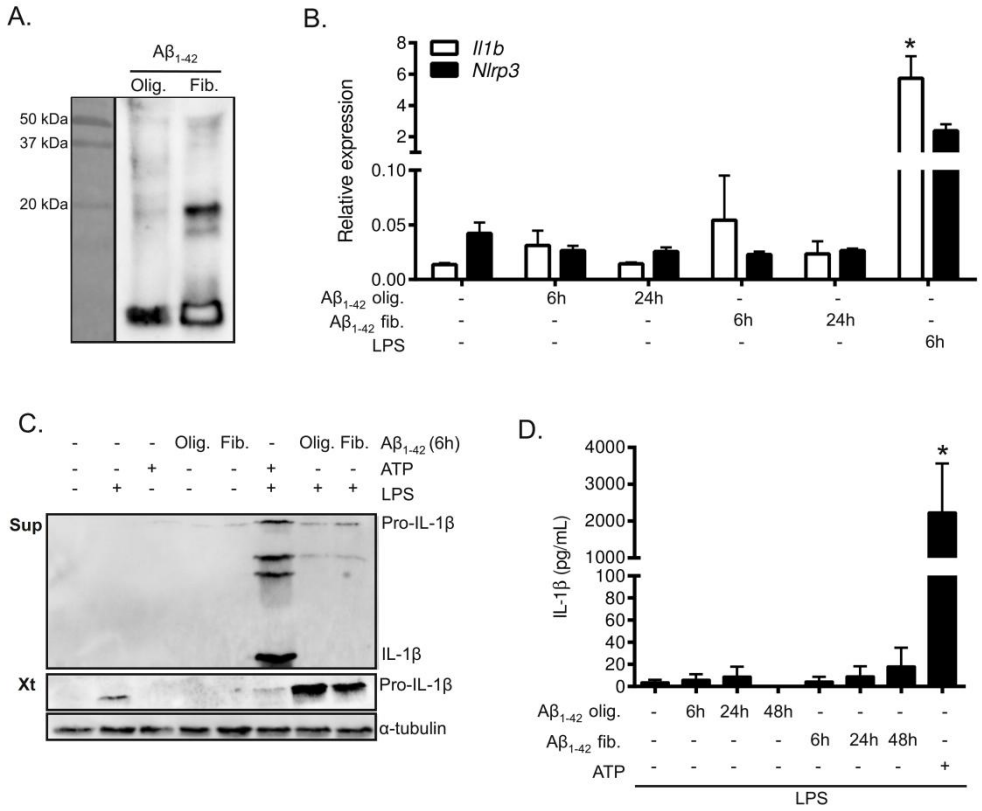


Fig. 39: Oligomeric and fibrillar Aβ1-42 fail to activate the inflammasome in microglia.

(A) Aβ1-42 (10 µM) was used under oligomeric form (Olig. = directly resuspended following manufacturer's instructions) or under fibrillar form (Fib. = obtained by heating at 37°C during 7 days). These different Aβ1-42 conformations were analysed by WB. **(B)** Microglia were stimulated for 6 h or 24 h with both forms of Aβ1-42 (10 µM) or for 6 h with LPS (10 ng/mL). Transcripts were analysed for the expression of *Nlrp3* and *Il1b* by RT-PCR. Data were normalized to *Rpl27*. **(C, D)** Untreated or LPS-primed microglia were stimulated for 6 h, 24 h or 48 h with both forms of Aβ1-42 (10 µM) or with ATP (1 mM, 30 min). **(C)** Cell free culture supernatants (SN) and cell lysates (XT) were analysed by WB for expression IL-1β. α-Tubulin was used as a loading control. **(D)** IL-1β production in culture supernatant was assessed by ELISA. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least independent experiments). *p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

In addition to Aβ peptide, related to AD, we had tested the ability of α-synuclein (α-syn), linked to PD, to trigger inflammasome activation. Some mutations in the *Snca* gene have been associated to familial forms of PD. Among them, the substitution of alanine to threonine at position 53 of the α-syn protein (A53T) was identified as causing a higher propensity to form α-syn fibrils. Therefore, we tested the human native and A53T mutant α-syn, in both oligomeric and fibrillar forms, for their ability to activate mouse microglial inflammasome. To obtain the fibrillar form, the peptides were incubated during 4 days at 57°C, under shaking conditions (see Materials and Methods, section 2.3.3.). WB analysis revealed the monomeric and dimeric forms, represented respectively by the 17 kDa and 37 kDa bands. Once heated, we observed additional bands at 50 kDa and around 150 kDa (Fig. 40A).

We investigated, by RT-PCR, whether α-syn could prime microglia. Indeed, the native human α-syn (WT α-syn) tended to increase the transcription of *Nlrp3* (4-fold for oligomeric preparation and 2-fold for fibrillar preparation, Fig. 40B). Interestingly, the mutant A53T α-syn seemed to be more potent than the WT α-syn, especially in its oligomeric form, since we were able to observe a stronger trend toward *Nlrp3* upregulation after the treatment (7.5-fold, Fig. 40B).

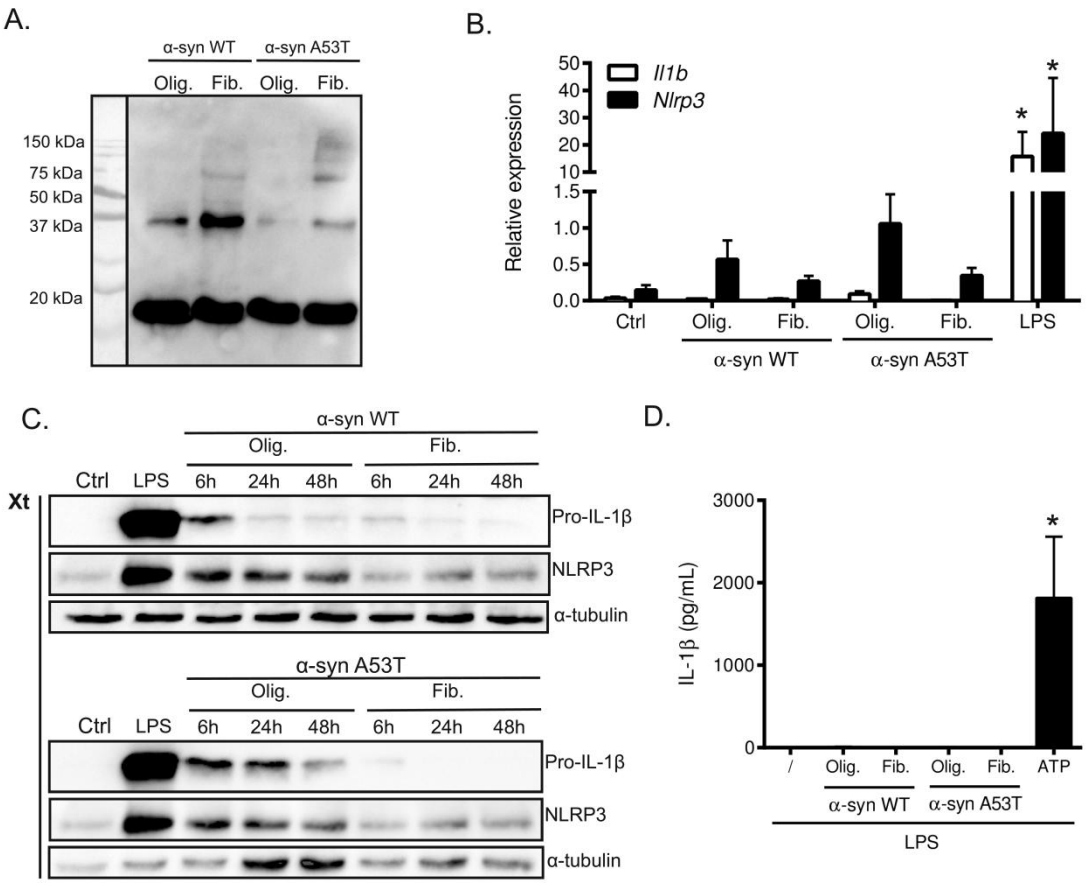


Fig. 40: WT and mutant (A53T) α-synuclein fail to activate the inflammasome.

(A) Wild-type (WT) or mutant (A53T) α-synuclein (α-syn) were used under oligomeric form (Olig. = directly resuspended in water) or under fibrillar form (Fib. = obtained by heating at 57°C during 4 days under shaking). These different conformations of α-syn were analysed by WB. **(B, C)** Microglia were stimulated for 6 h, 24 h or 48 h with both forms of WT and A53T α-syn (5 µM) or for 6 h with LPS (10 ng/mL). **(B)** Transcripts were analysed for the expression of *Nlrp3* and *Il1b* by RT-PCR. Data were normalized to *Rpl27*. **(C)** Cell lysates (XT) were analysed by WB for pro-IL-1β and NLRP3 expression. α-Tubulin was used as a loading control. **(D)** LPS-primed microglia were stimulated with both forms of WT or A53T α-syn (5 µM, 6 h) or with ATP (1 mM, 30 min). IL-1β production in culture supernatant was assessed by ELISA. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Even if these effects on transcripts were non-significant and remained largely below the effect of LPS, we were able to confirm such an upregulation by a WB analysis. Figure 40C shows that pro-IL-1β and NLRP3 protein were overexpressed after treatment with either human WT or A53T oligomeric α-syn (Fig. 40C). To study if both form could be able to activate the inflammasome, we used them on LPS-primed microglia. While ATP induced a strong release of IL-1β detected by ELISA, we could not observe IL-1β in the supernatant of cells treated with the different α-syn forms (Fig. 40D).

1.2 ADP and adenosine are able to prime the NLRP3 inflammasome in microglia.

During neurodegenerative diseases, dying neurons are thought to release ATP which might locally reach high concentrations in the brain parenchyma. It is already known that ATP is an efficient NLRP3 inflammasome activator, but much less is known about the potential of its metabolites, ADP and adenosine. Therefore, we decided to analyse their capacity to prime or activate the microglial NLRP3 inflammasome.

First, we activated microglia with 1 mM of adenosine, ADP or ATP for 3 h and analysed changes in gene expression by RT-PCR. Surprisingly, ADP, and to a lower extent adenosine, significantly upregulated *Nlrp3* and *Il1b* gene expressions, while ATP did not (Fig. 41A). The priming effect of ADP and adenosine also influenced other pro-inflammatory factors, such as *Cxcl10, Il6 or Tnf* (Suppl. Fig. 5).

To investigate whether ADP or adenosine would be able to activate NLRP3, we stimulated LPS-primed microglia with both compounds and quantified the IL-1β levels secreted in the supernatant. Interestingly, our ELISA analysis revealed that adenosine did not trigger any IL-1β release (Fig. 41B), whereas ADP led to a dose-dependent IL-1β secretion (Fig. 41C). Moreover, the quantified levels were similar to those observed after ATP exposure (Fig. 41C). To confirm that the ADP-induced IL-1β release did not rely on the ATP receptor P2X7, we performed the activation on *P2rx7*$^{-/-}$ cells. Surprisingly, we failed to detect IL-1β release in P2X7R-deficient cells (Fig. 41D). As ADP is not supposed to bind to this receptor, we decided to test whether ATP could be present in commercial ADP batches. These quantifications, based on a luminescent assay, revealed that they were indeed contaminated by 20% of ATP (Fig. 41E).

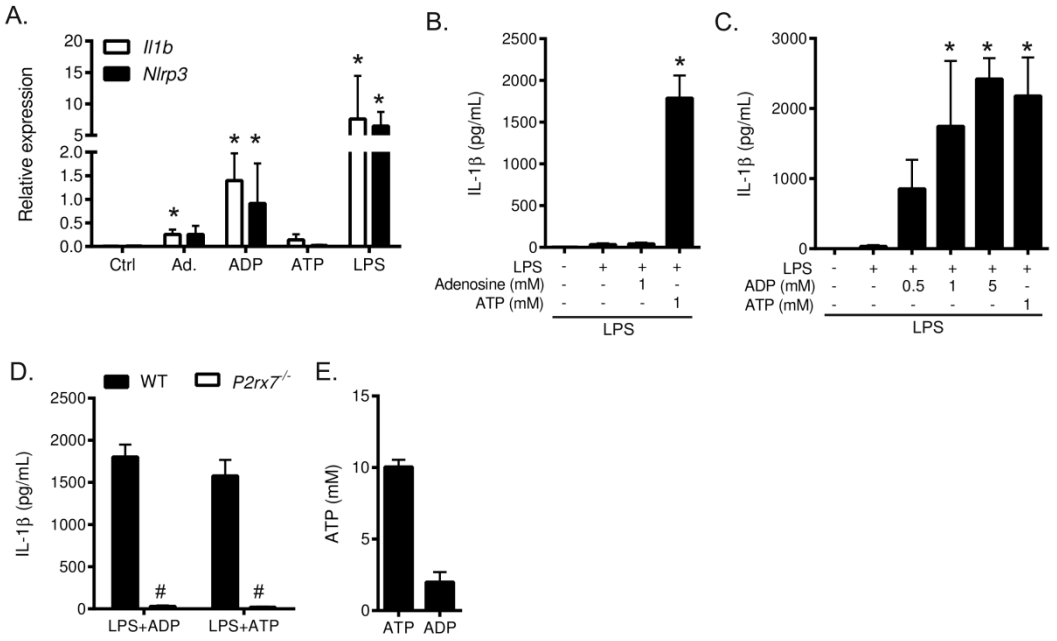


Fig. 41: ADP upregulates *Il1b* and *Nlrp3* genes and triggers IL-1β release via the P2X7R in microglia.

(A) Microglia were stimulated for 3 h with adenosine (Ad., 1 mM), ADP (1 mM), ATP (1 mM) or LPS (10 ng/mL). Transcripts were analysed for expression of *Nlrp3* and *Il1b* by RT-PCR. Data were normalized to *Rpl27*. **(B, C, D)** LPS-primed microglia were stimulated for 30 min with adenosine (1mM), ADP (0.5 mM, 1 mM, or 5 mM) or ADP (1mM). IL-1β production was assessed by ELISA in culture supernatant of wild-type (WT) **(B and C)** or *P2rx7*$^{-/-}$ **(D)** microglia. **(E)** ATP concentration in ADP and ATP samples was quantified using a luminescent assay. Data are mean ± SEM of at least three independent experiments. *p<0.05 compared to Ctrl, #p<0.05, KO compared to WT. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

1.3 IL-1β release triggered by rotenone is NLRP3 inflammasome-dependent but ROS- and ATP-independent.

Inflammasome and neurodegenerative diseases are both related to mitochondrial dysfunction. To study the implication of mitochondrial dysfunction in microglial inflammasome activity, we used rotenone, a specific mitochondrial complex I inhibitor. This product is also described as a causative agent of Parkinson's disease.

LPS-primed microglia were stimulated with different concentrations of rotenone (from 10 nM to 5 µM) for different incubation times (from 15 to 90 min). As shown in figure 42A, rotenone exposure induced a significant release of IL-1β after 90 min (100±46, 150±72 and 157±66 pg/mL after respectively 0.1, 1 and 5 µM of rotenone). This IL-1β secretion occurred in an NLRP3 inflammasome-dependent way, as its secretion was strongly decreased in supernatants of NLRP3-deficient microglia as compared to wild-type (respectively 4.3±1.3 pg/mL *vs* 161±57 pg/mL, Fig. 42B). In addition, we observed an impressive disruption of the IL-1β release from 466±144 pg/mL to 34±9 pg/mL when the cells were activated in presence of the caspase-1 inhibitor z-YVAD-FMK

(Fig. 42C). Then, we analysed the implication of ROS in the rotenone-triggered inflammasome activation. For this purpose, the antioxidant molecule NAC was used. Surprisingly, the secretion of IL-1β was not affected by this compound (Fig. 42D). In addition, we observed that rotenone did not induce ATP release in microglia, indicating that it acted in an ATP-independent way (Fig. 42E).

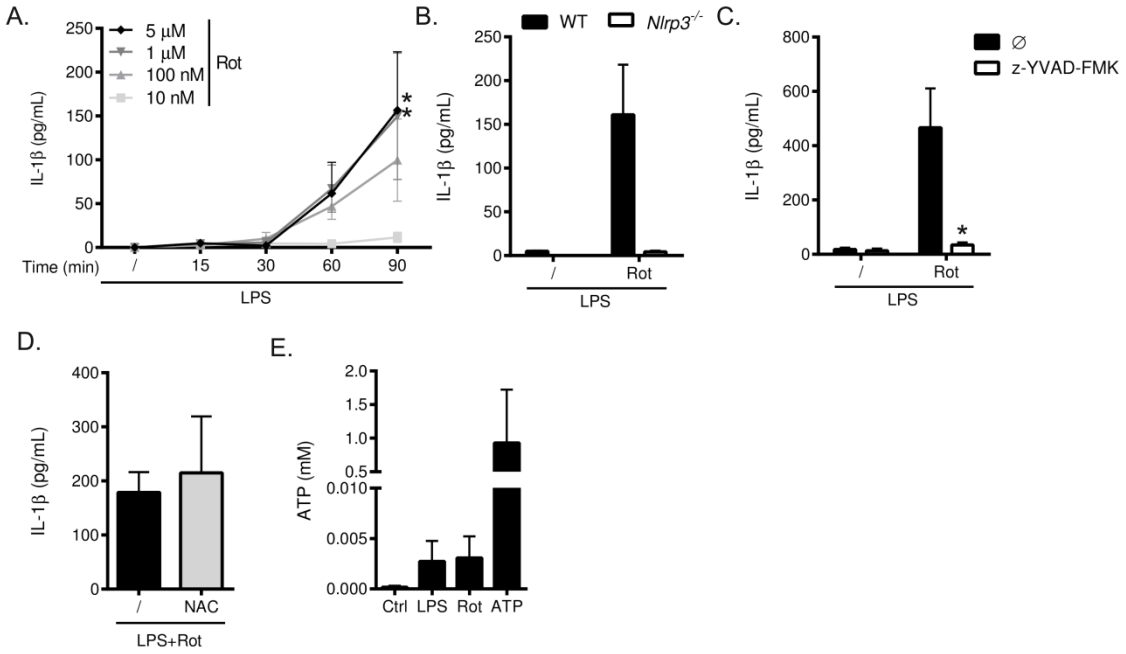


Fig. 42: IL-1β secretion is triggered by rotenone via the NLRP3 inflammasome activation.

(A) LPS-primed microglia were stimulated with rotenone (Rot) at different concentrations (from 10 nM to 5 µM) and different time points (between 15 to 90 min). Supernatants were analysed for IL-1β secretion by ELISA. **(B, C, D)** LPS-primed microglia were stimulated for 90 min with rotenone (1 mM). Secretion of IL-1β was quantified by ELISA in supernatant of wild-type (WT) and *Nlrp3*$^{-/-}$ microglia **(B)** or in presence of inhibitors, *i.e.* z-YVAD-FMK (20 nM, added 15 min before LPS) **(C)**, N-Acetyl Cysteine (NAC, 5 mM, 2 h) **(D). (F)** ATP release was quantified in cell supernatant using a luminescent assay and upon treatment with LPS (10 ng/mL, 6 h), rotenone (1 mM, 90 min), or ATP (1 mM, 30 min). Data are mean ± SEM of at least three independent experiments, *=p<0.05 compared to Ctrl, #=p<0.05 KO compared to WT. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

2. Preliminary results: Effect of NLRP3 deficiency in an *in vivo* model of Parkinson's disease.

In addition to *in vitro* studies, we decided to investigate the role of the NLRP3 inflammasome in an *in vivo* model of Parkinson's disease. For this purpose, we chose to study the effect of the NLRP3 deficiency on the neurodegenerative processes occurring in a mouse intra-striatal 6-OHDA injection model.

Most of this *in vivo* project was performed by Dr. Eric Koncina. I expanded the NLRP3 knockout mice colony, performed the genotyping and generated the first cohort of NLRP3-deficient KO mice.

The striata of the *Nlrp3*$^{-/-}$ mice and age-matched wild-type mice, were injected with 4 µg 6-OHDA or with the vehicle. Fourteen days after the injection, the mice were euthanized and the neurodegeneration, as well as the extent of microgliosis, were evaluated by immunostaining. To detect the presence of dopaminergic neurons, we used an antibody against Tyrosine hydroxylase (TH) whereas microgliosis was highlighted by the expression of CD68. We analysed these markers in two related anatomical structures: the substantia nigra pars compacta (SNpc, in midbrain), containing the dopaminergic neuron cell bodies, and the striatum, which is the target of their axonal projections. The summary of the results obtained are reported in the figure 43.

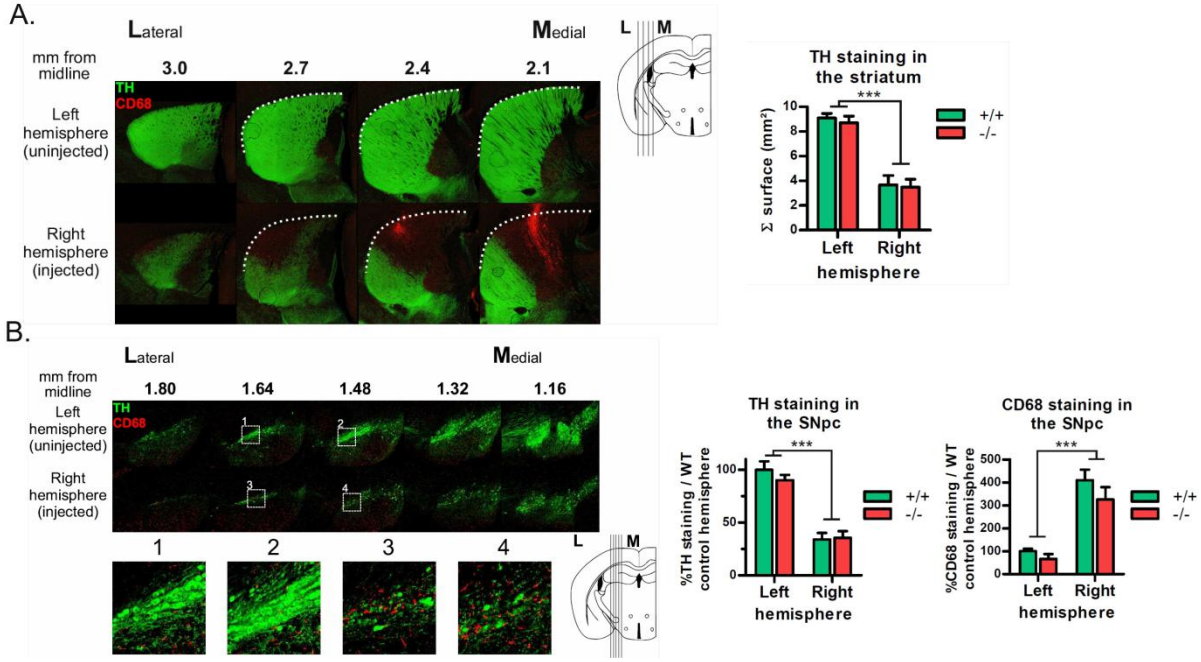


Fig. 43: 6-OHDA triggers similar neurodegenerescence and microgliosis in wild-type and in *Nlrp3*$^{-/-}$ mice.

Results obtained by Dr. Eric Koncina. 4 µg of 6-OHDA or vehicle was injected into the mouse striatum (at stereotaxic coordinates: AP: +0.5 mm, ML: +2.3 mm, DV: +3.2mm) of wild-type (+/+) and *Nlrp3*$^{-/-}$ (-/-) mice. Fourteen days after the injection, mice were sacrificed and brain collected. TH (Tyrosine hydroxylase) and CD68 staining were performed **(A)** on sagittal striatum sections, at 4 medio-lateral levels (3, 2.7, 2.4 and 2.1 mm from midline) and **(B)** on sagittal midbrain sections, at 5 medio-lateral levels (1.8, 1.64, 1.48, 1.32, 1.16 mm from midline) for both uninjected or injected hemispheres. The quantification of the TH and CD68 stained surface is represented by the sum of the 4 values for the striatum or the 5 values for the midbrain. n = 13 per group (WT and KO) *** = p<0.001 compared to left. Two-way ANOVA.

As expected, we can observe that in wild-type mice the TH staining was strongly reduced in the striatum of the injected hemisphere compared to the contralateral one (Fig. 43A). However, there was no significant difference in the degeneration between wild-type and

Nlrp3$^{-/-}$ mice (Fig. 43A). Similarly, we observed a significant TH+ neuron degeneration in the substantia nigra (Fig. 43B), but this neuronal cell loss was equivalent in wild-type and NLRP3-deficient mice (Fig. 43B). Microgliosis, which was characterized by the expression of CD68, appeared significantly upregulated in the injured substantia nigra but again regardless of the genotype of the mice (Fig. 43B).

The precise kinetic of the ongoing neuroinflammation in the 6-OHDA model is not very well defined. This is why we decided to characterize the time window in which selected pro-inflammatory markers, including IL-1β, are expressed in this model. To this purpose, we performed a kinetic transcript analysis of the pro-inflammatory factors expressed in response to 6-OHDA.

First, we have validated our dissection protocol of collecting samples from the mouse striatum and the substantia nigra. Figure 44 shows the expression profile of genes relevant to dopamine neurons: *Slc6a3* (DAT, (Dopamine Active Transporter), *Th* and *Slc18a2* (VMAT2, Vesicular Monoamine Transporter 2) in three collected structures: the cortex (Cx), the striatum (St) and the midbrain (Mb). As expected, we observed that these genes linked to dopamine neurons were mostly expressed in the midbrain (Fig. 44).

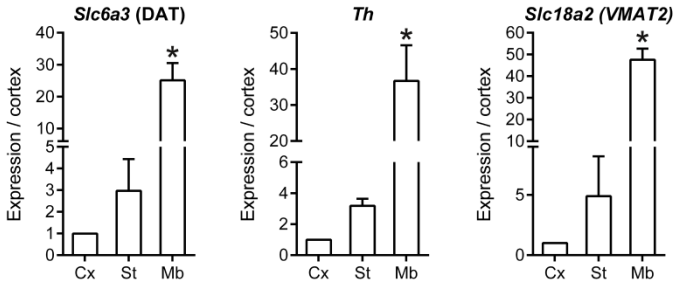


Fig. 44: Validation of the striatum and midbrain dissection protocol.

RNA were extracted from biopsies of the cortex (Cx), the striatum (St) and the midbrain (Mb) collected from wild-type mice. RT-PCR was performed to analyse the gene expression of *Slc6a3*, *Th* and *Slc18a2* respectively coding for DAT, TH and VMAT2. The expression ratio of these genes was calculated on the Cx. Data are mean ± SEM of six independent experiments. *p<0.05 compared to the Cx. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Once our dissection protocol was validated, we administered the vehicle or 6-OHDA into wild-type mice by stereotaxic injection (Fig. 45A). The mice were euthanized after 3, 7 or 14 days. The striatum and midbrain from both hemispheres (ipsi- and contralateral) were collected for RNA extraction. In addition, after 14 days, we isolated the brain from a mouse of each group (Vehicle or 6-OHDA) in order to check, by immunohistochemistry, whether the neurodegeneration was effective in this experiment.

The transcripts collected from the striatum were analysed by RT-PCR to investigate the expression of pro-inflammatory genes. For each animal, we calculated the ratio of the gene expression in the ipsilateral sample compared to the gene expression in the contralateral sample of the same animal. The results showed a modest, non-significant, trend in upregulation (around 2-fold) of the pro-inflammatory genes *Cxcl10*, *Tnf* and *Il1b* in the injected hemispheres, 3 or 7 days after the injection. (Fig. 45B). However, we were not able to detect a difference between the vehicle and 6-OHDA injected animals. The *Aif1* and *Gfap* genes also showed a weak and non-significant increase (around 1.8-fold) in expression after 3 days, which might indicate, respectively, the microgliosis and astrogliosis (Fig. 45B). As we were unable to observe a significant change in expression, we performed an immunohistochemistry staining on the dedicated brains (Fig. 45A). We were able to observe an intense upregulation of the GFAP, Iba1 and CD68 staining in the ipsilateral side of injected brains compared to the contralateral one (Fig. 45C). In addition, the TH staining in the striatum and substantia nigra were decreased in the 6-OHDA injected slices, confirming the efficacy of our injection (Fig. 45D).

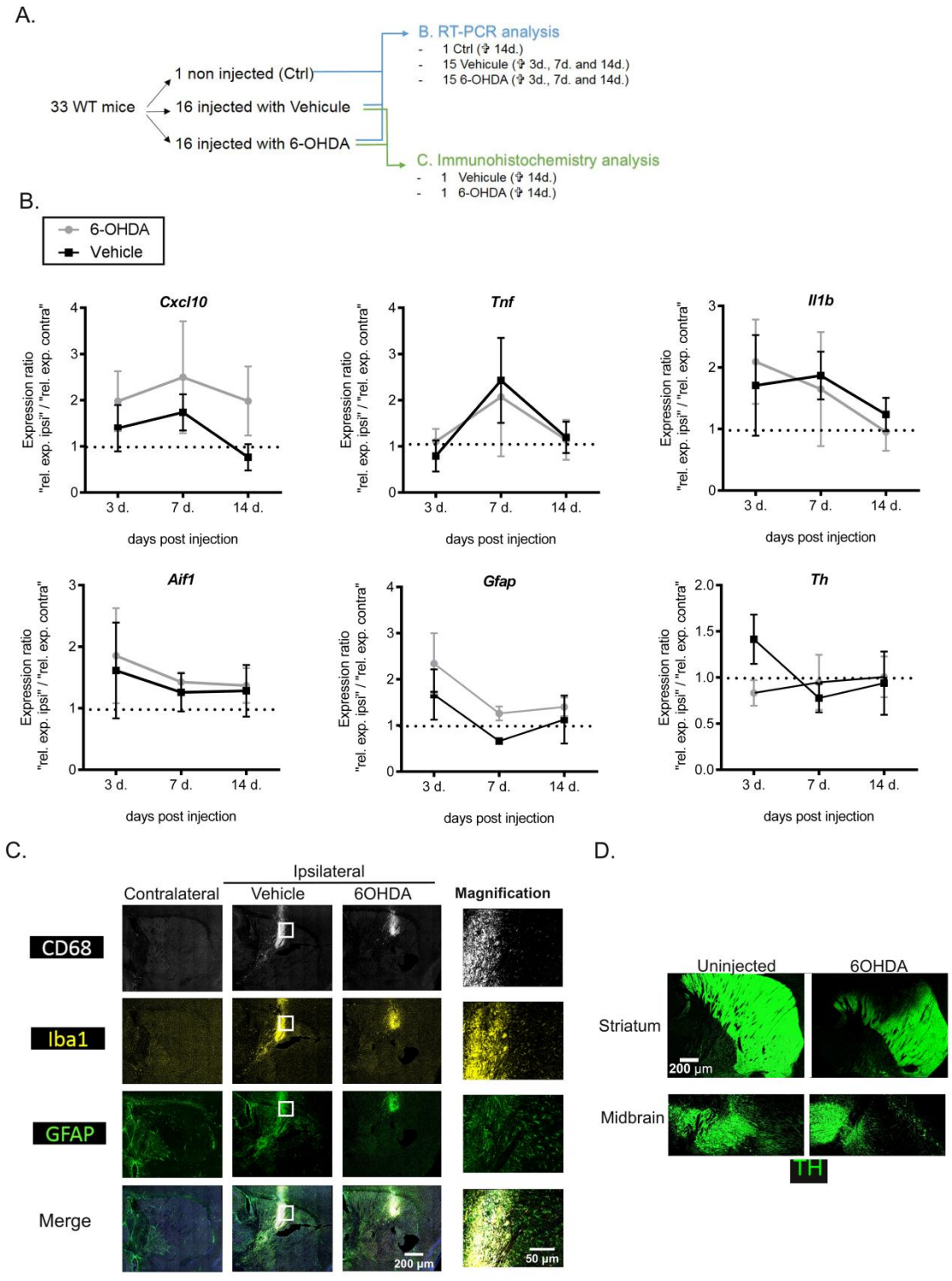


Fig. 45: Pro-inflammatory genes are modestly upregulated in the striatum of mice injected with vehicle or 6-OHDA.

(A) Plan of the kinetic transcript analysis performed **(B, C, D)** Striatum of wild-type mice were injected with 4 µg 6-OHDA or with vehicle. Mice were euthanized after 3, 7 or 14 days. **(B)** RT-PCR analysis of mRNA levels for *Cxcl10*, *Tnf*, *Il1b*, *Aif1*, *Gfap* and *Th* in striatum samples was performed. Three to five mice were analysed for each time point and for each experimental group. Data are expressed as ratio of the relative expression to *Rpl27* in ipsilateral site versus contralateral site from individual animal. Data are mean ± SEM. **(C)** Representative immunofluorescent histochemical staining for CD68 (white), Iba1 (yellow) and GFAP (green) in ipsilateral and contralateral striatum. **(D)** Representative immunofluorescent histochemical staining for TH (Tyrosine hydroxylase, green) in the striatum and midbrain of 6-OHDA injected mice.

V. Discussion

1. Characterization of inflammasome expression and activation in brain cells.

The inflammasome is a multiprotein complex which, once activated, results in the activation of caspase-1 and in the subsequent maturation and secretion of active IL-1β. IL-1β release has been linked to neuroinflammatory conditions and neurodegenerative diseases for a long time with both beneficial and detrimental effects described (Allan *et al*, 2005). However the potential role of the inflammasome in the local production of IL-1β in the brain is not very well understood. Recently, several reports started to investigate inflammasome activation in neuroinflammatory and neurodegenerative conditions but many fundamental questions remain. We therefore wanted to characterize in more detail the *in vitro* capacities of microglia and astrocytes to express and activate the inflammasome.

Inflammasome in microglia

Although microglia resemble hematopoietic macrophages both in phenotype and function, it has recently been discovered that they originate from a different progenitor (Ginhoux *et al*, 2010; Schulz *et al*, 2012; Lourbopoulos *et al*, 2015). Furthermore, microglia, which are isolated from the periphery by the BBB, exhibit differences with circulating immune cells (Schulz *et al*, 2012; Ginhoux *et al*, 2013). Thus, the investigations of inflammasome expression and activation in microglia have to be directly addressed in these cells rather than being extrapolated from the existing inflammasome knowledge in macrophages.

For this purpose, we have decided to perform our *in vitro* experiments on primary microglial cells, since our preliminary data showed that these cells exhibit a better response to the inflammasome activation compared to microglial cells lines. It is also well admitted and described that primary cells better reflect the physiology of *in situ* microglia.

Our results showed that primary microglia express the NLRP3 inflammasome core components (NLRP3, ASC and Caspase-1) as well as other inflammasome genes, such as *Nlrp1, Nlrp6, Aim2, Nlrc4* or *Casp4 (Casp11)*. These findings are in accordance with other reports suggesting that microglia could form NLRC4 and NLRP3 inflammasome complexes (Walsh *et al*, 2014). In this work, we have mainly focused our attention on the NLRP3 inflammasome, since this specific complex assembles in response to DAMPs, such as ATP or misfolded proteins, which are strongly linked to neurodegenerative diseases.

First, we aimed to study if microglia respond to NLRP3 inflammasome activation in the same manner than macrophages, in terms of caspase-1 activation and cytokines secretion. Our findings indicated that the stimulation of LPS-primed microglia with the classical NLRP3 activators ATP, Nigericin and Alum led to the inflammasome complex assembly and to subsequent cleavage of caspase-1. We also showed that this caspase-1 activation triggered the maturation and release of IL-1β, IL-18, IL-1α and HMGB1 by microglia. Moreover, our observations of the activation of caspase-1, associated with an important release of LDH, might indicate that microglia undergo pyroptotic cell death following inflammasome activation (Rayamajhi *et al*, 2013).

In this work, we have used NLRP3- and Caspase-1 knockout mice to show the requirement of the NLRP3 inflammasome in these processes. Our results are in line with different reports showing that microglia possess the capacity to release IL-1β in an NLRP3- and Caspase-1-dependent way (Halle *et al*, 2008; Hafner-Bratkovič *et al*, 2012; Kaushik *et al*, 2012). However, the release of IL-18, IL-1α and HMGB1 by microglia after inflammasome activation is less described.

A previous publication showed that the microglial secretion of IL-18 in response to stimulation with *S.aureus* was NLRP3- and ASC-independent (Hanamsagar *et al*, 2011). Interestingly, we were able to show that in response to ATP, Nigericin or Alum treatment, the microglial secretion of IL-18 is, on the contrary, NLRP3 inflammasome-dependent. These divergent findings clearly open interesting questions concerning a possible differential production of IL-18 by microglia depending on the activation stimuli.

Our experiments also demonstrated that microglial inflammasome activation by ATP and Nigericin led to the release of IL-1α and HMGB1 and that this event was abrogated in NLRP3 and Caspase-1 knockout cells. It has been already demonstrated that macrophages can release IL-1α and HMGB1 after LPS+ATP or LPS+Nigericin treatment (Lamkanfi *et al*, 2010; Gross *et al*, 2012). However, our study is, to our knowledge, the first one which is addressing the involvement of NLRP3 inflammasome in the release of these alarmins in microglia. Furthermore, we showed that the caspase-1 protease activity was not required for the ATP- and Nigericin-mediated release of HMGB1 and IL-1α in microglia. These findings are also in line with previous results obtained from cultured macrophages (Gross *et al*, 2012).

Little is known about the signalling pathways required to trigger the inflammasome activation in the CNS (de Rivero Vaccari *et al*, 2014). First, it is unclear whether the two-step model of NLRP3 activation described in macrophages is operational in glial cells. Our results showed that microglia required a priming signal (LPS in our experiments)

before the exposure to an NLRP3 activator (here, ATP, Nigericin or Alum) in order to secrete IL-1β. Human monocytes for instance behave differently, as IL-1β can be released after a single stimulation with bacterial products alone (Rubartelli *et al*, 2011). Intriguingly, Burguillos and colleagues have shown in 2011 that, unlike in macrophages, the LPS priming of mouse microglia might require caspase 8 activation (Burguillos *et al*, 2011). Remarkably, despite several attempts, we were unable to detect the caspase-8 activity in microglia after LPS stimulation alone (data not shown).

After considering the requirement of such a signal 1 to prime the microglial inflammasome, we focused our study on the cellular cascades responsible for the specific activation of NLRP3. Our investigations showed that the main mechanisms described in macrophages — including ROS production, K+ efflux and endosomal rupture — also applied to NLRP3 activation within microglia, as supported by previous reports (Halle *et al*, 2008; Hoegen *et al*, 2011; Kaushik *et al*, 2012).

First, we were able to highlight the implication of the potassium efflux in the activation of NLRP3. Indeed, we observed that blocking this efflux, by adding KCl to the extracellular medium, strongly reduced IL-1β secretion by microglia. These results are in line with two reports showing a decrease of bacteria- and virus-triggered IL-1β secretion when microglia are cultured in high potassium conditions (Hoegen *et al*, 2011; Kaushik *et al*, 2012). However, these studies did not investigate the effect of KCl on the priming step of the inflammasome activation. Interestingly, we observed that blocking potassium efflux was linked to a strong decrease in NLRP3 and pro-IL-1β expression. Therefore, we suggest that K^+ efflux is well implicated in the NLRP3-dependent IL-1β secretion in microglia by playing, at least, upstream of the NLRP3 activation. Our observation is in opposition with a previous study using macrophages as the authors did not observe a modification of pro-IL-1β in KCl-enriched conditions (Pétrilli *et al*, 2007). These discrepant results between microglia and macrophages suggest a possible differential regulation level of potassium efflux in inflammasome activation in both cell types.

In addition, we showed that, with all the NLRP3 activators we tested, the presence of an antioxidant reduced the IL-1β secretion, suggesting a role for ROS in microglial inflammasome activation. It has been hypothesized that ROS inhibition does not directly affect the activation of the NLRP3 inflammasome. Instead, it might negatively regulate the priming step of NLRP3 inflammasome activation (Bauernfeind *et al*, 2011). However, in our experiments, the modulatory effect of the antioxidant NAC was independent of the priming as the exposure to it did not influence the expression of *Nlrp3* and *Il1b* genes. While we cannot exclude that this difference might be linked to the use of different antioxidants i.e. DPI in the study of Bauernfeind and colleagues *vs* NAC in ours, we

strongly believe that the redox state of microglia could affect the NLRP3 activity in microglia, independently of the priming. Indeed, it is noteworthy that these authors applied the antioxidant before the LPS treatment while we used NAC after the priming step.

Finally, we demonstrated that the Alum-induced IL-1β release relied on cathepsin B and phagocytosis. A similar mechanism has been demonstrated for Aβ (Halle *et al*, 2008). However, blocking phagocytosis and cathepsin B activity failed to abrogate the release of IL-1β triggered by ATP or Nigericin. Thus, our observations suggest that lysosomal damage might only be implicated in the particle-mediated NLRP3 activation in microglia, as described in peripheral myeloid cells (Hornung *et al*, 2008).

Taken together, our results on primary microglia indicate that they are able to form a functional NLRP3 inflammasome, through mechanisms close to those described in macrophages. In addition, we demonstrate that this inflammasome is responsible for the secretion of IL-1β, IL-1α, IL-18 and HMGB1.

In addition to the most studied NLRP3 inflammasome, other inflammasome complexes have been characterized. However, their contribution to the cerebral production of IL-1β has not been extensively addressed. Our transcript analysis showed that microglia express all core components of the AIM2 and NLRC4 inflammasome. Therefore, we started to investigate the microglial capacity to form these two complexes. In line with the hypothesis that these inflammasomes might be active in microglia, we showed that microglia release IL-1β following poly(dA:dT) or Flagellin that respectively activate the AIM2 and NLRC4 inflammasome in macrophages. As expected, this IL-1β secretion occurred independently of NLRP3. While a single publication already demonstrated that microglia could assemble the NLRC4 inflammasome (Jamilloux *et al*, 2013), the formation of the AIM2 inflammasome in CNS cells has until now only been described in neurons (Adamczak *et al*, 2014). Our findings also suggest that, in addition to the AIM2 and NLRC4 complexes, the non-canonical inflammasome pathway could be effective in microglia. Indeed, our results showed that LPS transfection triggered the secretion of IL-1β and initiated the release of LDH, a possible sign of pyroptosis. However, we need to clarify different points by additional experiments, and in particular: (i) confirm that our transfection protocol is efficient (ii) determine if the transfection is required (as the high LPS concentration was, in our conditions, sufficient to induce IL-1β) (iii) decipher the precise implication and interaction of caspase-1 and caspase-11 in microglia.

We are aware that our actual results on the functionality of AIM2, NLRC4 or the non-canonical inflammasome in microglia require further evidences and verifications to clarify if murine microglia could form other inflammasome complexes than NLRP3.

In summary, our results showed that the inflammasome is an important player of the inflammation triggered by primary murine microglia. However, we have to keep in mind that cultured microglia do not faithfully reflect the *in situ* properties of microglia in a normal, nonpathological brain (Kettenmann *et al*, 2011). Moreover, a recent study also underlines the fact that adult microglia significantly differ from neonatal microglia, that we used (Butovsky *et al*, 2014).

Inflammasome in astrocytes

Current evidence has shown astrocytes as important actors in neuroinflammation. During CNS injury, astrocytes become reactive, migrate to the damaged site, release numerous pro-inflammatory factors and form the glial scar (Pekny & Pekna, 2014). Despite their implication in immune response, only few studies have tried to characterize the inflammasomes in astrocytes.

Before analysing the inflammasome expression in astrocytes, we emphasized the importance of the purity of astrocyte cultures. Indeed, studying the pro-inflammatory role of astrocytes required some precautions. In the last few years, we and others have noticed that, even small amounts of highly reactive microglia could interfere with the astrocytic response and mitigate the experimental readout (Saura, 2007; Losciuto *et al*, 2012). This could even result in an erroneous attribution of inflammatory capacities to astrocytes in place of microglia. To avoid such misleading conclusions, we purified our astrocyte cultures by two successive passages on MACS columns, in order to deplete as many microglia as possible. This protocol of purification has been validated and published by our group (Losciuto *et al*, 2012, included in Appendixes).

In the literature, numerous publications described the expression and release of IL-1β by astrocytes following diverse stimuli and in particular the exposure to LPS (*e.g.* Fontana et al. 1983; Lau and Yu 2001; Ma et al. 2013). We failed, however, to detect any *Il1b* mRNA or pro-IL-1β in astrocyte-enriched cultures, although we tested several potential inducers, such as LPS, P3C or CCM, as well as longer exposure times. The lack of *Il1b* expression was also supported by our experiments on neurosphere-derived astrocytes, a model of astrocyte culture devoid of microglia. Moreover, our results are in

line with the recent findings of Facci and colleagues using the lysosomotropic agent L-leucyl-L-leucine methyl ester (Leu-Leu-OMe) to eradicate residual microglia from astrocyte cultures (Facci *et al*, 2014). In this study, classical astrocyte cultures released IL-1β into the medium while microglia-depleted cultures did not. By taking all these considerations together, we believe that many reports of IL-1β production by astrocytes could be due to microglial contaminations and that there is still no convincing evidence for IL-1β expression in astrocytes.

Even if pure astrocytes might not be able to express IL-1β, they nevertheless constitutively expressed IL-18 and HMGB1. As both need a functional inflammasome to be matured and/or released, we investigated the expression of NLRP3 inflammasome components by astrocytes. Despite the constitutive presence of caspase-1, we observed very weak levels of NLRP3 and ASC transcripts and almost no NLRP3 and ASC proteins, with or without priming. On the contrary, we were able to detect *Aim2* and *Nlrp6* expression in astrocytes. However, the stimulation of astrocytes with poly(dA:dT), an efficient AIM2 activator did not result in detectable IL-18 production by astrocytes. As the AIM2 inflammasome is dependent on ASC, the lack of ASC expression in murine astrocytes could explain this observation.

Together, these considerations suggest that the capacity of mouse astrocytes to form a functional inflammasome might be compromised. However, some hints suggest that, in opposition to the mouse cells, human astrocytes could present a different profile of inflammasome components expression. Indeed, some reports described the expression of NLRP3, ASC or IL-1β in human astrocytes (Zou & Crews, 2012; Kawana *et al*, 2013; Kaushal *et al*, 2015). Moreover, a publication described a functional NLRP2 inflammasome in human astrocytes (Minkiewicz *et al*, 2013). In mice, NLRP2 expression seems to be restricted to ovaries playing a role in early embryonic development as a maternal effect gene (Peng *et al*, 2012). Accordingly, we did not detect NLRP2 expression in our mouse astrocytes. Together, these observations suggest species-specific differences in inflammasome components expression in astrocytes.

Astrocytes and microglia represent key actors of the neuroinflammatory response. In case of brain injury, microglia are able to trigger an appropriate response involving secretion of cytokines and chemokines, resulting in the activation of astrocytes. Our results suggest that mouse microglia express a functional inflammasome contrarily to astrocytes and are thus the main glial cell type in the brain responsible for IL-1β and IL-18 secretion. However, we have to keep in mind that, even if astrocytes might not be able to express or release IL-1β and IL-18, they are a target of both cytokines. As such, they

certainly are an important element in the study of neuroinflammation triggered by the inflammasome. Therefore, it is crucial to analyse the crosstalk between microglia and astrocytes to fully appreciate the importance of the inflammasome function in the brain and the potential therapeutic applications in neurodegenerative diseases.

2. NLRP3 inflammasome in neurodegenerative diseases.

Activators of microglial NLRP3 inflammasome in neurodegenerative context

The relationship between the NLRP3 inflammasome and CNS diseases has emerged only recently. Several studies suggested that the NLRP3 inflammasome might be involved in acute brain infections, multiple sclerosis or in neurodegenerative diseases (Walsh *et al*, 2014). So far, the molecular and cellular events responsible for the activation of NLRP3 in these pathological conditions have been poorly investigated. Our purpose is to investigate and discuss the events that might facilitate an effective inflammasome activation during neurodegenerative pathologies, with a special regard to Parkinson's disease. To this aim, we have explored the role of neurological disease-relevant molecules in the NLRP3 inflammasome activation, such as neurodegenerative disease-associated misfolded proteins, ATP and its metabolites or mitochondrial inhibitors.

Inflammasome function has been linked to a number of diseases that are characterized by peptide aggregates or particulate structures, such as uric acid crystals in gout or islet amyloid polypeptide in type 2 diabetes (Masters *et al*, 2010; Robbins *et al*, 2014). The accumulation of protein aggregates is a hallmark of many neurodegenerative diseases such as α-synuclein in PD, Aβ in AD or prion proteins in prion disease. Recent studies showed that prion protein fibrils are able to induce inflammasome-dependent IL-1β secretion in microglia (Hafner-Bratkovič *et al*, 2012; Shi *et al*, 2012). Besides, several other reports demonstrated the capacity of different conformations of the Aβ peptide to trigger an inflammasome-dependent IL-1β secretion in macrophages and microglia (Halle *et al*, 2008; Parajuli *et al*, 2013; Wu *et al*, 2013b). In addition to these *in vitro* experiments, the *in vivo* relevance of the NLRP3 inflammasome in Alzheimer's disease has recently been demonstrated in the APP/PS1 mouse model where NLRP3-deficient mice show less severe symptoms (Heneka *et al*, 2013).

At the beginning of our project, in 2011, only one report addressed the possibility that Aβ could induce IL-1β secretion. Therefore, we aimed to confirm the activation of microglial NLRP3 by Aβ. To this purpose, we have used two different Aβ peptides: the shorter peptide A$\beta_{25\text{-}35}$ and the full-length A$\beta_{1\text{-}42}$. A$\beta_{25\text{-}35}$ is endogenously found in elderly people and has been described to be a more toxic form (Millucci *et al*, 2009). Moreover, the neurodegenerative properties of this peptide have been demonstrated *in vitro* (Pike *et*

al, 1993) and are supported by *in vivo* experiments demonstrating that the direct cerebral infusion of $A\beta_{25-35}$ impairs memory (Tohda *et al*, 2003; Yamada *et al*, 2005).

We were able to show that $A\beta_{25-35}$ could indeed activate the NLRP3 inflammasome in microglia, consistently to a previous report (Sanz *et al*, 2009). However, we observed that, in our conditions, IL-1β secretion occurred in a P2X7R-independent way, while Sanz and colleagues showed that the expression of P2X7 receptor seemed required for the release of IL-1β.

While the $A\beta_{25-35}$ peptide has been extensively used in previous *in-vitro* studies, we wanted to test the physiologically more relevant full length peptide $A\beta_{1-42}$ for its capacity to induce NLRP3 activation. Surprisingly, we were unsuccessful in triggering inflammasome activation by $A\beta_{1-42}$, contrarily to published studies (Halle *et al*, 2008; Parajuli *et al*, 2013; Wu *et al*, 2013b). We tested the oligomeric and fibrillar forms from different providers and tried multiple protocols but remained unable to detect IL-1β production in primary microglia. We believe it is likely that the preparation of Aβ may be a crucial step and explain these discrepancies. It is well admitted that the conformation of $A\beta_{1-42}$ peptide might be of great importance for immune activation, at least *in vitro*. For example, it has been described that $A\beta_{1-42}$ oligomers induce much greater microglial activation than insoluble fibrils (Sondag *et al*, 2009; Heurtaux *et al*, 2010; Paranjape *et al*, 2012). The importance of the conformation is sustained by the divergent results that we obtained with the $A\beta_{1-42}$ and $A\beta_{25-35}$ as the latter peptide is described as being more toxic and less prone to aggregation. Moreover, we have to consider that the Aβ peptide changes easily its conformation depending on various factors including the concentration, pH or solvent (Shahdat & Hashimoto, 2012). In line with these concerns, it is possible that our Aβ preparations contain less pro-inflammatory species than Aβ preparations used in published reports. This underlines the importance for us and the other authors to clearly characterize the different conformations of Aβ that they used.

α-synuclein (α-syn), the main component of Lewy Bodies in PD, can form fibrils with a cross–β-sheet structure. Therefore, we tested the capacity of oligomeric and fibrillar α-syn to activate the NLRP3 inflammasome in microglia. For this purpose, we used human native α-syn but also the human A53T mutant form, which occurs in familial forms of PD. α-syn treatment did not result in mature IL-1β secretion in our hands, although we tested different kinetics and concentrations of both forms. Recently, other reports described the ability of α-syn to trigger the inflammasome in human monocytes and THP1 cells (Codolo *et al*, 2013; Freeman *et al*, 2013). Again, as for Aβ, these discrepancies might be explained by a difference in aggregation forms. In addition, these studies were

based on human monocyte cell models and it has precisely been described that the NLRP3 inflammasome activation occurs through different mechanisms in mouse macrophages and human monocytes (Netea *et al*, 2014; Rubartelli, 2014). Even though we failed to characterize α-syn as a direct NLRP3 activator, it might still indirectly contribute to the activation of the inflammasome. Indeed, in the literature, numerous publications described α-syn as an important pro-inflammatory agent responsible for microglial activation (Béraud *et al*, 2013). This is also in accordance with our own observations where the human WT and A53T forms, in particular the oligomeric ones, upregulate the expression of IL-1β and NLRP3 at both transcript and protein levels. Thus, our results suggest that α-synuclein could at least participate in the priming step required for the NLRP3 inflammasome activation.

In view to the above discussed results, we hypothesized that microglial inflammasome might be preferentially activated in response to neuronal death and associated ATP release rather than by the presence of peptide aggregates during the course of neurodegenerative diseases. It has been reported that the intracellular ATP concentration varies between 1 and 10 mM depending on the cell type (Orriss *et al*, 2009), whereas the extracellular concentration is in the nanomolar range. However, after CNS injury the extracellular ATP concentration may reach 1 mM at the injury site (Pellegatti *et al*, 2005; Yin *et al*, 2007; Orriss *et al*, 2009). We and others have described that such an ATP concentrations are able to activate NLRP3 in cultured microglia (Halle *et al*, 2008; Hanamsagar *et al*, 2011; Hoegen *et al*, 2011; Facci *et al*, 2014). In this work, we wanted to perform a more extensive study and investigate the role of ATP metabolites during microglial activation. Indeed, in the brain, the released ATP can be rapidly hydrolysed (within 200 milliseconds) in a stepwise manner to ADP, AMP (Adenosine monophosphate) and adenosine by ecto-enzymes (Antonioli *et al*, 2013). Therefore, ADP and adenosine might be transiently present at high concentrations during neurodegeneration and could specifically interact with cells via their respective receptors, notably the P2 receptors, which are massively expressed by microglia (Koizumi *et al*, 2013).

Our results suggest that ADP and adenosine could participate in the establishment of neuroinflammation. Indeed, they induced the overexpression and secretion of different pro-inflammatory factors (*i.e.* CXCL10, TNFα, IL-6). Most interestingly, adenosine and ADP were able to prime microglia for a subsequent NLRP3 activation. Indeed, our experiments showed that microglia upregulate the expression of *Nlrp3* and *Il1b* genes following the exposure to adenosine or ADP. If the priming ability of adenosine has been recently published using THP1 cells (Baron et al. 2015), the pro-inflammatory effect of

ADP has, to our knowledge, not been described.

In 2013, a paper written by Gombault et al. proposed that multiple purinergic signalling pathways triggered by ATP, ADP and adenosine could be involved in the regulation of the NLRP3 inflammasome (Gombault 2013). Therefore, we wanted to test whether ADP or adenosine could activate the NLRP3 inflammasome in LPS-primed microglia. We failed to observe any IL-1β secretion even after stimulation with 1mM adenosine. These results confirmed a recent publication demonstrating that only millimolar concentrations of adenosine (> 3 mM) activate the NLRP3 inflammasome (Baron *et al*, 2015). On another hand, we were surprised to detect a strong ADP-induced IL-1β release, similar to the one observed after ATP treatment in LPS-primed microglia. Moreover, this ADP-triggered IL-1β release was abrogated in P2X7 receptor-deficient microglia. As ADP is not described as a ligand of P2X7 receptor, we suspected our commercial ADP sources of being contaminated by ATP. Indeed, the quantification of ATP levels in the ADP commercial batch (Sigma) clearly indicated the presence of 20% ATP. We ordered and tested ADP batches from alternative providers (Enzo Life Sciences and Jena Bioscience) which were contaminated too. Therefore, we cannot confirm or deny that ADP could be an NLRP3 activator, although this could provide new molecular insights on the mechanisms of inflammasome regulation during neurodegenerative processes.

The pesticide rotenone is known to be a causative agent of Parkinson's disease (Tanner *et al*, 2011). This compound act as a specific mitochondrial complex I inhibitor which enhances mitochondrial ROS production. Interestingly, mitochondrial defects and ROS generation have both been linked to neurodegenerative diseases (Hussain Bhat *et al*, 2015) but also to inflammasome signalling (Harijith *et al*, 2014). Therefore, we decided to study if rotenone could be a possible microglial NLRP3 activator. In the literature, rotenone has been described as a putative inducer of NLRP3 activation and IL-1β release in macrophages (Nakahira *et al*, 2011; Zhou *et al*, 2011). Accordingly, microglia exposed to rotenone released IL-1β in an NLRP3 inflammasome-dependent manner. Our observation confirmed a recent report demonstrating that BV2 cells respond to rotenone by activating caspase-1 and releasing IL-1β (Liang *et al*, 2015). However, the same authors also report that the antioxidant NAC could modulate this response, an observation that we were unable to reproduce. The causes of these discrepancies could be diverse: the different treatment durations, the dissimilar concentrations used for both rotenone and NAC, and also the different cell models. Indeed, they used a rat cell line instead of mouse primary cultures and the redox status of cell lines and primary cells could differ (Rubartelli

et al, 2011). Thus, our findings suggest that rotenone could activate the inflammasome in a ROS-independent way in primary microglia. As this result was rather unexpected, additional investigations are required to determine whether rotenone could activate the NLRP3 trough such an alternative activation mechanism.

Taken together, our *in vitro* results suggest that different hallmarks of neurodegenerative diseases could indeed participate in the activation of the NLRP3 inflammasome and lead to the subsequent secretion of IL-1β, a cytokine which can be observed in the brain of PD patients. We hypothesize that the abnormal deposition of misfolded proteins in the brain parenchyma could act as the signal 1 by activating and priming surrounding microglia. At the same time, mitochondrial dysfunction and ATP released by injured cells might play the role of the signal 2 and thus, could induce the inflammasome assembly and downstream release of cytokines and alarmins observed in neurodegenerative processes.

Parkinson's disease: a role for NLRP3 inflammasome?

Increasing evidence implies that immune dysregulation and neuroinflammation might sustain and exacerbate the loss of the dopaminergic neurons in PD (Brochard *et al*, 2009; Perry, 2012; Rodrigues *et al*, 2014). Among the factors driving neuroinflammation, IL-1β has been highlighted for its link to PD: (i) IL-1β is elevated in the CSF (Blum-Degen *et al*, 1995; Mogi *et al*, 1996) and in the serum of PD patients (Koziorowski *et al*, 2012; Dursun *et al*, 2015). (ii) *Il1b* gene polymorphisms have been associated with age-at-onset of sporadic PD (Nishimura *et al*, 2000; McGeer *et al*, 2002; Wahner *et al*, 2007). (iii) *In vivo*, the chronic expression of IL-1β by the introduction of adenoviral vectors into the substantia nigra of rats elicited diverse characteristics of PD, including dopaminergic cell death, akinesia and glial activation (Ferrari *et al*, 2006). (iv) Blocking IL-1β signalling by administration of IL-1Ra reduces the cell loss observed in the 6-OHDA PD model (Koprich *et al*, 2008; Pott Godoy *et al*, 2008). Taken together, these findings support our hypothesis that the release of IL-1β and thus the inflammasome could be involved in dopaminergic neuronal degeneration observed in PD.

Our *in vitro* results suggest that the NLRP3 inflammasome could be activated during the pathogenesis of PD. Thus, we decided to investigate the relevance of NLRP3 signalling in an *in vivo* PD mouse model. Among the different animal models of PD, we chose the broadly used 6-OHDA injection into the mouse striatum which presents several advantages, including a robust degeneration of TH neurons within 15 days. Moreover, the

presence of IL-1β in both striatum and substantia nigra have been observed in this model (Villar-Cheda *et al*, 2012; Machado-Filho *et al*, 2014; Munoz *et al*, 2014; Nadella *et al*, 2014).

Therefore, we analysed the PD-related features induced by the administration of 6-OHDA in wild-type compared to NLRP3 knockout mice. Our preliminary results suggest that the genetic ablation of NLRP3 did not exert any significant impact on the dopaminergic neuron degenerescence after the intra-striatal injection of 6-OHDA. Indeed, the absence of NLRP3 did not protect TH neurons from cell death. Moreover, the expression of CD68 was similar in wild-type and in NLRP3 KO mice, suggesting no modification in terms of microgliosis. Interestingly, a recent report shows that NLRP3 knockout mice appear less susceptible to MPTP-induced dopaminergic neuronal death than wild-type mice (Yan et al, 2015). This dissimilar result could be explained by two factors: the different PD model used (6-OHDA *vs* MPTP) as well as the time points at which the degeneration was evaluated. In fact, Yan et al quantified the TH neuron loss only 24 h after MPTP injection while we decided to perform our analysis 14 days after the injection, which could be considered as an endpoint measurement. Thus, we cannot exclude that the loss of NLRP3 might transiently delay the degeneration of dopamine neurons.

In order to explore this hypothesis, we aimed to determine the time frame during which IL-1β is present in the intra-striatal 6-OHDA model. For this purpose, we carried out a kinetic transcriptional analysis of the pro-inflammatory factors expressed after the 6-OHDA injection.

Our results showed that the expression of *Il1b* and other neuroinflammation-related genes were not significantly upregulated in the ipsilateral hemisphere compared to the contralateral control, at any tested time point (3, 7 or 14 days). While it might be possible than the pro-inflammatory gene expression was really not altered after the injection, we believe it is unlikely. Indeed, our immunohistochemistry analyses clearly showed a strong inflammation, at least due to the stab wound injury. This last observation supported by the bibliographic context argues that a technical failure prevented us from detecting the transcriptional overexpression of the pro-inflammatory genes. Thus, additional experiments are still required to determine the time frame of IL-1β expression and determine whether NLRP3 deficiency could delay the dopaminergic neuron degenerescence.

VI.
General conclusion & Perspectives

In the present study, we outlined the expression and regulation of the inflammasome in glial cells, emphasizing its role in neurodegenerative diseases.

The following points summarise our results:

- Astrocytes constitutively expressed IL-18 and HMGB1. However, we were unable to observe any constitutive or induced expression of IL-1β or IL-1α.
- Astrocytes appeared unable to form a functional NLRP3 inflammasome, as they did not express NLRP3 and ASC proteins, at least in our conditions.
- On the contrary, microglia expressed a functional NLRP3 inflammasome whose activation occurred through similar mechanisms than described in macrophages.
- The exposure of microglia to ATP, Nigericin or Alum triggered IL-1β, IL-1α, IL-18 and HMGB1 release in an NLRP3 inflammasome-dependent way.
- The PD relevant α-synuclein did not activate the microglial NLRP3 inflammasome but was able to prime it.
- We confirmed $A\beta_{25-35}$,but not $A\beta_{1-42}$, as a microglial inflammasome activator
- Rotenone and ATP induced microglial inflammasome activation.
- Genetic ablation of NLRP3 did not exert any impact on late stages of neurodegenerescence induced by intra-striatal injection of 6-OHDA.

A summarized view of these findings is depicted in Figure 46.

In conclusion, the experiments presented in this doctoral thesis expand our current knowledge of the expression and regulation of inflammasome components in the mouse CNS. This project also contributes to the current effort to determine whether NLRP3 is involved in neurodegenerative diseases and in particular in Parkinson's disease.

However, further experiments are required to complete this work. These include the characterization of AIM2, NLRC4 and non-canonical inflammasomes in microglia but also the clarification of how the purinergic signalling could activate the inflammasome in microglia.

In addition, our results suggest that microglia is the main glial cell type in the brain responsible for IL-1β and IL-18 secretion. However, it is essential to confirm that our observation remains true *in situ* with regard to the intercellular network and specific pathologic conditions.

Furthermore, we also have to pursue studies in view to better respond to the question: Is the NLRP3 inflammasome implicated in Parkinson's disease?

Our *in vivo* study showed that the deficiency of NLRP3 appeared not able to protect, at least permanently, dopamine neurons from the 6-OHDA-induced cell death. However,

in light to the recent report of Yan and colleagues showing that the loss of NLRP3 is neuroprotective at early time points in the MPTP model, we are now planning to investigate whether such an early difference might occur in the 6-OHDA model and thus, whether the loss of NLRP3 might provide a transient neuroprotection.

In addition, we showed in this study that α-synuclein, another major hallmark of PD, was at least able to prime the inflammasome in microglial cultures. It would be interesting to strengthen this observation using an *in vivo* mouse model and in particular to test whether the loss of NLRP3 is beneficial in a transgenic mice model overexpressing α-synuclein.

All these investigations could enlighten a potential therapeutic interest of the NLRP3 inflammasome for Parkinson's disease.

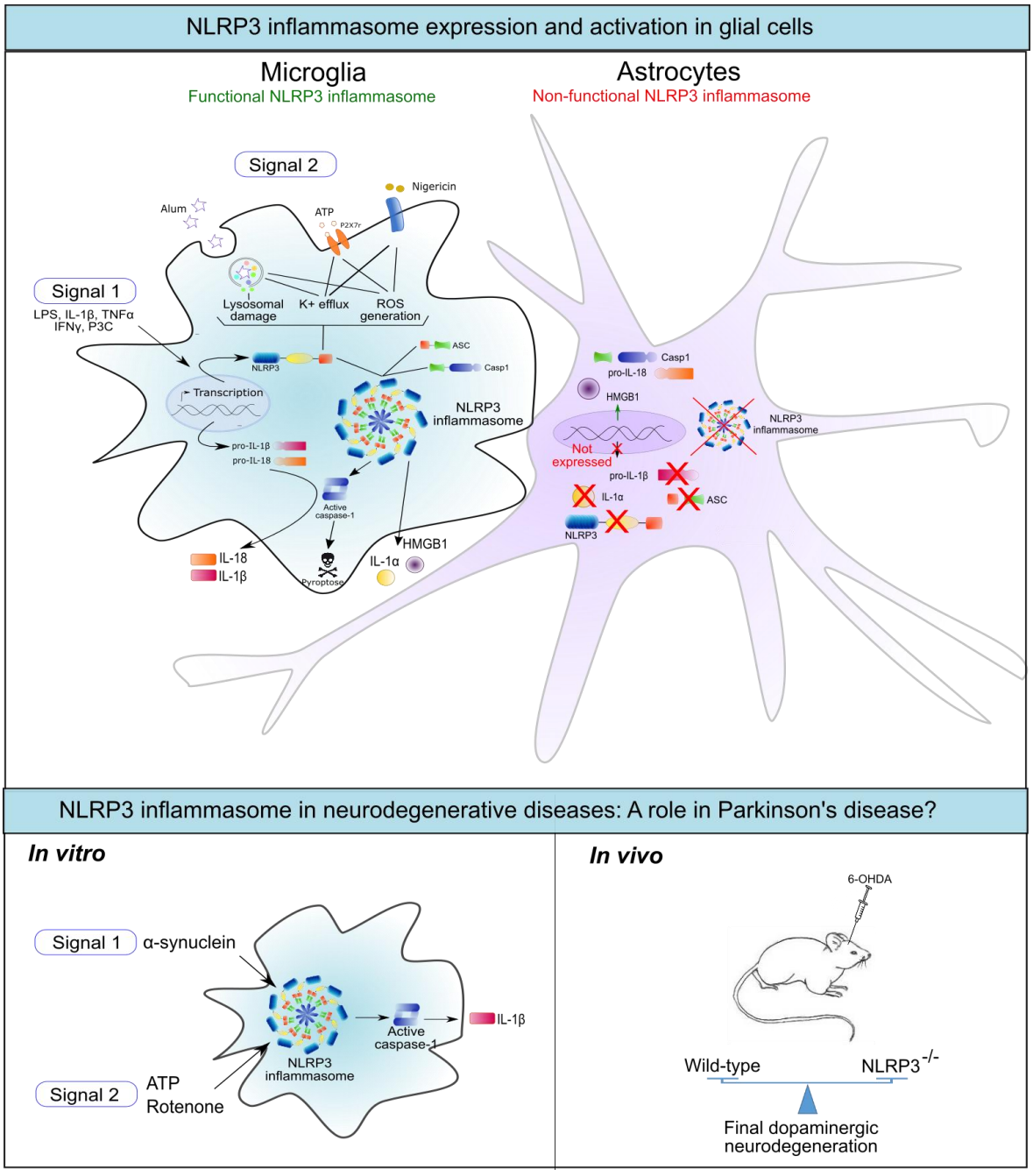


Fig. 46: Summary of our findings.

The capacity to form a functional NLRP3 inflammasome and secretion of IL-1β is limited to the microglial compartment in the mouse brain. Indeed, we were not able to detect the expression neither of IL-1β nor of all NLRP3 inflammasome components. In addition to IL-1β, microglia were able to secrete IL-18, IL-1α and HMGB1 in an NLRP3 inflammasome-dependent way and through mechanisms similar to those observed in macrophages. Moreover, our results suggest that microglial inflammasome can play a role in the neuroinflammation observed during Parkinson's disease. Indeed, microglia can be primed by α-synuclein exposure and microglial stimulation with Parkinson's disease-related products, such as rotenone or ATP, which results in the activation of the NLRP3 inflammasome and IL-1β release. However, our preliminary *in vivo* results tend to indicate that genetic ablation of NLRP3 did not exert any significant impact on neurodegenerative processes occurring in an *in vivo* model of Parkinson's disease s.

VII. References

Abbott NJ, Rönnbäck L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* **7**: 41–53, doi:10.1038/nrn1824.

Abdullah R, Basak I, Patil KS, Alves G, Larsen JP, Moller SG (2015) Parkinson's disease and age: the obvious but largely unexplored link. *Exp Gerontol* **68**: 33–38, doi:10.1016/j.exger.2014.09.014.

Abulafia DP, de Rivero Vaccari JP, Lozano JD, Lotocki G, Keane RW, Dietrich WD (2009) Inhibition of the inflammasome complex reduces the inflammatory response after thromboembolic stroke in mice. *J Cereb Blood Flow Metab* **29**: 534–544, doi:10.1038/jcbfm.2008.143.

Adamczak S, Dale G, de Rivero Vaccari JP, Bullock MR, Dietrich WD, Keane RW (2012) Inflammasome proteins in cerebrospinal fluid of brain-injured patients as biomarkers of functional outcome. *J Neurosurg* 1–7, doi:10.3171/2012.9.JNS12815.

Adamczak SE, De Rivero Vaccari JP, Dale G, Brand Iii FJ, Nonner D, Bullock MR, Dahl GP, Dietrich D, Keane RW (2014) Pyroptotic neuronal cell death mediated by the AIM2 inflammasome. *J Cereb Blood Flow Metab* **34236**: 621–629, doi:10.1038/jcbfm.2013.236.

Ahn H, Kim J, Jeung E-B, Lee G-S (2013) Dimethyl sulfoxide inhibits NLRP3 inflammasome activation. *Immunobiology* 1–8, doi:10.1016/j.imbio.2013.11.003.

Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T, Takakura-Yamamoto R, Torigoe K, Gu Y, Su MS-S, Fujii M, Satoh-Itoh M, Yamamoto K, Kohno K, Ikeda M, Kurimoto M (1997) Involvement of Caspase-1 and Caspase-3 in the Production and Processing of Mature Human Interleukin 18 in Monocytic THP.1 Cells. *J Biol Chem* **272**: 26595–26603, doi:10.1074/jbc.272.42.26595.

Alboni S, Cervia D, Sugama S, Conti B (2010) Interleukin 18 in the CNS. *J Neuroinflammation* **7**: 9, doi:10.1186/1742-2094-7-9.

Alfonso-Loeches S, Ureña-Peralta JR, Morillo-Bargues MJ, Oliver-De La Cruz J, Guerri C (2014) Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. *Front Cell Neurosci* **8**: 216, doi:10.3389/fncel.2014.00216.

Allan SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. *Nat Rev Immunol* **5**: 629–640, doi:10.1038/nri1664.

Allen IC, Scull M a, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, Guthrie EH, Pickles RJ, Ting JP-Y (2009) The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* **30**: 556–565, doi:10.1016/j.immuni.2009.02.005.

Allen IC, Wilson JE, Schneider M, Lich JD, Roberts R a, Arthur JC, Woodford R-MT, Davis BK, Uronis JM, Herfarth HH, Jobin C, Rogers AB, Ting JP-Y (2012) NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. *Immunity* **36**: 742–754, doi:10.1016/j.immuni.2012.03.012.

Allen NJ (2014) Astrocyte Regulation of Synaptic Behavior. *Annu Rev Cell Dev Biol* **30**: 439–463, doi:10.1146/annurev-cellbio-100913-013053.

Amer A, Franchi L, Kanneganti T-D, Body-Malapel M, Ozören N, Brady G, Meshinchi S, Jagirdar R, Gewirtz A, Akira S, Núñez G (2006) Regulation of Legionella phagosome maturation and infection through flagellin and host Ipaf. *J Biol Chem* **281**: 35217–35223, doi:10.1074/jbc.M604933200.

Andersson U, Tracey KJ (2011) HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu Rev Immunol* **29**: 139–162, doi:10.1146/annurev-immunol-030409-101323.

Andre R, Wheeler RD, Collins PD, Luheshi GN, Pickering-Brown S, Kimber I, Rothwell NJ, Pinteaux E (2003) Identification of a truncated IL-18Rβ mRNA: a putative regulator of IL-18 expressed in rat brain. *J Neuroimmunol* **145**: 40–45, doi:10.1016/j.jneuroim.2003.09.005.

Antonioli L, Pacher P, Vizi ES, Haskó G (2013) CD39 and CD73 in immunity and inflammation. *Trends Mol Med* **19**: 355–367, doi:10.1016/j.molmed.2013.03.005.

Banerjee S, Bond JS (2008) Prointerleukin-18 is activated by meprin beta in vitro and in vivo in intestinal inflammation. *J Biol Chem* **283**: 31371–31377, doi:10.1074/jbc.M802814200.

Banerjee S, Jin G, Bradley SG, Matters GL, Gailey RD, Crisman JM, Bond JS (2011) Balance of meprin A and B in mice affects the progression of experimental inflammatory bowel disease. **300**: 273–282, doi:10.1152/ajpgi.00504.2009.

Baron L, Gombault A, Fanny M, Villeret B, Savigny F, Guillou N, Panek C, Le Bert M, Lagente F, Rassendren F, Riteau N, Couillin I (2015) The NLRP3 inflammasome is activated by nanoparticles trough ATP, ADP and Adenosine. *Cell Death Dis* **6**: doi:10.1038/cddis.2014.576.

Bauernfeind F, Horvath G, Stutz A, Alnemri ES, Speert D, Fernandes-alnemri T, Wu J, G B, Fitzgerald KA, Hornung V, Latz E (2010) Nf-kB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol* **183**: 787–791, doi:10.4049/jimmunol.0901363.

Bauernfeind FG, Bartok E, Rieger A, Franchi L, Nunez G, Hornung V (2011) Cutting Edge: Reactive Oxygen Species Inhibitors Block Priming, but Not Activation, of the NLRP3 Inflammasome. *J Immunol* **187**: 613–617, doi:10.4049/jimmunol.1100613.

Bayraktar OA, Fuentealba LC, Alvarez-Buylla A, Rowitch DH (2015) Astrocytes Development and Heterogeneity. *Cold Spring Harb Perspect Biol* **7**: doi:10.1101/cshperspect.a020362 Cite.

Bendor J, Logan T, Edwards R (2013) The function of α-synuclein. *Neuron* **79**: 1044–1066, doi:10.1016/j.neuron.2013.09.004.

Béraud D, Hathaway H a, Trecki J, Chasovskikh S, Johnson D a, Johnson J a, Federoff HJ, Shimoji M, Mhyre TR, Maguire-Zeiss K a (2013) Microglial activation and antioxidant responses induced by the Parkinson's disease protein α-synuclein. *J Neuroimmune Pharmacol* **8**: 94–117, doi:10.1007/s11481-012-9401-0.

Bergsbaken T, Cookson BT (2007) Macrophage activation redirects yersinia-infected host cell death from apoptosis to caspase-1-dependent pyroptosis. *PLoS Pathog* **3**: e161, doi:10.1371/journal.ppat.0030161.

Bianchi ME, Manfredi AA (2009) Dangers In and Out. *Science (80-)* **323**: 1683–1684, doi:10.1126/science.1172794.

Biber K, Neumann H, Inoue K, Boddeke HWGM (2007) Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci* **30**: 596–602, doi:10.1016/j.tins.2007.08.007.

Blasi E, Barluzzi R, Bocchini V, Mazolla R, Bistoni F (1990) Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. *J Neuroimmunol* **27.**:

Blum-Degen D, Muller T, Khun W, Gerlach M, Przuntek H, Riederer Pe (1995) Interleukin-1b and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neurosci Lett* **202**: 17–20.

Booker CS, Grattan DR (2014) Identification of a truncated splice variant of IL-18 receptor alpha in the human and rat, with evidence of wider evolutionary conservation. *PeerJ* **2**: doi:10.7717/peerj.560.

Bossaller L, Chiang P-I, Schmidt-Lauber C, Ganesan S, Kaiser WJ, Rathinam V a K, Mocarski ES, Subramanian D, Green DR, Silverman N, Fitzgerald K a, Marshak-Rothstein A, Latz E (2012) Cutting edge: FAS (CD95) mediates noncanonical IL-1β and IL-18 maturation via caspase-8 in an RIP3-independent manner. *J Immunol* **189**: 5508–5512, doi:10.4049/jimmunol.1202121.

Boutin H, LeFeuvre R a, Horai R, Asano M, Iwakura Y, Rothwell NJ (2001) Role of IL-1alpha and IL-1beta in ischemic brain damage. *J Neurosci* **21**: 5528–5534, doi:21/15/5528 [pii].

Boyden ED, Dietrich WF (2006) Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat Genet* **38**: 240–244, doi:10.1038/ng1724.

Bribian A, Figueres-Onate M, Martin-Lopez E, Mascaraque-Lopez L (2015) Decoding astrocyte heterogeneity: new tools for clonal analysis. *Neuroscience* doi:10.1016/j.neuroscience.2015.04.036.

Briers TW, Desmaretz C, Vanmechelen E (1994) Generation and characterization of mouse microglial cell lines. *J Neuroimmunol* **52**: 153–164.

Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-berthat V, Bonduelle O, Alvarez-fischer D, Callebert J, Launay J, Duyckaerts C, Flavell RA, Hirsch EC, Hunot S (2009) Infiltration of CD4 + lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest* **119**: doi:10.1172/JCI36470.

Broz P, von Moltke J, Jones JW, Vance RE, Monack DM (2010) Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* **8**: 471–483, doi:10.1016/j.chom.2010.11.007.

Broz P, Monack DM (2013) Noncanonical inflammasomes: caspase-11 activation and effector mechanisms. *PLoS Pathog* **9**: e1003144, doi:10.1371/journal.ppat.1003144.

Bruey JM, Bruey-Sedano N, Newman R, Chandler S, Stehlik C, Reed JC (2004) PAN1/NALP2/PYPAF2, an inducible inflammatory mediator that regulates NF-kappaB and caspase-1 activation in macrophages. *J Biol Chem* **279**: 51897–51907, doi:10.1074/jbc.M406741200.

Bruggen R Van, Ko MY, Jansen M, Houdt M Van, Roos D, Kuijpers TW (2010) Human NLRP3 inflammasome activation is Nox1-4 independent. *Blood* **115**: 5398–5401, doi:10.1182/blood-2009-10-250803.

Brunette RL, Young JM, Whitley DG, Brodsky IE, Malik HS, Stetson DB (2012) Extensive evolutionary and functional diversity among mammalian AIM2-like receptors. *J Exp Med* **209**: 1969–1983, doi:10.1084/jem.20121960.

Burda JE, Sofroniew M V. (2014) Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron* **81**: 229–284, doi:10.1016/j.neuron.2013.12.034.

Burguillos M a, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL, Joseph B (2011) Caspase signalling controls microglia activation and neurotoxicity. *Nature* **472**: 319–324, doi:10.1038/nature09788.

Burm SM, Zuiderwijk-Sick EA, Jong AEJ, Van Der Putten C, Veth J, Kondova I, Bajramovic JJ (2015) Inflammasome-induced IL-1b secretion in microglia is characterized by delayed kinetics and is only

partially dependent on inflammatory caspases. *J Neurosci* **35**: 678–687, doi:10.1523/JNEUROSCI.2510-14.2015.

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, Fanek Z, Liu L, Chen Z, Rothstein JD, Ransohoff RM, Gygi SP, Antel JP, Weiner HL (2014) Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat Neurosci* **17**: 131–143, doi:10.1038/nn.3599.

Caldero J, Brunet N, Ciutat D, Hereu M, E. EJ (2009) Development of microglia in the chick embryo spinal cord: implications in the regulation of motoneuronal survival and death. *J Neurosci Res* **87**: 2447–2466, doi:10.1002/jnr.22084.

Calero M, Gómez-Ramos A, Calero O, Soriano E, Avila J, Medina M (2015) Additional mechanisms conferring genetic susceptibility to Alzheimer's disease. *Front Cell Neurosci* **9**: 138, doi:10.3389/fncel.2015.00138.

Case CL (2011) Regulating Caspase-1 During Infection: Roles of NLRs, AIM2, and ASC. *Yale J Biol Med* **84**: 333–343.

Chang Y-P, Ka S-M, Hsu W-H, Chen A, Chao LK, Lin C-C, Hsieh C-C, Chen M-C, Chiu H-W, Ho C-L, Chiu Y-C, Liu M-L, Hua K-F (2014) Resveratrol inhibits NLRP3 inflammasome activation by preserving mitochondrial integrity and augmenting autophagy. *J Cell Physiol* **230**: 1567–1579, doi:10.1002/jcp.24903.

Chen C-J, Kono H, Golenbock D, Reed G, Akira S, Rock KL (2007) Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med* **13**: 851–856, doi:10.1038/nm1603.

Chen H, Zhao EJ, Zhang W, Lu Y, Liu R, Huang X, Ciesielski-Jones A, Justice MA, Cousins DS, Peddada S (2015) Meta-analyses on prevalence of selected Parkinson's non-motor symptoms before and after diagnosis. *Transl Neurodegener* **4**: doi:10.1186/2047-9158-4-1.

Chiu IM, Morimoto ETA, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, Muratet M, Carroll MC, Levy S, Tavazoie S, Myers RM, Maniatis T (2013) A neurodegeneration-specific gene expression signature and immune profile of acutely isolated microglia from ALS mouse model. *cell Rep* **25**: 385–401, doi:10.1016/j.celrep.2013.06.018.

Chu J, Thomas LM, Watkins SC, Franchi L, Núñez G, Salter RD (2009) Cholesterol-dependent cytolysins induce rapid release of mature IL-1beta from murine macrophages in a NLRP3 inflammasome and cathepsin B-dependent manner. *J Leukoc Biol* **86**: 1227–1238, doi:10.1189/jlb.0309164.

Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, de Bernard M (2013) Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. *PLoS One* **8**: e55375, doi:10.1371/journal.pone.0055375.

Coeshott C, Ohnemus C, Pilyavskaya a, Ross S, Wieczorek M, Kroona H, Leimer a H, Cheronis J (1999) Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc Natl Acad Sci U S A* **96**: 6261–6266, doi:10.1073/pnas.96.11.6261.

Coll RC, O'Neill L a J (2011) The cytokine release inhibitory drug CRID3 targets ASC oligomerisation in the NLRP3 and AIM2 inflammasomes. *PLoS One* **6**: doi:10.1371/journal.pone.0029539.

Coll RC, Robertson A a B, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, Vetter I, Dungan LS, Monks BG, Stutz A, Croker DE, Butler MS, Haneklaus M, Sutton CE, Nunez G, Latz E, Kastner DL, Mills KHG, Masters SL, Schroder K, Cooper M a, O'Neill L a J (2015) A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med* **21**: 248–255, doi:10.1038/nm.3806.

Compan V, Baroja-Mazo A, López-Castejón G, Gomez AI, Martínez CM, Angosto D, Montero MT, Herranz AS, Bazán E, Reimers D, Mulero V, Pelegrín P (2012) Cell volume regulation modulates NLRP3 inflammasome activation. *Immunity* **37**: 487–500, doi:10.1016/j.immuni.2012.06.013.

Conway K a., Harper JD, Lansbury PT (2000) Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* **39**: 2552–2563, doi:10.1021/bi991447r.

Cox DJ, Field RH, Williams DG, Baran M, Bowie AG, Cunningham C, Dunne A (2015) DNA sensors are expressed in astrocytes and microglia in vitro and are upregulated during gliosis in neurodegenerative disease. *Glia* **63**: 812–825, doi:10.1002/glia.22786 Published.

Crocker SJ, Frausto RF, Whitton JL, Milner R (2008) A novel method to establish microglia-free astrocyte cultures: Comparison of matrix metalloproteinase expression profiles in pure cultures of astrocytes and microglia. *Glia* **56**: 1187–1198, doi:10.1002/glia.20689.

Cruz CM, Rinna A, Forman HJ, Ventura ALM, Persechini PM, Ojcius DM (2007) ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J Biol Chem* **282**: 2871–2879, doi:10.1074/jbc.M608083200.

Dahlstrand J, Lardelli M, Lendahl U (1995) Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. *Dev Brain Res* **84**: 109–129, doi:10.1016/0165-3806(94)00162-S.

Denoble AE, Huffman KM, Stabler T V., Kelly SJ, S. HM, McDaniel GE, Coleman RE, Kraus VB (2011) Uric acid is a danger signal of incresaing risk for osteoarthritis through inflammasome activation. *PNAS* **105**: 2088–2093, doi:10.1073/pnas.1012743108.

Dinarello C a (2011) Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* **117**: 3720–3732, doi:10.1182/blood-2010-07-273417.

Dinarello C a, Fantuzzi G (2003) Interleukin-18 and host defense against infection. *J Infect Dis* **187 Suppl** : S370–S384, doi:10.1086/374751.

Dinarello C a, Simon A, van der Meer JWM (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* **11**: 633–652, doi:10.1038/nrd3800.

Dinarello CA (1999) IL-18 : A TH1-inducing , proinflammatory cytokine and new member of the IL-1 family. *Mol Mech allergy Clin Immunol* **103**: 11–24.

Dinarello CA, van der Meer JWM (2013) Treating inflammation by blocking interleukin-1 in humans. *Semin Immunol* **25**: 469–484, doi:10.1016/j.smim.2013.10.008.

Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. *Nature* **11**: 98–107, doi:10.1038/nri2925 Published.

Dong Y, Benveniste EN (2001) Immune Function of Astrocytes. **190**: 180–190, doi:10.1002/glia.1107.

Dooley CT, Li L, Misler J a., Thompson JH (2012) Toxicity of 6-hydroxydopamine: Live cell imaging of cytoplasmic redox flux. *Cell Biol Toxicol* **28**: 89–101, doi:10.1007/s10565-011-9209-3.

Dostert C, Guarda G, Romero JF, Menu P, Gross O, Tardivel A, Suva M-L, Stehle J-C, Kopf M, Stamenkovic I, Corradin G, Tschopp J (2009) Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS One* **4**: e6510, doi:10.1371/journal.pone.0006510.

Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* **320**: 674–677, doi:10.1126/science.1156995.

Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nuñez G, Schnurr M, Espevik T, Lien E, Fitzgerald K a, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E (2010) NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* **464**: 1357–1361, doi:10.1038/nature09316.

Duncan J a, Gao X, Huang MT-H, O'Connor BP, Thomas CE, Willingham SB, Bergstralh DT, Jarvis G a, Sparling PF, Ting JP-Y (2009) Neisseria gonorrhoeae activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J Immunol* **182**: 6460–6469, doi:10.4049/jimmunol.0802696.

Dursun E, Gezen-Ak D, Hanagasi H, Bilgic B, Lohmann E, Ertan S, Atasoy IL, Alaylioglu M, Selin Araz O, Onal B, Gunduz A, Apaydin H, Kiziltan G, Ulutin T, Gurvit H, Yilmazer S (2015) The interleukin-1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *J Neuroimmunol* **383**: 50–57, doi:10.1016/jneuroim.2015.04.014.

Eigenbrod T, Park J-H, Harder J, Iwakura Y, Nunez G (2008) Critical role for mesothelial cells in necrosis-induced inflammation trough the recognition of IL-1a released from dying cells. *J Immunol* **181**: 8194–8198, doi:10.4049/jimmunol.181.12.8194.

Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell R a (2008) Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* **453**: 1122–1126, doi:10.1038/nature06939.

Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss C a, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell R a (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* **145**: 745–757, doi:10.1016/j.cell.2011.04.022.

Elmore MMP, Najafi AR, Koike NN, Dagher NN, Spangenberg EE, Rice RA, Kitazawa M, Matusow B, Nguyen H, West BL, Green KN (2014) Colony-Stimulating Factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron* **82**: 380–397, doi:10.1016/j.neuro.2014.02.040.

Facci L, Barbierato M, Marinelli C, Argentini C, Skaper SD, Giusti P (2014) Toll-like receptors 2, -3 and -4 prime microglia but not astrocytes across central nervous system regions for ATP-dependent interleukin-1β release. *Sci Rep* **4**: 6824, doi:10.1038/srep06824.

Fang P, Schachner M, Shen Y-Q (2012) HMGB1 in development and diseases of the central nervous system. *Mol Neurobiol* **45**: 499–506, doi:10.1007/s12035-012-8264-y.

Fann DY-W, Lee SY, Manzanero S, Tang SC, Gelderblom M, Chunduri P, Bernreuther C, Glatzel M, Cheng YL, Thundyil J, Widiapradja a, Lok KZ, Foo SL, Wang YC, Li YI, Drummond GR, Basta M, Magnus T, Jo DG, Mattson MP, Sobey CG, Arumugam T V (2013) Intravenous immunoglobulin suppresses NLRP1 and NLRP3 inflammasome-mediated neuronal death in ischemic stroke. *Cell Death Dis* **4**: e790, doi:10.1038/cddis.2013.326.

Fantuzzi G, George K, Harding MW, Livingston DJ, Sipe JD (1997) Response to local Inflammation of IL-1beta-Converting Enzyme-Deficient Mice'. *J Immunol* **158**: 1818–1824.

Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. *Trends Immunol* **28**: 138–145, doi:10.1016/j.it.2007.01.005.

Farrer M, Wavrant-Devrieze F, Crook R, Boles L, Perez-Tur J, Hardy J, Johnson WG, Steele J, Maraganore D, Gwinn K, Lynch T (1998) Low frequency of alpha-synuclein mutations in familial Parkinson's disease. *Ann Neurol* **43**: 394–397, doi:10.1002/ana.410430320.

Faustin B, Lartigue L, Bruey J-M, Luciano F, Sergienko E, Bailly-Maitre B, Volkmann N, Hanein D, Rouiller I, Reed JC (2007) Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol Cell* **25**: 713–724, doi:10.1016/j.molcel.2007.01.032.

Felderhoff-Mueser U, Schmidt OI, Oberholzer A, Bührer C, Stahel PF (2005) IL-18: a key player in neuroinflammation and neurodegeneration? *Trends Neurosci* **28**: 487–493, doi:10.1016/j.tins.2005.06.008.

Feldman N, Rotter-Maskowitz A, Okun E (2015) DAMPs as mediators of sterile inflammation in aging-related pathologies. *Ageing Res Rev* doi:10.1016/j.arr.2015.01.003.

Felten DL (2006) Atlas De Neurosciences Humaines De Netter.

Fernandes-Alnemri T, Yu J-W, Datta P, Wu J, Alnemri ES (2009) AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* **458**: 509–513, doi:10.1038/nature07710.

Fernandes-Alnemri T, Yu J-W, Juliana C, Solorzano L, Kang S, Wu J, Datta P, McCormick M, Huang L, McDermott E, Eisenlohr L, Landel CP, Alnemri ES (2010) The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. *Nat Immunol* **11**: 385–393, doi:10.1038/ni.1859.

Ferrari CC, Pott Godoy MC, Tarelli R, Chertoff M, Depino AM, Pitossi FJ (2006) Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1beta in the substantia nigra. *Neurobiol Dis* **24**: 183–193, doi:10.1016/j.nbd.2006.06.013.

Fettelschoss A, Kistowska M, Leibundgut-Landmann S, Beer H-D, Johansen P, Senti G, Contassot E, Bachmann MF, French LE, Oxenius A, Kündig TM (2011) Inflammasome activation and IL-1{beta} target IL-1{alpha} for secretion as opposed to surface expression. *Proc Natl Acad Sci U S A* **108**: 18055–18060, doi:10.1073/pnas.1109176108.

Fink SL, Bergsbaken T, Cookson BT (2008) Anthrax lethal toxin and Salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms.

Fink SL, Cookson BT (2006) Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell Microbiol* **8**: 1812–1825, doi:10.1111/j.1462-5822.2006.00751.x.

Fiszer D, Rozwadowska N, Rychlewski L, Kosicki W, Kurpisz M (2007) Identification of IL-18RAP mRNA truncated splice variants in human testis and the other human tissues. *Cytokine* **39**: 178–183, doi:10.1016/j.cyto.2007.07.186.

Fontana A, Weber E, GRob P, Lim R, Miller J (1983) Dual effect of glia maturation factor on astrocytes. Differentiation and release of interleukin-1 like factors. *J Neuroimmunol* **5**: 261–269.

Franchi L, Eigenbrod T, Núñez G (2009) Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. *J Immunol* **183**: 792–796, doi:10.4049/jimmunol.0900173.

Franchi L, Muñoz-Planillo R, Núñez G (2012) Sensing and reacting to microbes through the inflammasomes. *Nat Immunol* **13**: 325–332, doi:10.1038/ni.2231.

Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, Marvin S, Burrage AM, Sudholt S, Rana A, O'Connor C, Wiethoff CM, Campbell EM (2013) Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. *PLoS One* **8**: e62143, doi:10.1371/journal.pone.0062143.

Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T (2002) A-synuclein is phosphorylated in synucleinopathy lesions. *Nat cell Biol* **4**: doi:10.1038/ncb748.

Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny M V, Dawson TM, Dawson VL, El-Deiry WS, Fulda S, Gottlieb E, Green DR, Hengartner MO, Kepp O, Knight R a, Kumar S, Lipton S a, Lu X, Madeo F, Malorni W, Mehlen P, Nuñez G, Peter ME, Piacentini M, Rubinsztein DC, Shi Y, Simon H-U, Vandenabeele P, White E, Yuan J, Zhivotovsky B, Melino G, Kroemer G (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ* **19**: 107–120, doi:10.1038/cdd.2011.96.

Gardella S, Andrei C, Ferrera D, Lotti L V, Torrisi MR, Bianchi ME, Rubartelli A (2002) The nuclear protein HMGB1 is secreted by secretory pathway. *EMBO Rep* **3**: 995–1001.

Garlanda C, Dinarello C a, Mantovani A (2013) The interleukin-1 family: back to the future. *Immunity* **39**: 1003–1018, doi:10.1016/j.immuni.2013.11.010.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**: 841–845, doi:10.1126/science.1194637.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. *Front Cell Neurosci* **7**: doi:10.3389/fncel.2013.00045.

Gomez-Nicola D, Perry VH (2015) Microglial Dynamics and Role in the Healty and Diseased Brain: A Paradigm of Functional Plasticity. *Neuroscientist* **21**: 169–184, doi:10.1177/1073858414530512.

Götz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* **6**: 777–788, doi:10.1038/nrm1739.

Grandbarbe L, Bouissac J, Rand M, Hrabé de Angelis M, Artavanis-Tsakonas S, Mohier E (2003) Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. *Development* **130**: 1391–1402, doi:10.1242/dev.00374.

Grenier JM, Wang L, Manji G a., Huang WJ, Al-Garawi A, Kelly R, Carlson A, Merriam S, Lora JM, Briskin M, DiStefano PS, Bertin J (2002) Functional screening of five PYPAF family members identifies PYPAF5 as a novel regulator of NF-kB and caspase-1. *FEBS Lett* **530**: 73–78, doi:10.1016/S0014-5793(02)03416-6.

Gringhuis SI, Kaptein TM, Wevers B a, Theelen B, van der Vlist M, Boekhout T, Geijtenbeek TBH (2012) Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. *Nat Immunol* **13**: 246–254, doi:10.1038/ni.2222.

Gris D, Ye Z, Iocca H a, Wen H, Craven RR, Gris P, Huang M, Schneider M, Miller SD, Ting JP-Y (2010) NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. *J Immunol* **185**: 974–981, doi:10.4049/jimmunol.0904145.

Gross O, Poeck H, Bscheider M, Dostert C, Hannesschläger N, Endres S, Hartmann G, Tardivel A, Schweighoffer E, Tybulewicz V, Mocsai A, Tschopp J, Ruland J (2009) Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* **459**: 433–436, doi:10.1038/nature07965.

Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, Guarda G, Quadroni M, Drexler SK, Tschopp J (2012) Inflammasome activators induce interleukin-1α secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* **36**: 388–400, doi:10.1016/j.immuni.2012.01.018.

Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, Farlik M, Decker T, Du Pasquier R a, Romero P, Tschopp J (2011) Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity* **34**: 213–223, doi:10.1016/j.immuni.2011.02.006.

Guarda G, Dostert C, Staehli F, Cabalzar K, Castillo R, Tardivel A, Schneider P, Tschopp J (2009) T cells dampen innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes. *Nature* **460**: 269–273, doi:10.1038/nature08100.

Guicciardi ME, Leist M, Gores GJ (2004) Lysosomes in cell death. *Oncogene* **23**: 2881–2890, doi:10.1038/sj.onc.1207512.

Guma M, Ronacher L, Liu-Bryan R, Takai S, Karin M, Corr M (2009) Caspase-1 independent IL-1b activation in neutrophil dependent inflammation. *Arthritis Rheum* **60**: 3642–3650, doi:10.1002/art.24959.Caspase-1.

Gurung P, Lukens JR, Kanneganti T-D (2014) Mitochondria: diversity in the regulation of the NLRP3 inflammasome. *Trends Mol Med* 1–9, doi:10.1016/j.molmed.2014.11.008.

Haass C, Kaether C, Gopal T, Sisodia S (2012) Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med* **2**: doi:10.1101/cshperspect.a006270.

Hachiya a., Kobayashi T, Takema Y, Imokawa G (2001) Biochemical Characterization of Endothelin-converting Enzyme-1 in Cultured Skin-derived Cells and Its Postulated Role in the Stimulation of Melanogenesis in Human Epidermis. *J Biol Chem* **277**: 5395–5403, doi:10.1074/jbc.M105874200.

Hafner-Bratkovič I, Benčina M, Fitzgerald K a, Golenbock D, Jerala R (2012) NLRP3 inflammasome activation in macrophage cell lines by prion protein fibrils as the source of IL-1β and neuronal toxicity. *Cell Mol Life Sci* **69**: 4215–4228, doi:10.1007/s00018-012-1140-0.

Hagar JA, Powell DA, Aachoui Y, Ernst RK, Miao EA (2013) Cytoplasmic LPS Activates Caspase-11: Implications in TLR4-Independent Endotoxic Shock. *Science (80-)* **341**: 1250–1253, doi:10.1126/science.1240988.

Halassa MM, Fellin T, Haydon PG (2007) The tripartite synapse: roles for gliotransmission in health and disease. *Trends Mol Med* **13**: 54–63, doi:10.1016/j.molmed.2006.12.005.

Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald K a, Latz E, Moore KJ, Golenbock DT (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol* **9**: 857–865, doi:10.1038/ni.1636.

Hamby ME, Sofroniew M V (2010) Reactive astrocytes as therapeutic targets for CNS disorders. *Neurotherapeutics* **7**: 494–506, doi:10.1016/j.nurt.2010.07.003.

Hanamsagar R, Torres V, Kielian T (2011) Inflammasome activation and IL-1β/IL-18 processing are influenced by distinct pathways in microglia. *J Neurochem* **119**: 736–748, doi:10.1111/j.1471-4159.2011.07481.x.

Hanisch U-K, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* **10**: 1387–1394, doi:10.1038/nn1997.

Hannum CH, Wilcox CJ, Arend WP, Fenneke GJ, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC (1990) Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* **343**: 336–340.

Hari A, Zhang Y, Tu Z, Detampel P, Stenner M, Ganguly A, Shi Y (2014) Activation of NLRP3 inflammasome by crystalline structures via cell surface contact. *Sci Rep* **4**: 7281, doi:10.1038/srep07281.

Harijith A, Ebenezer DL, Natarajan V (2014) Reactive oxygen species at the crossroads of inflammasome and inflammation. *Front Physiol* **5**: 352, doi:10.3389/fphys.2014.00352.

He X, Mekasha S, Mavrogiorgos N, Fitzgerald K a, Lien E, Ingalls RR (2010) Inflammation and fibrosis during Chlamydia pneumoniae infection is regulated by IL-1 and the NLRP3/ASC inflammasome. *J Immunol* **184**: 5743–5754, doi:10.4049/jimmunol.0903937.

He Y, Varadarajan S, Muñoz-Planillo R, Burberry A, Nakamura Y, Nunez G (2014) 3.4-Methylenedioxy-b-nitrostyrene inhibits NLRP3 Inflammasome Activation by Blocking Assembly of the Inflammasome. *J Biol Chem* **289**: 1142–1150.

Heneka MT, Kummer MP, Latz E (2014) Innate immune activation in neurodegenerative disease. *Nat Rev Immunol* **14**: 463–477, doi:10.1038/nri3705.

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng T-C, Gelpi E, Halle A, Korte M, Latz E, Golenbock DT (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* **493**: 674–678, doi:10.1038/nature11729.

Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. *Nature* **16**: 358–372, doi:10.1038/nrn3880.

Herrero M-T, Estrada C, Maatouk L, Vyas S (2015) Inflammation in Parkinson's disease: role of glucocorticoids. *Front Neuroanat* **9**: doi:10.3389/fnana.2015.00032.

Herzog C, Haun RS, Kaushal V, Mayeux PR, Shah S V, Kaushal GP (2009) Meprin A and meprin alpha generate biologically functional IL-1beta from pro-IL-1beta. *Biochem Biophys Res Commun* **379**: 904–908, doi:10.1016/j.bbrc.2008.12.161.

Heurtaux T, Michelucci A, Losciuto S, Gallotti C, Felten P, Dorban G, Grandbarbe L, Morga E, Heuschling P (2010) Microglial activation depends on beta-amyloid conformation: Role of the formylpeptide receptor 2. *J Neurochem* **114**: 576–586, doi:10.1111/j.1471-4159.2010.06783.x.

Hickman SE, Kingery ND, Ohsumi TK, Borowsky M, Wang L-C, Means TK, El Khoury J (2013) The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci* **16**: 1896–1907, doi:10.1038/nn.3554.

Hise AG, Tomalka J, Ganesan S, Patel K, Hall BA, Brown D, Fitzgerald KA (2010) An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen, Candida albicans. *Cell Host Microbe* **5**: 487–497, doi:10.1016/j.chom.2009.05.002.An.

Hoegen T, Tremel N, Klein M, Angele B, Wagner H, Kirschning C, Pfister H-W, Fontana A, Hammerschmidt S, Koedel U (2011) The NLRP3 Inflammasome Contributes to Brain Injury in Pneumococcal Meningitis and Is activated Through ATP-Dependent Lysosomal Cathpsin B release. *J Immunol* **187**: 5440–5451.

Hoffman HM, Mueller JL, Broide DH, Wanderer a a, Kolodner RD (2001) Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat Genet* **29**: 301–305, doi:10.1038/ng756.

Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald K a, Latz E (2008) Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* **9**: 847–856, doi:10.1038/ni.1631.

Hussain Bhat A, Bashir Dar K, Anees S, Afzal Zargar M, Masood A, Ahmad Sofi M, Ahmad Ganie S (2015) Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. *Biomed Pharmacother* **74**: 101–110, doi:10.1016/j.biopha.2015.07.025.

Ichinohe T, Pang IK, Iwasaki A (2010) Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat Immunol* **11**: 404–410, doi:10.1038/ni.1861.

Irwin DJ, Lee VM-Y, Trojanowski JQ (2013) Parkinson's disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies. *Nat Rev Neurosci* **14**: 626–636, doi:10.1038/nrn3549.

Ito M, Yanagi Y, Ichinohe T (2012) Encephalomyocarditis virus viroporin 2B activates NLRP3 inflammasome. *PLoS Pathog* **8**: e1002857, doi:10.1371/journal.ppat.1002857.

Iyer SS, He Q, Janczy JR, Elliott EI, Zhong Z, Olivier AK, Sadler JJ, Knepper-Adrian V, Han R, Qiao L, Eisenbarth SC, Nauseef WM, Cassel SL, Sutterwala FS (2013) Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity* **39**: 311–323, doi:10.1016/j.immuni.2013.08.001.

Jamilloux Y, Pierini R, Querenet M, Juruj C, Fauchais AL, Jauberteau MO, Jarraud S, Lina G, Etienne J, Roy CR, Henry T, Davoust N, Ader F (2013) Inflammasome activation restricts Legionella pneumophila replication in primary microglial cells through flagellin detection. *Glia* **61**: 539–549, doi:10.1002/glia.22454.

Jensen PH, Nielsen, M. S. Jakes R, Dotti CG, Goedert M (1998) Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. *J Biol Chem* **273**: 26292–26294, doi:10.1074/jbc.273.41.26292.

Jha MK, Seo M, Kim J-H, Kim B-G, Cho J-Y, Suk K (2013) The secretome signature of reactive glial cells and its pathological implications. *Biochim Biophys Acta* **1834**: 2418–2428.

Jha S, Srivastava SY, Brickey WJ, Iocca H, Toews a., Morrison JP, Chen VS, Gris D, Matsushima GK, Ting JP-Y (2010) The Inflammasome Sensor, NLRP3, Regulates CNS Inflammation and Demyelination via Caspase-1 and Interleukin-18. *J Neurosci* **30**: 15811–15820, doi:10.1523/JNEUROSCI.4088-10.2010.

Jiang X, Nardelli J (2015) Cellular and molecular introduction to brain development. *Neurobiol Dis* doi:10.1016/j.nbd.2015.07.007.

Jin C, Frayssinet P, Pelker R, Cwirka D, Hu B, Vignery A, Eisenbarth SC, Flavell R a (2011) NLRP3 inflammasome plays a critical role in the pathogenesis of hydroxyapatite-associated arthropathy. *Proc Natl Acad Sci U S A* **108**: 14867–14872, doi:10.1073/pnas.1111101108.

Jin T, Perry A, Jiang J, Smith P, Curry J a, Unterholzner L, Jiang Z, Horvath G, Rathinam V a, Johnstone RW, Hornung V, Latz E, Bowie AG, Fitzgerald K a, Xiao TS (2012) Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* **36**: 561–571, doi:10.1016/j.immuni.2012.02.014.

Jin T, Perry A, Smith P, Jiang J, Xiao TS (2013) Structure of the absent in melanoma 2 (AIM2) pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly. *J Biol Chem* **288**: 13225–13235, doi:10.1074/jbc.M113.468033.

Johann S, Heitzer M, Kanagaratnam M, Goswami A, Rizo T, Weis J, Troost D, Beyer C (2015) NLRP3 Inflammasome is expressed by Astrocytes in the SOD1 Mouse Model of ALS and in Human Sporadic ALS Patients. *Glia* **63**: doi:10.1002/glia.22891.

Joly S, Ma N, Sadler JJ, Soll DR, Cassel SL, Sutterwala FS (2009) Cutting edge: Candida albicans hyphae formation triggers activation of the Nlrp3 inflammasome. *J Immunol* **183**: 3578–3581, doi:10.4049/jimmunol.0901323.

Joosten L a B, Netea MG, Fantuzzi G, Koenders MI, Helsen MM a, Sparrer H, Pham CT, van der Meer JWM, Dinarello C a, van den Berg WB (2009) Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. *Arthritis Rheum* **60**: 3651–3662, doi:10.1002/art.25006.

Juliana C, Fernandes-Alnemri T, Kang S, Farias A, Qin F, Alnemri ES (2012) Non-transcriptional priming and deubiquitination regulate NLRP3 Inflammasome Activation. *J Biol Chem* **287**: 36617–36622, doi:10.1074/jbc.M112.407130.

Juliana C, Fernandes-Alnemri T, Wu J, Datta P, Solorzano L, Yu J-W, Meng R, Quong AA, Latz E, Scott CP, Alnemri ES (2010) Anti-inflammatory coumpounds Parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome. *J Biol Chem* **285**: 9792–9802, doi:10.1074/jbc.M109.082305.

Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R (2003) APP Processing and Synaptic Function. *Neuron* **37**: 925–937, doi:10.1016/S0896-6273(03)00124-7.

Kashayar J, Liu LF, Wang JC (1978) Nonhistone Proteins HMG1 and HMG2 change the DNA helical structure. *Science (80-)* **199.**:

Kaushal V, Dye R, Pakavathkumar P, Foveau B, Flores J, Hyman B, Ghetti B, Koller BH, LeBlanc AC (2015) Neuronal NLRP1 inflammasome activation of Caspase-1 coordinately regulates inflammatory interleukin-1-beta production and axonal degeneration-associated Caspase-6 activation. *Cell Death Differ* **22**: 1676–1686, doi:10.1038/cdd.2015.16.

Kaushik DK, Gupta M, Kumawat KL, Basu A (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. *PLoS One* **7**: e32270, doi:10.1371/journal.pone.0032270.

Kawana N, Yamamoto Y, Ishida T, Saito Y, Konno H, Arima K, Satoh J (2013) Reactive astrocytes and perivascular macrophages express NLRP3 inflammasome in active demyelinating lesions of multiple sclerosis and necrotic lesions of neuromyelitis optica and cerebral infarction. *Clin Exp Neuroimmunol* **4**: 296–304, doi:10.1111/cen3.12068.

Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, Newton K, Qu Y, Liu J, Heldens S, Zhang J, Lee WP, Roose-Girma M, Dixit VM (2011) Non-canonical inflammasome activation targets caspase-11. *Nature* **479**: 117–121, doi:10.1038/nature10558.

Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-takamura S, Miyake K, Zhang J, Lee WP, Forsberg LS, Carlson RW, Dixit VM (2013) Independent of TLR4. **130**: 1246–1250, doi:10.5061/dryad.bt51g.

Keller M, Rüegg A, Werner S, Beer H-D (2008) Active caspase-1 is a regulator of unconventional protein secretion. *Cell* **132**: 818–831, doi:10.1016/j.cell.2007.12.040.

Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A (2011) Physiology of Microglia. *Physiol Rev* **91**: 461–553, doi:10.1152/physrev.00011.2010.

Keyel P a. (2014) How is inflammation initiated? Individual influences of IL-1, IL-18 and HMGB1. *Cytokine* **69**: 136–145, doi:10.1016/j.cyto.2014.03.007.

Khakh BS, Sofroniew M V (2015) Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci* **18**: 942–952, doi:10.1038/nn.4043.

Kiely AP, Asi YT, Kara E, Limousin P, Ling H, Lewis P, Proukakis C, Quinn N, Lees AJ, Hardy J, Revesz T, Houlden H, Holton JL (2013) A-synucleinopathy associated with G51D SNCA mutation: A link between Parkinson's disease and multiple system atrophy? *Acta Neuropathol* **125**: 753–769, doi:10.1007/s00401-013-1096-7.

Kigerl K a., de Rivero Vaccari JP, Dietrich WD, Popovich PG, Keane RW (2014) Pattern recognition receptors and central nervous system repair. *Exp Neurol* **258**: 5–16, doi:10.1016/j.expneurol.2014.01.001.

Kim J-B, Sig Choi J, Yu Y-M, Nam K, Piao C-S, Kim S-W, Lee M-H, Han P-L, Park J-S, Lee J-K (2006) HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. *J Neurosci* **26**: 6413–6421, doi:10.1523/JNEUROSCI.3815-05.2006.

Kim S, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, Dinarello CA (2000) Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *PNAS* **97**: 1190–1195.

Kofoed EM, Vance RE (2011) Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* **477**: 592–595, doi:10.1038/nature10394.

Koizumi S, Ohsawa K, Inoue K, Kohsaka S (2013) Purinergic receptors in microglia: functional modal shifts of microglia mediated by P2 and P1 receptors. *Glia* **61**: 47–54, doi:10.1002/glia.22358.

Koprich JB, Reske-Nielsen C, Mithal P, Isacson O (2008) Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. *J Neuroinflammation* **5**: 8, doi:10.1186/1742-2094-5-8.

Kostura MJ, Tocci MJ, Limjuco G, Chin J, Cameron P, Hillman AG, Chartrain NA, Schmidt JA (1989) Identification of a monocyte specific pre-interleukin 1 beta convertase activity. *Proc Natl Acad Sci* **86**: 5227–5231.

Kovarova M, Hesker PR, Jania L, Nguyen M, Snouwaert JN, Xiang Z, Lommatzsch SE, Huang MT, Ting JP-Y, Koller BH (2012) NLRP1-dependent pyroptosis leads to acute lung injury and morbidity in mice. *J Immunol* **189**: 2006–2016, doi:10.4049/jimmunol.1201065.

Koziorowski D, Tomasiuk R, Szlufik S, Friedman A (2012) Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. *Cytokine* **60**: 762–766, doi:10.1016/j.cyto.2012.07.030.

Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA (1995) Altered Cytokine Export and Apoptosis in Mice Deficient in Interleukin-1 beta Converting Enzyme. *Science (80-)* **267**: 2000–2003.

Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, van Bruggen R, Tschopp J (2007) Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J Histochem Cytochem* **55**: 443–452, doi:10.1369/jhc.6A7101.2006.

Lamkanfi M (2011) Emerging inflammasome effector mechanisms. *Nat Rev Immunol* **11**: 213–220, doi:10.1038/nri2936.

Lamkanfi M, Amer A, Kanneganti T-D, Muñoz-Planillo R, Chen G, Vandenabeele P, Fortier A, Gros P, Nu G (2007) The Nod-Like Receptor Family Member Naip5/Birc1e Restricts Legionella pneumophila Growth Independently of Caspase-1 Activation. *J Immunol* **178**: 8022–8027.

Lamkanfi M, Mueller JL, Vitari AC, Misaghi S, Fedorova A, Deshayes K, Lee WP, Hoffman HM, Dixit VM (2009) Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. *J Cell Biol* **187**: 61–70, doi:10.1083/jcb.200903124.

Lamkanfi M, Sarkar A, Vande Walle L, Vitari AC, Amer AO, Wewers MD, Tracey KJ, Kanneganti T-D, Dixit VM (2010) Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J Immunol* **185**: 4385–4392, doi:10.4049/jimmunol.1000803.

Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY (2007) Interleukin-1-Receptor Antagonist in Type 2 Diabetes Mellitus. *N Engl J Med* **356.**:

Latz E (2010) NOX-free inflammasome activation Sometimes a cigar is just a cigar. *Blood* **116**: 1393–1394, doi:10.1182/blood-2009-12-257063.12.

Lau L, Yu A (2001) Astrocytes produce and release interleukin-1, interleukin-6, tumor necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. *J Neurotrauma* **18**: 351–359.

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**: 151–170, doi:10.1016/0306-4522(90)90229-W.

Lee E-J, Woo M-S, Moon P-G, Beak M-C, Choi I-Y, Kim W, Junn E, Kim H (2010) a-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. *J Immunol* **185**: 615–623, doi:10.4049/jimmunol.0903480.

Lee H-M, Kang J, Lee SJ, Jo E-K (2013) Microglial activation of the NLRP3 inflammasome by the priming signals derived from macrophages infected with mycobacteria. *Glia* **61**: 441–452, doi:10.1002/glia.22448.

Lee KM (2015) New advances on glial activation in health and disease. *World J Virol* **4**: 42–55, doi:10.5501/wjv.v4.i2.42.

Lee M, Jantaratnotai N, McGeer E, McLarnon JG, McGeer PL (2011) Mg2+ ions reduce microglial and THP-1 cell neurotoxicity by inhibiting Ca2+ entry through purinergic channels. *Brain Res* **1369**: 21–35, doi:10.1016/j.brainres.2010.10.084.

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang P-W, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* **487**: 443–448, doi:10.1038/nature11314.

Lent R, Azevedo FAC, Andrade-Mororaes CH, Pinto AVO (2012) How many neuron do you have? Some dogmas of quantitative neuroscience under revision. *Eur J Neurosci* **35**: 1–9, doi:10.1111/j.1460-9568.2011.07923.

Li Q, Tian Y, Wang ZF, Liu SB, Mi WL, Ma HJ, Wu GC, Wang J, Yu J, Wang YQ (2013) Involvement of the spinal NALP1 inflammasome in neuropathic pain and aspirin-triggered-15-epi-lipoxin A4 induced analgesia. *Neuroscience* **254**: 230–240, doi:10.1016/j.neuroscience.2013.09.028.

Liang Y, Jing X, Zeng Z, Bi W, Chen Y, Wu X, Yang L, Liu J, Xiao S, Liu S, Lin D, Tao E (2015) Rifampicin attenuates rotenone-induced inflammation via suppressing NLRP3 inflammasome activation in microglia. *Brain Res* 43–50, doi:10.1016/j.brainres.2015.06.008.

Limón ID, Díaz A, Mendieta L, Chamorro G, Espinosa B, Zenteno E, Guevara J (2009) Amyloid-β25-35 impairs memory and increases NO in the temporal cortex of rats. *Neurosci Res* **63**: 129–137, doi:10.1016/j.neures.2008.11.006.

Litteljohn D, Mangano E, Clarke M, Bobyn J, Moloney K, Hayley S (2010) Inflammatory mechanisms of neurodegeneration in toxin-based models of Parkinson's disease. *Parkinsons Dis* **2011**: 713517, doi:10.4061/2011/713517.

London A, Cohen M, Schwartz M (2013) Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair. *Front Cell Neurosci* **7**: 34, doi:10.3389/fncel.2013.00034.

Long-smith M, Sullivan AM, Nolan YM (2009) Progress in Neurobiology The influence of microglia on the pathogenesis of Parkinson ' s disease. *Prog Neurobiol* **89**: 277–287, doi:10.1016/j.pneurobio.2009.08.001.

Losciuto S, Dorban G, Gabel S, Gustin A, Hoenen C, Grandbarbe L, Heuschling P, Heurtaux T (2012) An efficient method to limit microglia-dependent effects in astroglial cultures. *J Neurosci Methods* **207**: 59–71, doi:10.1016/j.jneumeth.2012.03.010.

Lourbopoulos A, Ertürk A, Hellal F (2015) Microglia in action: how aging and injury can change the brain's guardians. *Front Cell Neurosci* **9**: doi:10.3389/fncel.2015.00054.

Lu M, Sun XL, Qiao C, Liu Y, Ding JH, Hu G (2014) Uncoupling protein 2 deficiency aggravates astrocytic endoplasmic reticulum stress and nod-like receptor protein 3 inflammasome activation. *Neurobiol Aging* **35**: 421–430, doi:10.1016/j.neurobiolaging.2013.08.015.

Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D (2014) Neuroinflammation: The role and consequences. *Neurosci Res* **79**: 1–12, doi:10.1016/j.neures.2013.10.004.

Ma D, Jin S, Li E, Doi Y, Parajuli B, Noda M, Sonobe Y, Mizuno T, Suzumura A (2013) The neurotoxic effect of astrocytes activated with toll-like receptor ligands. *J Neuroimmunol* **254**: 10–18, doi:10.1016/j.jneuroim.2012.08.010.

Machado-Filho JA, Correia AC, Brilhantes Aires Montenegro A, Pareira Nobre ME, Cerqueira GS, Tavares Neves KR, Naffah-Mazzacoratti M da G, Abrao Cavalheiro E, De Castro Brito GA, De Barros Viana GS (2014) Caffeine neuroprotective effects on 6-OHDA-lesioned rats are mediated by several factors, including pro-inflammatory cytokines and histone deacetylase inhibitions. *Behav Brain Res* **264**: 116–125.

Man SM, Tourlomousis P, Hopkins L, Monie TP, Fitzgerald K a, Bryant CE (2013) Salmonella infection induces recruitment of Caspase-8 to the inflammasome to modulate IL-1β production. *J Immunol* **191**: 5239–5246, doi:10.4049/jimmunol.1301581.

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440**: 228–232, doi:10.1038/nature04515.

Maroteaux L, Campanelli JT, Scheller RH (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J Neurosci* **8**: 2804–2815.

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, doi:10.1038/nature04516.

Mason JL, Suzuki K, Chaplin DD, Matsushima GK (2001) Interleukin-1beta promotes repair of the CNS. *J Neurosci* **21**: 7048–7052.

Masters SL, Dunne A, Subramanian SL, Hull RL, Gillian M, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Kingston HG, Mok KH, Newsholme P, Nuñez G, Yodoi J, Kahn SE, Lavelle EC, O'Neill L a J (2010) Activation of the Nlrp3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1b in type 2 diabetes. *Nat Imm* **11**: 897–904, doi:10.1038/ni.1935.

Mawhinney LJ, de Rivero Vaccari JP, Dale G a, Keane RW, Bramlett HM (2011) Heightened inflammasome activation is linked to age-related cognitive impairment in Fischer 344 rats. *BMC Neurosci* **12**: 123, doi:10.1186/1471-2202-12-123.

Mayor A, Martinon F, De Smedt T, Pétrilli V, Tschopp J (2007) A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* **8**: 497–503, doi:10.1038/ni1459.

McGeer PL, Yasojima K, McGeer EG (2002) Association of interleukin-1beta polymorphisms with idiopathic Parkinson's disease. *Neurosci Lett* **326**: 67–69, doi:10.1016/S0304-3940(02)00300-2.

Meissner F, Molawi K, Zychlinsky A (2010) Mutant superoxide dismutase 1-induced IL-1β accelerates ALS pathogenesis. *PNAS* **107**: 13046–13050, doi:10.1073/pnas.1002396107.

Miao E a, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, Aderem A (2006) Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat Immunol* **7**: 569–575, doi:10.1038/ni1344.

Miller a. CK, Schattenberg DG, Malkinson AM, Ross D (1994) Decreased content of the IL1alpha processing enzyme calpain in murine bone marrow-derived macrophages after treatment with the benzene metabolite hydroquinone. *Toxicol Lett* **74**: 177–184, doi:10.1016/0378-4274(94)90096-5.

Miller FD, Gauthier AS (2007) Timing Is Everything: Making Neurons versus Glia in the Developing Cortex. *Neuron* **54**: 357–369, doi:10.1016/j.neuron.2007.04.019.

Millucci L, Ghezzi L, Bernardini G, Santucci A (2009) Conformations and biological activities of amyloid beta peptide 25-35. *Curr Protein Pept Sci* **10**: doi:10.2174/138920310790274626.

Minkiewicz J, de Rivero Vaccari JP, Keane RW (2013) Human astrocytes express a novel NLRP2 inflammasome. *Glia* **61**: 1113–1121, doi:10.1002/glia.22499.

Mitchell AnJ, Yau B, McQuillan JA, Ball HJ, Khoon Too L, Abtin A, Hertzog P, Leib SL, Jones CA, Gerega SK, Weninger W, Hunt NH (2012) Inflammasome-dependent IFN-y drives pathogenesis in Streptococcus pneumoniae Meningitis. *J Immunol* **189**: 4970–4980, doi:10.4049/jimmunol.1201687.

Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T (1996) Interleukin (IL)-1beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett* **211**: 13–16.

Molofsky A V., Krenick R, Ullian E, Tsai H, Deneen B, Richardson WD, Barres B a, Rowitch DH (2012) Astrocytes and disease: a neurodevelopmental perspective. *genes Dev* **26**: 891–907, doi:10.1101/gad.188326.112.

Molofsky AB, Byrne BG, Whitfield NN, Madigan C a, Fuse ET, Tateda K, Swanson MS (2006) Cytosolic recognition of flagellin by mouse macrophages restricts Legionella pneumophila infection. *J Exp Med* **203**: 1093–1104, doi:10.1084/jem.20051659.

Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, Greene WC (2014) IFI16 DNA Sensor Is Required for Death of Lymphoid CD4 T Cells Abortively Infected with HIV. *Science (80-)* **343**: 428–432, doi:10.1126/science.1243640.

Moriwaki K, Bertin J, Gough PJ, Chan FK-M (2015) A RIPK3-Caspase 8 Complex Mediates Atypical Pro-IL-1β Processing. *J Immunol* **194**: 1938–1944, doi:10.4049/jimmunol.1402167.

Mosley B, Dower SK, Gillis S, Cosman D (1987) Determination of the minimum polypeptide lengths of the functionally active sites of human interleukins 1 alpha and 1 beta. *PNAS* **84**: 4572–4576.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**: 55.

Munoz A, Garrido-Gil P, Dominguez-Meijide A, Labandeira-Garcia JL (2014) Angiotensin type 1 receptor blockage reduces L-dopa-induced dyskinesia in the 6-OHDA model of Parkinson's disease. Involvement of vascular endothelial growth factor and interleukin-1b. *Exp Neurol* **261**: 720–732.

Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith BL, Rajendiran TM, Núñez G (2013) K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* **38**: 1142–1153, doi:10.1016/j.immuni.2013.05.016.

Murakami T, Ockinger J, Yu J, Byles V, Mccoll A, Hofer AM, Horng T (2012) Critical role for calcium mobilization in activation of the NLRP3 inflammasome. *PNAS* **109**: 11282–11287, doi:doi: 10.1073/pnas.1117765109.

Murphy N, Grehan B, Lynch M a (2014) Glial uptake of amyloid beta induces NLRP3 inflammasome formation via cathepsin-dependent degradation of NLRP10. *Neuromolecular Med* **16**: 205–215, doi:10.1007/s12017-013-8274-6.

Nadella R, Voutilainen MH, Saarma M, Gonzales-Barrios JA, Leon-Chavez BA, Dues Jimnez JM, Dues Jimnez SH, Escobedo L, Martinez-Fong D (2014) Transient transfection of human CDNF gene reduces the 6-hydroxydopamine-induced neuroinflamamtion in the rat substantia nigra. *J Neuroinflammation* **11**: doi:10.1186/s12974-014-0209-0.

Nagelhus EA, P. OO (2013) Physiological roles of aquaporin-4 in brain. *Physiol Rev* **93**: 1543–1562, doi:10.1152/physrev.00011.2013.

Nakahira K, Haspel JA, Rathinam V a K, Lee S-J, Dolinay T, Lam HC, Englert J a, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald K a, Ryter SW, Choi AMK (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol* **12**: 222–230, doi:10.1038/ni.1980.

Nelson N (1993) Presynaptic events involved in neurotransmission. *J Physiol* **87**: 171–178.

Netea MG, van de Veerdonk FL, van der Meer JWM, Dinarello C a, Joosten L a B (2014) Inflammasome-Independent Regulation of IL-1-Family Cytokines. *Annu Rev Immunol* 1–29, doi:10.1146/annurev-immunol-032414-112306.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting Microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science (80-)* **308**: 1314–1318, doi:10.1126/science.1110647.

Nishimura M, Mizuta I, Mizuta E, Yamasaki S, Ohta M, Kuno S (2000) Influence of interleukin-1b gene polymorphisms on age-at-onset of sporadic Parkinson's disease. *Neurosci Lett* **284**: 73–76.

Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello C a., Rubinstein M (1999) Interleukin-18 binding protein: A novel modulator of the Th1 cytokine response. *Immunity* **10**: 127–136, doi:10.1016/S1074-7613(00)80013-8.

Nuvolone M, Sorce S, Schwarz P, Aguzzi A (2015) Prion Pathogenesis in the Absence of NLRP3/ASC Inflammasomes. *PLoS One* **10**: doi:10.1371/journal.pone.0117208.

Oberheim NA, Goldman S a., Nedergaard M (2012) Heterogeneity of astrocytic form and function. *Methods Mol Biol* **814**: 23–45, doi:10.1007/978-1-61779-452-0_3.

Obermeier B, Daneman R, Ransohoff RM (2013) Development, maintenance and disruption of the blood-brain barrier. *Nat Med* **19**: 1584–1596, doi:10.1038/nm.3407.

Oliveira Da Cruz JF, Robin LM, Drago F, Marsicano G, Metna-Laurent M (2015) Astroglial type-1 cannabinoid receptor (CB1): a new player in the tripartite synapse. *Neuroscience* doi:doi: 10.1016/j.neuroscience.2015.05.002.

Omoto Y, Yamanaka K, Tokime K, Kitano S, Kakeda M, Akeda T, Kurokawa I, Gabazza EC, Tsutsui H, Katayama N, Yamanishi K, Nakanishi K, Mizutani H (2010) Granzyme B is a novel interleukin-18 converting enzyme. *J Dermatol Sci* **59**: 129–135, doi:10.1016/j.jdermsci.2010.05.004.

Van Opdenbosch N, Gurung P, Vande Walle L, Fossoul A, Kanneganti T-D, Lamkanfi M (2014) Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. *Nat Commun* **5**: 3209, doi:10.1038/ncomms4209.

Orlowski GM, Colbert JD, Sharma S, Bogyo M, Robertson SA, Rock KL (2015) Multiple Cathepsins promote Pro-IL-1b Synthesis and NLRP3-mediated IL-1b activation. *J Immunol* **195**: 1685–1697, doi:10.4049/jimmunol.1500509.

Orriss IR, Knight GE, Utting JC, Taylor SEB, Burnstock G, Arnett TR (2009) Hypoxia Stimulates Vesicular ATP Release From Rat Ostoeoblasts. *J Cell Physiol* **220**: 155–162, doi:10.1002/jcp.21745.

Parajuli B, Sonobe Y, Horiuchi H, Takeuchi H, Mizuno T, Suzumura a (2013) Oligomeric amyloid β induces IL-1β processing via production of ROS: implication in Alzheimer's disease. *Cell Death Dis* **4**: e975, doi:10.1038/cddis.2013.503.

Paranjape GS, Gouwens LK, Osborn DC, Nichols MR (2012) Isolated Amyloid-β(1−42) Protofibrils, But Not Isolated Fibrils, Are Robust Stimulators of Microglia. *ACS Chem Neurosci* **3**: 302–311, doi:10.1021/cn2001238.

Paridaen JT, Huttner WB (2014) Neurogenesis during development of the vertebrate central nervous system. *EMBO Rep* **15**: 351–364, doi:10.1002/embr.201438447.

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates III JR, Lafaille JJ, Hempstead BL, Littman DR, Gan W-B (2013) Microglia promote Learning-Dependent Synapse Formation Through Brain-Derived Neurotrophic Factor. *Cell* **155**: 1596–1609, doi:10.1016/j.cell.2013.11.030.

Pasanen P, Myllykangas L, Siitonen M, Raunio A, Kaakkola S, Lyytinen J, Tienari P, Poyhonen M, Paetay A (2014) A novel alpha-synuclein mutation 153E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. *Neurobiol Aging* **35**: 2180e1–e2180e5.

Pekny M, Nilsson M (2005) Astrocyte Activation and Reactive Gliosis. *Glia* **50**: 427–434, doi:10.1002/glia.20207.

Pekny M, Pekna M (2014) Astrocyte reactivity and reactive astrogliosis: costs and benefits. *Physiol Rev* **94**: 1077–1098, doi:10.1152/physrev.00041.2013.

Pellegatti P, Falzoni S, Pinton P, Rizzuto R, Di Virgilio F (2005) A novel recombinant plasma membrane-targeted luciferase reveals a new pathway for ATP secretion. *Mol Biol Cell* **16**: 3659–3665, doi:10.1091/mbc.E05-03-0222.

Peng H, Chang B, Lu C, Su J, Wu Y, Lv P, Wang Y, Liu J, Zhang B, Quan F, Guo Z, Zhang Y (2012) Nlrp2, a maternal effect gene required for early embryonic development in the mouse. *PLoS One* **7**: doi:10.1371/journal.pone.0030344.

Perea G, Sur M, Araque A, Parpura V, Rusakov DA (2014) Neuron-glia networks: integral gear of brain function. *Front Cell Neurosci* **8**: doi:10.3389/fncel.2014.00378.

Perry VH (2012) Innate Inflammation in Parkinson's Disease. *Cold Spring Harb Perspect Med* **2**: doi:10.1101/cshperspect.a009373.

Pétrilli V, Papin S, Dostert C, Mayor a, Martinon F, Tschopp J (2007) Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ* **14**: 1583–1589, doi:10.1038/sj.cdd.4402195.

Phani S, Loike JD, Przedborski S (2012) Neurodegeneration and inflammation in Parkinson's disease. *Parkinsonism Relat Disord* **18**: S207–S209, doi:10.1016/S1353-8020(11)70064-5.

Piccioli P, Rubartelli A (2013) The secretion of IL-1β and options for release. *Semin Immunol* **25**: 425–429, doi:10.1016/j.smim.2013.10.007.

Pike CJ, Burdick D, Walencewicz a J, Glabe CG, Cotman CW (1993) Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci* **13**: 1676–1687.

Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, Hannesschläger N, Schlee M, Rothenfusser S, Barchet W, Kato H, Akira S, Inoue S, Endres S, Peschel C, Hartmann G, Hornung V, Ruland J (2010) Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol* **11**: 63–69, doi:10.1038/ni.1824.

Pott Godoy MC, Tarelli R, Ferrari CC, Sarchi MI, Pitossi FJ (2008) Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain* **131**: 1880–1894, doi:10.1093/brain/awn101.

Poyet J-L, Srinivasula SM, Tnani M, Razmara M, Fernandes-Alnemri T, Alnemri ES (2001) Identification of Ipaf, a human Caspase-1-activating Protein releated to Apaf-1. *J Biol Chem* **276**: 28309–28313.

Pradhan T, Jung HS, Jang JH, Kim TW, Kang C, Kim JS (2014) Chemical sensing of neurotransmitters. *Chem Soc Rev* **43**: 4684–4713.

Prat A, Biernacki K, Wosik K, Antel JP (2001) Glial cell influence on the human blood-brain barrier. *Glia* **36**: 145–155, doi:10.1002/glia.1104.

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat Rev Neurosci* **15**: 300–312, doi:10.1038/nrn3722.

Prinz M, Priller J, Sisodia SS, Ransohoff RM (2011) Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat Neurosci* **13**: 1227–1235, doi:10.1038/nn.2923.

Proell M, Gerlic M, Mace PD, Reed JC, Riedl SJ (2013) The CARD plays a critical role in ASC foci formation and inflammasome signalling. *Biochem J* **449**: 613–621, doi:10.1042/BJ20121198.

Proukakis C, Dudzik CG, Brier T, MacKay DS, Cooper JM, Millhauser GL, Houlden H, Schapira AH (2013) A novel α-synuclein missense mutation in Parkinson disease. *Neurology* **80**: 1062–1064, doi:10.1212/WNL.0b013e31828727ba.

Puren AJ, Fantuzzi G, Dinarello C a (1999) Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Proc Natl Acad Sci U S A* **96**: 2256–2261, doi:DOI 10.1073/pnas.96.5.2256.

Py BF, Kim MS, Vakifahmetoglu-Norberg H, Yuan J (2013) Deubiquitination of NLRP3 by BRCC3 Critically Regulates Inflammasome Activity. *Mol Cell* **49**: 331–338, doi:10.1016/j.molcel.2012.11.009.

Querfurth HW, LaFerla FM (2010) Alzheimer's Disease. *N Engl J Med* **362**: 329–344, doi:10.1056/NEJMra0909142.

Rajan J V, Rodriguez D, Miao E a, Aderem A (2011) The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. *J Virol* **85**: 4167–4172, doi:10.1128/JVI.01687-10.

Ramaswamy V, Walsh JG, Sinclair DB, Johnson E, Tang-Wai R, Wheatley BM, Branton W, Maingat F, Snyder T, Gross DW, Power C (2013) Inflammasome induction in Rasmussen's encephalitis: cortical and associated white matter pathogenesis. *J Neuroinflammation* **10**: 152, doi:10.1186/1742-2094-10-152.

Ramos HJ, Lanteri MC, Blahnik G, Negash A, Suthar MS, Brassil MM, Sodhi K, Treuting PM, Busch MP, Norris PJ, Gale M (2012) IL-1b Signaling Promotes CNS-Intrinsic Immune Control of West Nile Virus Infection. *PLoS Pathog* **8**: doi:10.1371/journal.ppat.1003039.

Ransohoff RM, Brown MA (2012) Innate immunity in the central nervous system. **122**: doi:10.1172/JCI58644.

Ransohoff RM, Katsumoto A, Lu H, Miranda AS (2015) System Macrophages Ontogeny and Functions of Central Nervous Ontogeny and Functions of Central Nervous System Macrophages. *J Immunol* **193**: 2615–2621, doi:10.4049/jimmunol.1400716.

Rathinam V a K, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, Vanaja SK, Monks BG, Ganesan S, Latz E, Hornung V, Vogel SN, Szomolanyi-Tsuda E, Fitzgerald K a (2010) The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat Immunol* **11**: 395–402, doi:10.1038/ni.1864.

Rathinam V a K, Vanaja SK, Waggoner L, Sokolovska A, Becker C, Stuart LM, Leong JM, Fitzgerald K a (2012) TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. *Cell* **150**: 606–619, doi:10.1016/j.cell.2012.07.007.

Rayamajhi M, Zhang Y, Miao E a. (2013) Detection of pyroptosis by measuring released lactate dehydrogenase activity. *Methods Mol Biol* **1040**: 85–90, doi:10.1007/978-1-62703-523-1-7.

Reuss B, Dono R, Unsicker K (2003) Functions of fibroblast growth factor (FGF)-2 and FGF-5 in astroglial differentiation and blood-brain barrier permeability: evidence from mouse mutants. *J Neurosci* **23**: 6404–6412.

De Rivero Vaccari JP, Dietrich WD, Keane RW (2014) Activation and regulation of cellular inflammasomes: gaps in our knowledge for central nervous system injury. *J Cereb Blood Flow Metab* **34**: 369–375, doi:10.1038/jcbfm.2013.227.

De Rivero Vaccari JP, Dietrich WD, Keane RW (2015) Therapeutics targeting the inflammasome after central nervous system injury. *Transl Res* doi:10.1016/j.trsl.2015.05.003.

De Rivero Vaccari JP, Lotocki G, Alonso OF, Bramlett HM, Dietrich WD, Keane RW (2009) Therapeutic neutralization of the NLRP1 inflammasome reduces the innate immune response and improves histopathology after traumatic brain injury. *J Cereb Blood Flow Metab* **29**: 1251–1261, doi:10.1038/jcbfm.2009.46.

De Rivero Vaccari JP, Lotocki G, Marcillo AE, Dietrich WD, Keane RW (2008) A molecular platform in neurons regulates inflammation after spinal cord injury. *J Neurosci* **28**: 3404–3414, doi:10.1523/JNEUROSCI.0157-08.2008.

Rivest S (2009) Regulation of innate immune responses in the brain. *Nat Rev Immunol* **9**: 429–439, doi:10.1038/nri2565.

Robbins GR, Wen H, Ting JPY (2014) Inflammasomes and metabolic disorders: Old genes in modern diseases. *Mol Cell* **54**: 297–308, doi:10.1016/j.molcel.2014.03.029.

Rodrigues MCO, Sanberg PR, Cruz LE, Garbuzova-Davis S (2014) The innate and adaptive immunological aspects in neurodegenerative diseases. *J Neuroimmunol* **269**: 1–8, doi:10.1016/j.jneuroim.2013.09.020.

Roodveldt C, Labrador-garrido A, Gonzalez-rey E, Fernandez-montesinos R, Caro M, Lachaud CC, Waudby CA, Delgado M, Dobson CM, Pozo D (2010) Glial Innate Immunity Generated by Non-Aggregated Alpha-Synuclein in Mouse : Differences between Wild-type and Parkinson ' s Disease-Linked Mutants. *Immunity* **5**: doi:10.1371/journal.pone.0013481.

Rossi D (2015) Astrocyte physiopathology: At the crossroads of intercellular networking, inflammation and cell death. *Prog Neurobiol* **130**: 86–120, doi:10.1016/j.pneurobio.2015.04.003.

Rossol M, Pierer M, Raulien N, Quandt D, Meusch U, Rothe K, Schubert K, Schöneberg T, Schaefer M, Krügel U, Smajilovic S, Bräuner-Osborne H, Baerwald C, Wagner U (2012) Extracellular Ca2+ is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors. *Nat Commun* **3**: 1329, doi:10.1038/ncomms2339.

Rothwell NJ, Luheshi GN (2000) Interleukin 1 in the brain: Biology, pathology and therapeutic target. *Trends Neurosci* **23**: 618–625, doi:10.1016/S0166-2236(00)01661-1.

Rubartelli A (2014) DAMP-Mediated activation of NLRP3-Inflammasome in brain sterile inflammation: The fine line between healing and neurodegeneration. *Front Immunol* **5**: doi:10.3389/fimmu.2014.00099.

Rubartelli A, Cozzolinol F, Talio M, Sitia R (1990) A novel secretory pathway for interleukin-1b, a protein lacking a signal sequence. **9**: 1503–1510.

Rubartelli A, Gattorno M, Netea MG, Dinarello CA (2011) Interplay between redox status and inflammasome activation. *Trends Immunol* **32**: 559–566, doi:10.1016/j.it.2011.08.005.

Rubio-Perez JM, Morillas-Ruiz JM (2012) A review: inflammatory process in Alzheimer's disease, role of cytokines. *ScientificWorldJournal* **2012**: 756357, doi:10.1100/2012/756357.

Sanz JM, Chiozzi P, Ferrari D, Colaianna M, Idzko M, Falzoni S, Fellin R, Trabace L, Di Virgilio F (2009) Activation of microglia by amyloid {beta} requires P2X7 receptor expression. *J Immunol* **182**: 4378–4385, doi:10.4049/jimmunol.0803612.

Sauer JD, Witte CE, Zemansky J, Hanson B, Lauer P, Portnoy D a. (2010) Listeria monocytogenes triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* **7**: 412–419, doi:10.1016/j.chom.2010.04.004.

Saura J (2007) Microglial cells in astroglial cultures: a cautionary note. *J Neuroinflammation* **4**: 26, doi:10.1186/1742-2094-4-26.

Savage CD, Lopez-Castejon G, Denes A, Brough D (2012) NLRP3-Inflammasome Activating DAMPs Stimulate an Inflammatory Response in Glia in the Absence of Priming Which Contributes to Brain Inflammation after Injury. *Front Immunol* **3**: 288, doi:10.3389/fimmu.2012.00288.

Saxena M, Yeretssian G (2014) NOD-like receptors: master regulators of inflammation and cancer. *Front Immunol* **5**: doi:10.3389/fimmu.2014.0032.

Schitine C, Nogaroli L, Costa MR, Hedin-Pereira C (2015) Astrocyte heterogeneity in the brain: from development to disease. *Front Cell Neurosci* **9**: doi:10.3389/fncel.2015.00076 Astrocyte.

Schober A (2004) Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell Tissue Res* **318**: 215–224, doi:10.1007/s00441-004-0938-y.

Scholze a. R, Barres B a. (2012) A Nogo signal coordinates the perfect match between myelin and axons. *Proc Natl Acad Sci* **109**: 1003–1004, doi:10.1073/pnas.1120301109.

Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SEW, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science (80-)* **336**: 86–90, doi:10.1126/science.1219179.

Shahdat H, Hashimoto M (2012) Mechanism of Neurotoxicity of Amyloid-beta peptide and the protective efects of docosahexaenoic acid. In Amyloids: Composition, Functions and Pathology, p. Chapter 4.

Shi F, Yang L, Kouadir M, Yang Y, Wang J, Zhou X, Yin X, Zhao D (2012) The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. *J Neuroinflammation* **9**: 73, doi:10.1186/1742-2094-9-73.

Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* **514**: 187–192, doi:10.1038/nature13683.

Shimada K, Crother TR, Karlin J, Chen S, Chiba N, Ramanujan VK, Vergnes L, Ojcius DM, Arditi M (2011) Caspase-1 dependent IL-1β secretion is critical for host defense in a mouse model of Chlamydia pneumoniae lung infection. *PLoS One* **6**: e21477, doi:10.1371/journal.pone.0021477.

Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, Ramanujan VK, Wolf AJ, Vergnes L, Ojcius DM, Rentsendorj A, Vargas M, Guerrero C, Wang Y, Fitzgerald K a, Underhill DM, Town T, Arditi M (2012) Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* **36**: 401–414, doi:10.1016/j.immuni.2012.01.009.

Sibille J, Pannash U, Rouach N (2013) Astroglial potassium clearance contributes to short-term plsticity of synaptically evoked currents at the tripartite synapse. *J Physiol*.

Sierra A, Encinas JM, Deudero JJP, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**: 483–495, doi:10.1016/j.stem.2010.08.014.

Silverman WR, de Rivero Vaccari JP, Locovei S, Qiu F, Carlsson SK, Scemes E, Keane RW, Dahl G (2009) The pannexin 1 channel activates the inflammasome in neurons and astrocytes. *J Biol Chem* **284**: 18143–18151, doi:10.1074/jbc.M109.004804.

Simi A, Tsakiri N, Wang P, Rothwell NJ (2007) Interleukin-1 and inflammatory neurodegeneration. *Biochem Soc Trans* **35**: doi:10.1042/BST0351122.

Singh VV, Kerur N, Bottero V, Dutta S, Chakraborty S, Ansari MA, Paudel N, Chikoti L, Chandran B (2013) Kaposi's sarcoma-associated herpesvirus latency in endothelial and B cells activates gamma

interferon-inducible protein 16-mediated inflammasomes. *J Virol* **87**: 4417–4431, doi:10.1128/JVI.03282-12.

Singhal G, Jaehne EJ, Corrigan F, Toben C, Baune BT (2014) Inflammasomes in neuroinflammation and changes in brain function: a focused review. *Front Neurosci* **8**: 315, doi:10.3389/fnins.2014.00315.

Sofroniew M V., Vinters H V. (2010) Astrocytes: Biology and pathology. *Acta Neuropathol* **119**: 7–35, doi:10.1007/s00401-009-0619-8.

Sondag CM, Dhawan G, Combs CK (2009) Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia. *J Neuroinflammation* **6**: 1, doi:10.1186/1742-2094-6-1.

Srinivasula SM, Poyet J-L, Razmara M, Datta P, Zhang Z, Alnemri ES (2002) The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. *J Biol Chem* **277**: 21119–21122, doi:10.1074/jbc.C200179200.

Stepanichev MY, Moiseeva Y V, Lazareva N a, Gulyaeva N V (2005) Studies of the effects of fragment (25-35) of beta-amyloid peptide on the behavior of rats in a radial maze. *Neurosci Behav Physiol* **35**: 511–518.

Sugawara S, Uehara a., Nochi T, Yamaguchi T, Ueda H, Sugiyama a., Hanzawa K, Kumagai K, Okamura H, Takada H (2001) Neutrophil Proteinase 3-Mediated Induction of Bioactive IL-18 Secretion by Human Oral Epithelial Cells. *J Immunol* **167**: 6568–6575, doi:10.4049/jimmunol.167.11.6568.

Suzuki T, Núñez G (2007) A role for Nod-like receptors in autophagy induced by Shigella infection. *Autophagy* **4**: 73–75, doi:10.4161/auto.5101.

Tan M-S, Tan L, Jiang T, Zhu X-C, Wang H-F, Jia C-D, Yu J-T (2014) Amyloid-β induces NLRP1-dependent neuronal pyroptosis in models of Alzheimer's disease. *Cell Death Dis* **5**: e1382, doi:10.1038/cddis.2014.348.

Tan M-S, Yu J-T, Jiang T, Zhu X-C, Wang H-F, Zhang W, Wang Y-L, Jiang W, Tan L (2013) NLRP3 polymorphisms are associated with late-onset Alzheimer's disease in Han Chinese. *J Neuroimmunol* **265**: 91–95, doi:10.1016/j.jneuroim.2013.10.002.

Tang D, Kang R, Livesey KM, Kroemer G, Billiar TR, Van Houten B, Zeh HJ, Lotze MT (2011) High-Mobility Group Box 1 is essential for mitochondrial quality control. *cell Metab* **13**: 701–711, doi:10.1016/j.cmet.2011.04.008.

Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW (2011) Rotenone, Paraquat, and Parkinson's disease. *Environ Health Perspect* **119**: 866–872, doi:10.1289/ehp.1002839.

Terada K, Yamada J, Hayashi Y, Wu Z, Uchiyama Y, Peters C, Nakanishi H (2010) Involvement of cathepsin B in the processing and secretion of interleukin-1beta in chromogranin A-stimulated microglia. *Glia* **58**: 114–124, doi:10.1002/glia.20906.

Tezel G, Yang X, Luo C, Cai J, Powell DA (2012) An Astrocyte-Specific Proteomic Approach to Inflammatory Response in Experimental Rat Glaucoma. *Glaucoma* **53**: doi:10.1167/iovs.11-9101.

Thomas PG, Dash P, Aldridge JR, Ellebedy AH, Reynolds C, Funk AJ, Martin WJ, Lamkanfi M, Webby RJ, Boyd KL, Doherty PC, Kanneganti T-D (2009) The intracellular sensor NLRP3 mediates key innate

and healing responses to influenza A virus via the regulation of caspase-1. *Immunity* **30**: 566–575, doi:10.1016/j.immuni.2009.02.006.

Ting JP-Y, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, Flavell R a, Girardin SE, Godzik A, Harton J a, Hoffman HM, Hugot J-P, Inohara N, Mackenzie A, Maltais LJ, Nunez G, Ogura Y, Otten L a, Philpott D, Reed JC, Reith W, Schreiber S, Steimle V, Ward P a (2008) The NLR gene family: a standard nomenclature. *Immunity* **28**: 285–287, doi:10.1016/j.immuni.2008.02.005.

Tohda C, Tamura T, Komatsu K (2003) Repair of amyloid beta(25-35)-induced memory impairment and synaptic loss by a Kampo formula, Zokumei-to. *Brain Res* **990**: 141–147, doi:S0006899303034498 [pii].

Toma C, Higa N, Koizumi Y, Nakasone N, Ogura Y, McCoy AJ, Franchi L, Uematsu S, Sagara J, Taniguchi S, Tsutsui H, Akira S, Tschopp J, Núñez G, Suzuki T (2010) Pathogenic Vibrio activate NLRP3 inflammasome via cytotoxins and TLR/nucleotide-binding oligomerization domain-mediated NF-kappa B signaling. *J Immunol* **184**: 5287–5297, doi:10.4049/jimmunol.0903536.

Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. *PLoS Biol* **8**: doi:10.1371/journal.pbio.1000527.

Triantafilou K, Hughes TR, Triantafilou M, Morgan BP (2013) The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. *J Cell Sci* **126**: 2903–2913, doi:10.1242/jcs.124388.

Tsacopoulos M, Magistretti PJ (1996) Metabolic coupling between glia and neurons. *J Neurosci* **16**: 877–885.

Tschopp J, Schroder K (2010) NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* **10**: 210–215, doi:10.1038/nri2725.

Tsung A, Tohme S, Billiar TR (2014) High-mobility group box-1 in sterile inflammation. *J Intern Med* **276**: 425–443.

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. *Nat Neurosci* **16**: 543–551, doi:10.1038/nn.3358.

Vajjhala PR, Mirams RE, Hill JM (2012) Multiple binding sites on the pyrin domain of ASC protein allow self-association and interaction with NLRP3 protein. *J Biol Chem* **287**: 41732–41743, doi:10.1074/jbc.M112.381228.

Veeranki S, Duan X, Panchanathan R, Liu H, Choubey D (2011) IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. *PLoS One* **6**: e27040, doi:10.1371/journal.pone.0027040.

Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, Liu J, Antonelli A, Preti A, Raeli L, Samadi Shams S, Yang H, Varani L, Andersson U, TRacey KJ, Bachi A, Uguccioni M, Bianchi ME (2012) Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J Exp Med* **209**: 1519–1528, doi:10.1084/jem.20120189.

Villar-Cheda B, Valenzuela R, Rodriguez-Perez AI, Guerra MJ, Labandeira-Garcia JL (2012) Aging-related changes in the nigral angiotensin system enhances proinflammatory and pro-oxidative markers and

6-OHDA-induced dopaminergic degeneration. *Neurobiol Aging* **33**: doi:10.1016/j.neurobiolaging.2010.08.006.

Vladimer GI, Weng D, Paquette SWM, Vanaja SK, Rathinam V a K, Aune MH, Conlon JE, Burbage JJ, Proulx MK, Liu Q, Reed G, Mecsas JC, Iwakura Y, Bertin J, Goguen JD, Fitzgerald K a, Lien E (2012) The NLRP12 inflammasome recognizes Yersinia pestis. *Immunity* **37**: 96–107, doi:10.1016/j.immuni.2012.07.006.

Wahner AD, Sinsheimer JS, Bronstein JM, Ritz B (2007) Inflammatory cytokine gene polymorphisms and increased risk of Parkinson disease. *Arch Neurol* **64**: 836–840.

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci* **29**: 3974–3980, doi:10.1523/JNEUROSCI.4363-08.2009.

Walsh JG, Muruve D a, Power C (2014) Inflammasomes in the CNS. *Nat Rev Neurosci* **15**: 84–97, doi:10.1038/nrn3638.

Wang H, Bloom O, Zhang M, Vishnubjakat JM, Michael O, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ (1999) HMG-1 as a late Mediator of endotoxin lethality in mice. *Science (80-)* **285.**:

Wang L, Manji G a, Grenier JM, Al-Garawi A, Merriam S, Lora JM, Geddes BJ, Briskin M, DiStefano PS, Bertin J (2002) PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NF-kappa B and caspase-1-dependent cytokine processing. *J Biol Chem* **277**: 29874–29880, doi:10.1074/jbc.M203915200.

Wheeler RD, Boutin H, Touzani O, Luheshi GN, Takeda K, Rothwell NJ (2003) No role for interleukin-18 in acute murine stroke-induced brain injury. *J Cereb Blood Flow Metab* **23**: 531–535, doi:10.1097/01.WCB.0000059587.71206.BA.

Willingham SB, Allen IC, Bergstralh DT, Brickey WJ, Huang MT-H, Taxman DJ, Duncan J a, Ting JP-Y (2009) NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol* **183**: 2008–2015, doi:10.4049/jimmunol.0900138.

Wiltfang J, Esselmann H, Bibl M, Smirnov A, Otto M, Paul S, Schmidt B, Klafki H-W, Maler M, Dyrks T, Bienert M, Beyermann M, Rüther E, Kornhuber J (2002) Highly conserved and disease-specific patterns of carboxyterminally truncated Ab peptides 1-37/38/39 in addition to 1-40/42 in Alzheimer's disease and in patients with chronic neuroinflammation. *J Neurochem* **81**: 481–496.

Wu Z, Sun L, Hashioka S, Yu S, Schwab C, Okada R, Hayashi Y, McGeer PL, Nakanishi H (2013a) Differential pathways for interleukin-1β production activated by chromogranin A and amyloid β in microglia. *Neurobiol Aging* **34**: 2715–2725, doi:10.1016/j.neurobiolaging.2013.05.018.

Wu Z, Sun L, Hashioka S, Yu S, Schwab C, Okada R, Hayashi Y, McGeer PL, Nakanishi H (2013b) Differential pathways for interleukin-1β production activated by chromogranin A and amyloid β in microglia. *Neurobiol Aging* **34**: 2715–2725, doi:10.1016/j.neurobiolaging.2013.05.018.

Xing L, Goswami M, Trudeau VL (2014) Radial glia cell: Critical functions and new perspective as a steroid synthetic cell. *Gen Comp Endocrinol* **203**: 181–185, doi:10.1016/j.ygcen.2014.03.010.

Xu S-L, Chan P (2015) Interaction between neuromelenin and alpha-synuclein in Parkinson's disease. *Biomolecules* **5**: 1122–1142, doi:10.3390/biom5021122.

Yamada M, Chiba T, Sasabe J, Nawa M, Tajima H, Niikura T, Terashita K, Aiso S, Kita Y, Matsuoka M, Nishimoto I (2005) Implanted cannula-mediated repetitive administration of Aβ25-35 into the mouse cerebral ventricle effectively impairs spatial working memory. *Behav Brain Res* **164**: 139–146, doi:10.1016/j.bbr.2005.03.026.

Yang F, Wang Z, Wei X, Han H, Meng X, Zhang Y, Shi W, Li F, Xin T, Pang Q, Yi F (2014) NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke. *J Cereb Blood Flow Metab* **34**: doi:10.1038/jcbfm.2013.242.

Yang Q-W, Lu F-L, Zhou Y, Wang L, Zhong Q, Lin S, Xiang J, Li J-C, Fang C-Q, Wang J-Z (2011) HMBG1 mediates ischemia-reperfusion injury by TRIF-adaptor independent Toll-like receptor 4 signaling. *J Cereb Blood Flow Metab* **31**: 593–605, doi:10.1038/jcbfm.2010.129.

Yazdi AS, Drexler SK (2013) Regulation of interleukin 1α secretion by inflammasomes. *Ann Rheum Dis* **72 Suppl 2**: ii96–ii99, doi:10.1136/annrheumdis-2012-202252.

Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, Couillin I, Tschopp J (2010) Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β. *Proc Natl Acad Sci U S A* **107**: 19449–19454, doi:10.1073/pnas.1008155107.

Ye Z, Ting JP-Y (2008) NLR, the nucleotide-binding domain leucine-rich repeat containing gene family. *Curr Opin Immunol* **20**: 3–9, doi:10.1016/j.coi.2008.01.003.

Yin J, Xu K, Zhang J, Kumar A, Yu F-SX (2007) Wound-induced ATP release and EGF receptor activation in epithelial cells. *J Cell Sci* **120**: 815–825.

Yuan J, Ledoux S, Ellis SHM, Horvitzt HR (1993) The C . elegans Cell Death Gene ted-3 Encodes a Protein Similar to Mammalian Interleukin-1 beta-Converting Enzyme. *Cell* **75**: 641–652.

Zaki MH, Vogel P, Malireddi RKS, Body-Malapel M, Anand PK, Bertin J, Green DR, Lamkanfi M, Kanneganti T-D (2011) The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. *Cancer Cell* **20**: 649–660, doi:10.1016/j.ccr.2011.10.022.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres B a (2012) Genomic analysis of reactive astrogliosis. *J Neurosci* **32**: 6391–6410, doi:10.1523/JNEUROSCI.6221-11.2012.

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Lorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yebenes JG (2004) The New mutation, E46K, of alpha-synuclein causes Parkinson and Lewy Body Dementia. *Ann Neurol* **55**: 164–173.

Zeis T, Allaman I, Gentner M, Schroder K, Tschopp J, Magistretti PJ, Schaeren-Wiemers N (2015) Metabolic gene expression changes in astrocytes in Multiple Sclerosis cerebral cortex are indicative of immune-mediated signaling. *Brain Behav Immun* doi:10.1016/j.bbi.2015.04.013.

Zhang W, Cai Y, Xu W, Yin Z, Gao X, Xiong S (2013) AIM2 facilitates the apoptotic DNA-induced systemic lupus erythematosus via arbitrating macrophage functional maturation. *J Clin Immunol* **33**: 925–937, doi:10.1007/s10875-013-9881-6.

Zhang W, Wang T, Pai Z, Miller, David SWu X, Block ML, Wilson B, Zhang W, Zhou Y, Jau-Shyong H, Zhang J (2005) Aggregated a-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* **19**: 533–542, doi:10.1096/fj.04-2751com.

Zhang Y, Barres B a. (2010) Astrocyte heterogeneity: An underappreciated topic in neurobiology. *Curr Opin Neurobiol* **20**: 588–594, doi:10.1016/j.conb.2010.06.005.

Zhong Z, Zhai Y, Liang S, Mori Y, Han R, Sutterwala FS, Qiao L (2013) TRPM2 links oxidative stress to NLRP3 inflammasome activation. *Nat Commun* **4**: 1611, doi:10.1038/ncomms2608.

Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol* **11**: 136–140, doi:10.1038/ni.1831.

Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**: 221–225, doi:10.1038/nature09663.

De Zoete MR, Palm NW, Zhu S, Flavell R a (2014) Inflammasomes. *Cold Spring Harb Perspect Biol* **6**: doi:10.1101/cshperspect.a016287.

Zou J, Crews FT (2012) Inflammasome-IL-1β Signaling Mediates Ethanol Inhibition of Hippocampal Neurogenesis. *Front Neurosci* **6**: 77, doi:10.3389/fnins.2012.00077.

Zoupi L, Savvaki M, Karagogeos D (2011) Axons and Myelinating Glia: an Intimate Contact. *IUBMB Life* **63**: 730–735, doi:10.1002/iub.513.

VIII.
Acknowledgements

Une fois n'est pas coutume, je voudrais commencer par remercier ma famille et mes amis. Sans vous, ce travail n'aurait jamais vu le jour ! Vous m'avez toujours soutenue, encouragée et surtout, vous avez forgé mon caractère qui me pousse aujourd'hui à toujours aller au bout des choses. Merci Maman, Merci Ludo, Merci Arthur, Merci Mamy et Merci Gilles, Lauranne, Aurore, Tom, Zoé, Mathys, Lio, Josianne, Charly, Parrain, Marraine, Bruno, Valérie, Nadine, Fabienne, Amé, Seb, Raymond, Caroline, Biskot, Caroline, Florence, Julien, Julie, Greg, Aline et les autres d'avoir été présents. Vous avez été ma bulle d'oxygène ces dernières années.

Je remercie plus particulièrement mon compagnon, Xavier, pour son soutien quotidien indéfectible. Tu as toujours été là pour me rassurer et me rappeler de regarder au-delà des difficultés. Grâce à toi et à tes encouragements, je n'ai jamais dévié de mon objectif. Je te remercie aussi d'être un super Papa pour notre merveilleux petit garçon. Merci de t'en être si bien occupé dans les moments où le travail me tenait éloignée de vous.

Ensuite, je tiens évidemment à remercier toutes les personnes qui m'ont accompagnée dans l'élaboration de ce travail.

Je tiens à remercier mes superviseurs, le Dr. Catherine Dostert et le Prof. Paul Heuschling. Merci Paul de m'avoir acceptée comme PhD dans ce laboratoire. Je vous remercie pour votre confiance et pour la liberté que vous m'avez laissée durant ces années. Merci Catherine d'avoir écrit et établi le projet « inflammasome » ainsi que de m'avoir donné l'opportunité de participer à son avancement.

Merci ensuite aux personnes ayant directement contribué au projet « inflammasome » :

Merci Eric ! Merci pour ta participation au projet mais surtout pour tout ce que tu m'as appris : la rigueur scientifique, les notions de statistiques et certains concepts de neurologie. Merci pour tes critiques et commentaires qui m'ont permis de grandir scientifiquement, d'améliorer mon travail (surtout ce manuscrit) et d'élargir mes connaissances. Mon seul regret est de n'avoir travaillé que si peu de temps avec toi.

Merci Mélanie d'avoir fait avancer le projet, surtout lorsque j'étais absente. Merci pour nos discussions scientifiques, pour ta motivation, pour ton soutien et ton dynamisme. Au-delà du travail, tu es devenue une vraie amie que j'admire beaucoup !

Merci Paul pour ton aide et tes connaissances techniques. Tu as toujours répondu présent lorsque le travail s'accumulait et je ne l'oublierai pas. Merci également pour ton ouverture sur le monde extérieur qui apporte un vent d'air frais dans le laboratoire ☺.

J’adresse aussi mes remerciements aux autres membres du laboratoire :

Tony ! Merci pour tes conseils, tes suggestions et tes encouragements. Tu as toujours été là pour répondre à mes questions et cela compte beaucoup. Merci aussi pour ton éternelle bonne humeur si précieuse pour le laboratoire.

Merci Cindy ☺ Merci pour ton amitié, pour nos discussions scientifiques (ou non scientifiques) et pour les bons moments partagés. Je te souhaite une fin de thèse agréable (hum, est-ce possible ? Pas trop difficile serait peut-être plus sensé). A très vite au Basic fit !

Luc, merci pour ta culture générale débordante et ta disponibilité à débattre de sujets divers.

Je tiens également à remercier les anciens du LNB : Mike, Gauthier, Seb, Xavier, Leila et surtout Sophie. Merci Sophie pour tes conseils et encouragements en tant que thésarde, post-doc et amie.

Concerning all former and present members of the Life Science Research Unit, all of them somehow participated to this thesis, in terms of answers, questions, ideas, suggestions, mental support or others: Thank you !

I am grateful to Ass.-Prof. Greta Guarda, Prof. Iris Behrmann, Prof. Mohamed Lamkanfi and Prof. Thierry Oster. I would like to thank you for your agreement to critically read my manuscript and to come to Luxembourg University as members of the thesis defence committee.

I thank the members of my “Comité d’encadrement de thèse”, Prof. Iris Behrmann and Prof. Rudy Balling for having accompanied me during the last years. Our feedback was always helpful during the CET sessions.

I also acknowledge Magali Guillaume, Dóra Bereti and Prof. Serge Hann for the general organization of the Doctoral School and for their assistance in administrative matters.

Last but not least, this thesis would not have been possible without the “Fond National de la Recherche Luxembourg”, which offered the generous funding for my PhD position. Huge thanks equally to the “Fondation du Pélican de Mie et Pierre Hippert-Faber” for the fellowship allowing me to participate in international conferences and summer schools and to interact with fellow researchers.

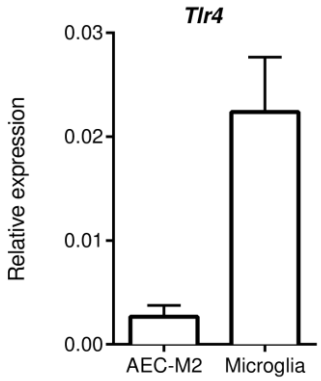
IX. Appendixes

Appendix I: Supplemental figures

Suppl. Table 1. Expression levels of inflammasome-related genes in untreated or LPS-primed microglia.

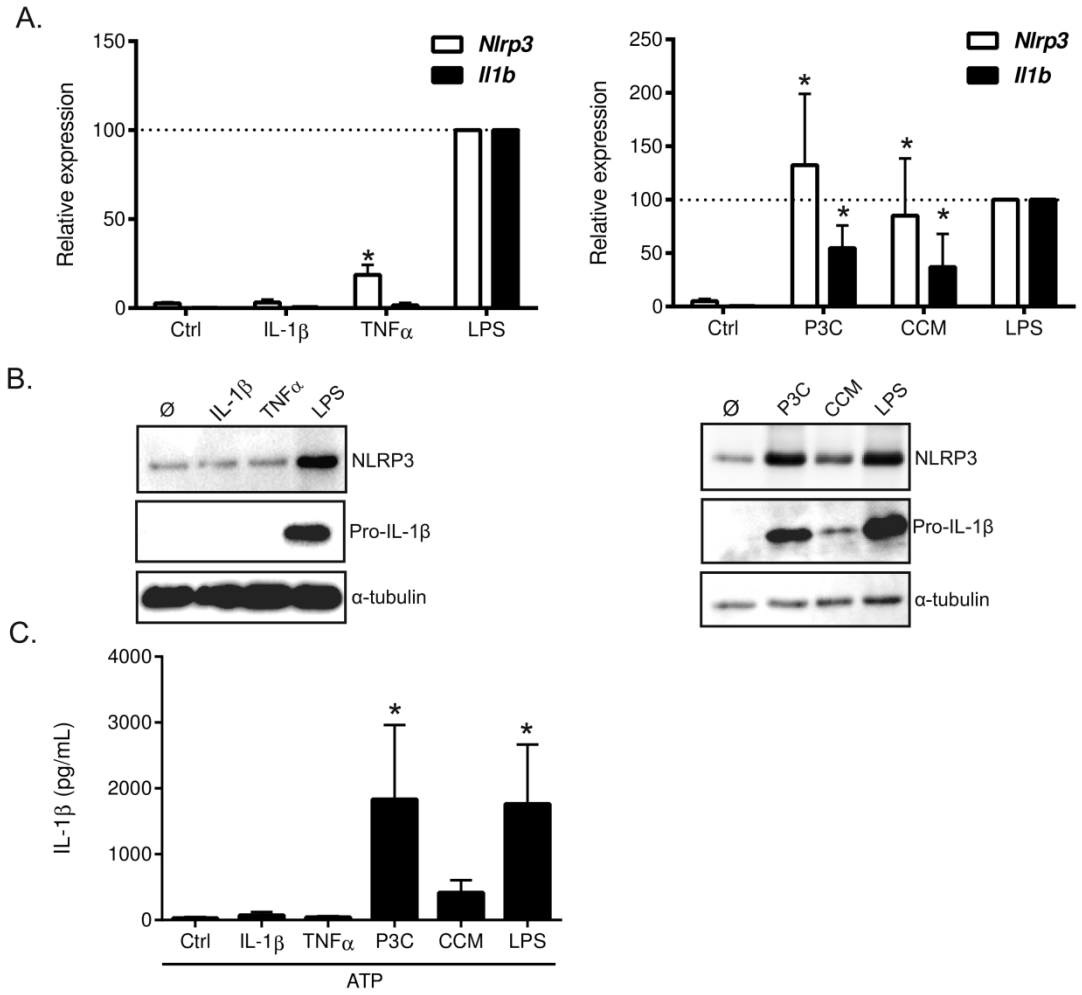
Nlrp1, Nlrp2, Nlrp3, Nlrp6, Nlrp12, Aim2, Nlrc4, Pycard, Casp1 and *Casp4* gene expressions were analysed by RT-PCR. Data are given as means of Ct ± SD and are representative of at least three independent experiments.

	Nlrp1	*Nlrp2*	*Nlrp3*	*Nlrp6*	*Nlrp12*	*Aim2*	*Nlrc4*	*Pycard*	*Casp1*	*Casp4*
Ctrl	30.2 ± 1.9	33.9 ± 2.3	26.3 ± 1.9	29.6 ± 1.5	33.6 ± 2.2	20.5 ± 0.6	27.5 ± 1.5	24.1 ± 2.0	24.8 ± 2.7	24.8 ± 2.2
LPS	31.4 ± 1.6	34.9 ± 1.4	21.1 ± 1.3	30.2 ± 1.3	33.6 ± 1.9	20.2 ± 0.4	29.8 ± 1.5	24.4 ± 1.5	22.5 ± 1.9	21.1 ± 0.2



Suppl. Fig. 1: *Tlr4* is lower expressed in AEC-M2 than in microglial cultures.

Gene expression of *Tlr4* was analysed in untreated AEC-M2 and microglial cultures by RT-PCR. Results were normalized to *Rpl27*. Data are mean ± SEM of at least three independent experiments



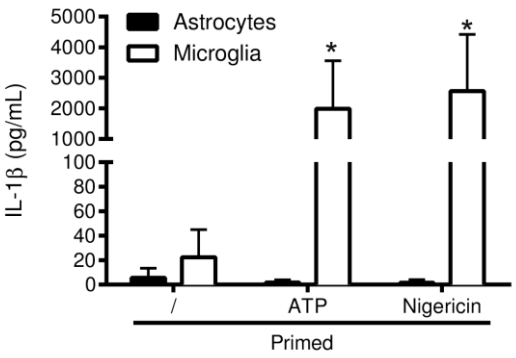
Suppl. Fig. 2: P3C and CCM efficiently primed microglia for subsequent NLRP3 inflammasome activation.

(A, B) Microglia were treated for 6 h with LPS (10 ng/mL), P3C (10 ng/mL), IL-1β (10 ng/mL), TNF-α (10 ng/mL), IFNγ (10 ng/mL) or CCM (10 ng/mL IL-1β + 10 ng/mL TNFα + 20 ng/mL IFNγ). **(A)** The gene expression level of *Il1b* and *Nlrp3* were analysed by RT-PCR and normalized to *Rpl27*. Data are shown as a percentage of the LPS-induced gene expression (100%). **(B)** Cell lysates (Xt) were analysed by WB for the expression of IL-1β and NLRP3 proteins. α-Tubulin was used as a loading control. **(C)** Microglia were treated for 6 h with LPS (10 ng/mL), P3C (10 ng/mL), IL-1β (10 ng/mL), TNF-α (10 ng/mL), IFNγ (10 ng/mL) or CCM (10 ng/mL IL-1β + 10 ng/mL TNFα + 20 ng/mL IFNγ) and then stimulated with ATP (1mM, 30 min). IL-1β secretion in supernatant were analysed by ELISA. Data are mean ± SEM of at least three independent experiments, except for WB (one representative experiment of at least 3 independent experiments). *=$p<0.05$ compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Suppl. Table 2. Expression levels of pro-inflammatory genes and inflammasome-related genes in primed astrocytes.

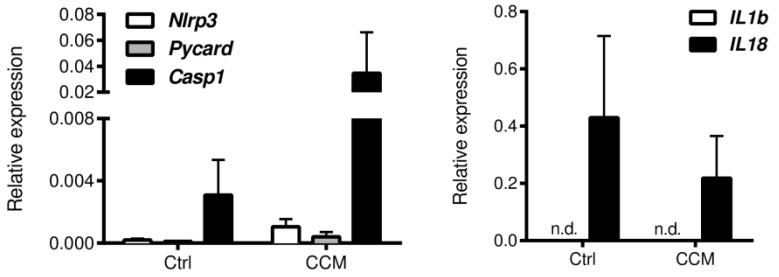
Primary astrocytes (AEC M2) were primed for 6 h with LPS (10 ng/mL), P3C (10 ng/mL), IL-1β (10 ng/mL), TNF-α (10 ng/mL), IFNγ (10 ng/mL) or CCM (10 ng/mL IL-1β + 10 ng/mL TNFα + 20 ng/mL IFNγ). Primary microglia were untreated or primed with LPS (10ng/mL, 6 h) and served as control. RNA were extracted and analysed by RT-PCR for expression of the pro-inflammatory genes *Il6, Nos2, Ptgs2, Cxcl10* and *Tnf* but also the expression of NLRP3 inflammasome-related genes (*i.e. Il1b, Il1a, Il18, Hmgb1, Nlrp3, Pycard* and *Asc)*. Data are given as means of Ct ± SD and are representative of at least three independent experiments.

		Il6	*Nos2*	*Ptgs2*	*Cxcl10*	*Tnf*	*Il1b*	*Il1a*	*Il18*	*Hmgb1*	*Nlrp3*	*Pycard*	*Casp1*
Astrocytes	Ctrl	32.3 ± 2.9	34.0 ± 2.7	26.5 ± 1.1	27.7 ± 3.5	34.6 ± 1.4	35.2 ± 1.9	35.0 ± 0.7	24.7 ± 2.3	20.5 ± 1.8	35.6 ± 1.4	31.3 ± 2.0	29.3 ± 1.7
	LPS	32.6 ± 1.9	32.3 ± 3.1	24.8 ± 1.6	27.7 ± 4.1	33.7 ± 2.2	34.2 ± 3.0	32.3 ± 4.0	24.0 ± 2.0	20.1 ± 1.1	34.6 ± 1.7	31.0 ± 2.3	27.3 ± 2.0
	P3C	29.8 ± 3.0	33.8 ± 2.4	25.2 ± 1.4	23.0 ± 3.2	33.8 ± 0.3	34.1 ± 2.0	34.0 ± 2.1	24.6 ± 2.7	19.3 ± 0.3	34.3 ± 1.8	30.1 ± 0.0	27.1 ± 0.1
	IL-1β	25.9 ± 3.3	27.7 ± 2.8	23.3 ± 2.3	21.3 ± 5.2	32.0 ± 0.0	35.1 ± 2.7	35.4 ±0.9	24.8 ± 2.7	21.5 ± 3.1	30.0 ± 0.6	34.2 ± 1.1.	28.7 ± 1.1
	TNFα	25.3 ± 1.7	27.6 ± 2.5	24.1 ± 0.9	19.5 ± 4.1	33.3 ± 0.9	34.7 ± 2.1	36.0 ± 1.2	23.9 ± 2.5	19.3 ± 0.5	32.7 ± 0.4	33.9 ± 0.9	26.6 ± 0.2
	IFNγ	29.5 ± 3.2	30.3 ± 2.2	25.1 ± 1.0	21.5 ± 2.7	36.0 ± 1.7	36.3 ± 2.1	36.3 ± 1.7	28.6 ± 0.1	19.3 ± 1.3	38.1 ± 0.7	34.6 ± 1.0	28.7 ± 0.5
	CCM	21.3 ± 3.6	22.7 ± 2.1	20.6 ± 1.9	17.5 ± 3.9	28.5 ± 1.7	35.8 ± 2.3	34.4 ± 2.0	23.4 ± 2.0	20.4 ± 1.1	26.9 ± 1.4	28.8 ± 3.4	24.9 ± 0.9
Microglia	Ctrl	35.8 ± 1.9	35.7 ± 2.5	26.8 ± 2.5	32.3 ± 3.6	27.4 ± 2.2	28.6 ± 3.9	30.7 ± 3.0	25.1 ± 2.3	22.1 ± 1.9	26.3 ± 2.1	24.1 ± 2.0	24.8 ± 2.7
	LPS	27.2 ± 2.0	27.0 ± 3.5	21.0 ± 3.8	21.6 ± 4.3	19.9 ± 3.5	19.8 ± 2.7	21.9 ± 4.3	21.7 ± 2.2	24.4 ± 1.3	21.1 ± 1.3	24.4 ± 1.5	22.5 ± 1.9



Suppl. Fig. 3: Astrocytes do not release IL-1β following NLRP3 inflammasome activation.

IL-1β secretion was assayed by ELISA on supernatant of CCM-primed astrocytes and LPS-microglia treated with ATP (1 mM, 30 min) or Nigericin (Nig, 1.34 µM, 2 h). Data are mean ± SEM of at least three independent experiments. *=p<0.05 compared to Ctrl. Kruskal-Wallis test followed by Dunn's multiple comparisons test.



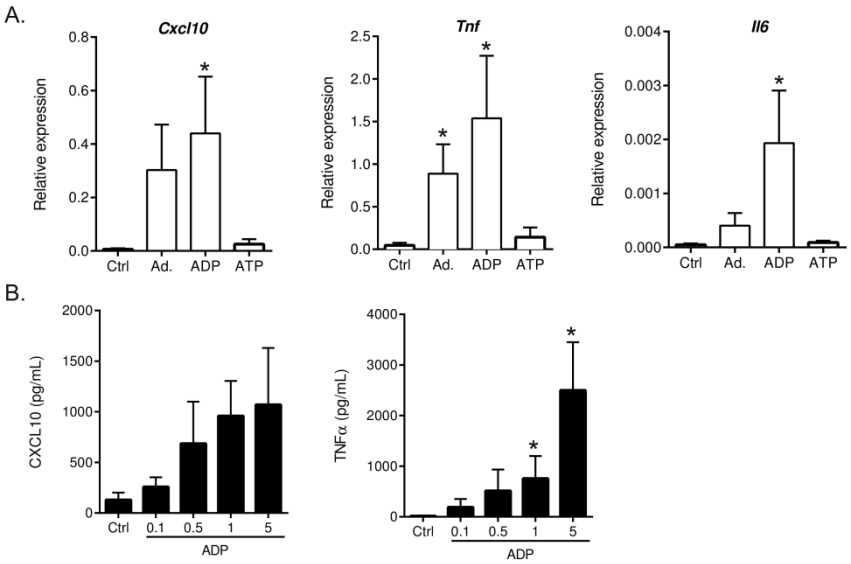
Suppl. Fig. 4: Neurosphere-derived astrocytes poorly express *Il1b*, *Pycard* and *Nlrp3* genes.

Gene expressions of *Nlrp3*, *Pycard*, C*asp1*, *Il1b* and *Il18* were analysed in neurosphere-derived astrocyte cultures stimulated for 6 h with CCM by RT-PCR. Data are normalized to *Rpl27* and are mean ± SEM values of at least three independent experiments.

Suppl. Table 3. Expression levels of other inflammasome-related genes in untreated or primed astrocytes.

Primary astrocytes were untreated or primed with CCM (10ng/mL, 6 h). Primary microglia were untreated or primed with LPS (10ng/mL, 6 h) and served as comparison. RNA were extracted and analysed for gene expression of *Nlrp1, Nlrp2, Nlrp6, Nlrp12, Aim2, Nlrc4* and *Casp4* by RT-PCR. Data are Ct mean ± SEM and are representative of at least three independent experiments.

		Nlrp1	*Nlrp2*	*Nlrp6*	*Nlrp12*	*Aim2*	*Nlrc4*	*Casp4*
Astrocytes	Ctrl	32.6 ± 2.2	34.2 ± 2.0	29.9 ± 1.9	33.2 ± 0.7	22.0 ± 0.7	35.3 ± 1.8	34.1 ± 1.7
	CCM	33.0 ± 2.4	32.9 ± 2.4	29.8 ± 2.1	33.1 ± 0.9	21.8 ± 0.9	34.2 ± 0.7	30.2 ± 3.1
Microglia	Ctrl	30.2 ± 1.9	33.9 ± 2.3	29.6 ± 1.5	33.6 ± 2.2	20.5 ± 0.6	27.5 ± 1.5	24.8 ± 2.2
	LPS	31.4 ± 1.6	34.9 ± 1.4	30.2 ± 1.3	33.6 ± 1.9	20.2 ± 0.4	29.8 ± 1.5	21.1 ± 0.2



Suppl. Fig. 5: ADP and adenosine seems to be pro-inflammatory factors.

(A) Microglia were stimulated for 3 h with adenosine (Ad., 1mM), ADP (1mM) or ATP (1mM). Transcripts were analysed for expression of *Cxcl10*, *Tnf* and *Il6* by RT-PCR. Data were normalized to *Rpl27*. **(B)** Microglia were stimulated for 3 h with ADP at 0.5 mM, 1 mM, or 5 mM. CXCL10 and TNFα secretions were assessed by ELISA in culture supernatant of wild-type (WT) **(B)** or P2rx7$^{-/-}$ **(D)** microglia. Data are mean ± SEM of at least three independent experiments. *p<0.05 compared to Ctrl, #p<0.05, KO compared to WT. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

Appendix II: List of grants, meetings and others activities

Grants

Fellowship, Pelican foundation of Pierre et Mie Hippert Faber. October, 2012
20.000€ to finance research stays in external Laboratories and travel support

AFR, Fonds National de la Recherche Luxembourg. March 2011
Aide à la Formation-Recherche, PhD Grant Scheme provides funding for PhD research training projects in Luxembourg and abroad for up to 4 years.

Presentations

"Glia in health and disease". Cold Spring Harbor, NYC, USA. July 19-23, 2012
Poster: "β-amyloid and α-synuclein fail to activate the inflammasome in microglia".
Audrey Gustin, Sophie Losciuto, Tony Heurtaux, Paul Heuschling and Catherine Dostert.

"Life Sciences PhD Days Luxembourg". September 11-12, 2012
Oral Presentation: "Characterization of inflammasome components and activation in brain cells". Audrey Gustin.

"Autophagy, Inflammation and Immunity". Quebec, Canada. February 17-22, 2013.
Poster: "Inflammasome activation in microglia in the context of neurodegenerative diseases".
Audrey Gustin, Paul Heuschling and Catherine Dostert.

"Inflammasome in health and disease". Boston, USA. June 24-25, 2013.

"Cytokines Down Under". Melbourne, Australia. October 26-29, 2014

"Glial Cells in Health and Disease". Bilbao, Spain. July 15-18, 2015.
Poster: "NLRP3 inflammasome is expressed and functional in brain microglia but not in astrocytes". Audrey Gustin, Mélanie Kirchmeyer, Eric Koncina, Paul Felten, Paul Heuschling and Catherine Dostert.

"IL-1 family members and the inflammasome conference". Dublin, Ireland. September 15-16, 2011.
Poster: "Characterization of inflammasome components and activation in glial cells."
Audrey Gustin, Sophie Losciuto, Eleonora Morga, Paul Heuschling and Catherine Dostert.

Courses & Workshops

Formation en science et technique des animaux de laboratoire.
August 29 – September 9, 2011. Université Libre de Bruxelles. Bruxelles, Belgium.

Advanced Proteomics.
July 2-6, 2012. Dr. Bruno Domon University of Luxembourg, Luxembourg.

Computational Systems Biology.
February 13-17, 2012. Dr. Antonio Del Sol Mesa. University of Luxembourg, Luxembourg.

Imaging-based systems biology.
January 21-35, 2013. Dr. Alexander Skupin. University of Luxembourg, Belval.

Presentation skills for scientific conferences.
April 18-19, 2013. Dr. Birgit Michel-Dittgen. University of Luxembourg, Luxembourg

Good Scientific Practice.
March 5-6, 2015. Dr. Michael Gommel. University of Luxembourg, Luxembourg.

Teaching with digital media and technologies in higher education.
March 9-11, 2015. Dr. Robert Reuter. University of Luxembourg, Luxembourg

Others

Teaching Assistant, University of Luxembourg (Luxembourg). October, 2011- December, 2014. 1st year Bachelor Practical Courses in Biology.

Internship. Sulzer Lab, Columbia University, NYC, USA. June 26 – July 5, 2013.
Learning dopaminergic neuronal cultures protocol. Prof. David Sulzer and Ellen Kanter.

Organization of "Life Sciences PhD Days Luxembourg", September 9-10, 2013.

Supervision of Master Students: Xiang Hi Dong (Uni.lu) and Alix Verbeke (FUNDP, BE)

This list of attendances have allowed to obtain the 20 ECTS required to successfully complete the Doctoral School in Systems and Molecular Biomedicine program.

Appendix III: Publications

"An efficient method to limit microglia-dependent effects in astroglial cultures"

Losciuto S., Dorban G., Gabel S., **Gustin A.**, Hoenen C., Grandbarbe L., Heuschling P., Heurtaux T.

Journal of Neuroscience Methods, 2012. 207, 59– 71

"NLRP3 Inflammasome Is Expressed and Functional in Mouse Brain Microglia but Not in Astrocytes"

Audrey Gustin, Mélanie Kirchmeyer, Eric Koncina, Paul Felten, Sophie Losciuto, Tony Heurtaux, Aubry Tardivel, Paul Heuschling, Catherine Dostert

PlOs One, 2015. 10 (6).

"Alpha-synuclein peptides promote pro-inflammatory cascades in microglia: stronger effects of the A53T mutant"

Claire Hoenen, **Audrey Gustin**, Cindy Birck, Mélanie Kirchmeyer, Nicolas Beaume, Luc Grandbarbe, Paul Heuschling, Tony Heurtaux

(*In preparation - J. Neuroinflammation*)