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MINI-SYMPOSIUM: Role of the Inflammasome in Brain Pathogenesis: A Potential Therapeutic Target?

Inflammasome activation and innate immunity in Alzheimer's disease

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Abstract

Activation of innate immunity and the assembly of microglial cells at sites of Alzheimer disease pathology has long been regarded as bystander phenomenon, which does not actively contribute to disease pathogenesis and progression. Recent data emerging from genetics, clinical imaging and animal experimentation point to an intimate and mutual interaction of innate immune mechanisms and neurodegenerative processes. NOD-like receptor (NLR) family, pyrin domain containing 3 and 1 inflammasomes, present in myeloid cells and neurons, respectively, represent key components of the innate immune reaction observed in Alzheimer patient brains. Inhibition of inflammasome activation just begins to prove beneficial and protective from cognitive deficits and neuronal death in cell culture and animal models of Alzheimer's disease, thereby opening a new avenue for therapeutic intervention.

INNATE IMMUNE ACTIVATION IN ALZHEIMER'S DISEASE

Accumulation and deposition of beta-amyloid peptides (Aβ) represent one of the major hallmarks of Alzheimer's disease (AD) pathology. Aβ is generated by sequential processing of the amyloid precursor protein by two proteases and usually removed from the brain by export into the cerebrospinal fluid (CSF), the blood vessels and local degradation by microglial cells, the brain's innate immune system (9). While CSF and blood drainage of Aβ each account for approximately 25% of its removal, the remaining 50% is taken up and degraded by microglia. In principle, microglia can take up Aβ by phagocytosis, but likewise secrete enzymes such as insulin-degrading enzyme, which is capable of degrading extracellular Aβ. It is likely that the described ways to remove Aβ can compensate for each other to a certain extent. In case one of these removal routes becomes compromised and fails, another one may step in and increase their particular clearance capacity. However, once the Aβ concentration has risen above a critical threshold, Aβ oligomers form and deposit in so called senile plaques begins. At this stage, accumulated Aβ and in particular oligomeric or aggregated fibrilar forms act as danger associated molecular patterns (DAMP), binding to and activating the innate immune control of the brain, namely the microglial cells. The latter have been equipped by nature with various surface receptors (so called pattern recognition receptors, PRRs), which are able to sense oligomeric or fibrillar Aβ and initiate an immune response upon ligation. One may speculate whether this system is in place, since several bacteria including *Salmonella typhimurium* and *Escherichia coli* present amyloid fibrils on their surface, very similar to those Aβ species that accumulate in the human AD brain (1). Innate immune cells such as microglia, which are capable of recognizing Aβ may therefore misread this signal and interpret those endogenously generated Aβ fibrills as bacterial presence. In contrast to a real bacterial infection, however, microglia may never fully succeed in removing the "detrimental pathogen," since there is a constant production of Aβ in the brain. Immune activation and response may thus contribute to the establishment of a chronic long lasting type of sterile inflammation in AD.

Of note, recent data from human longitudinal Aβ-PET analysis suggest that deposition of Aβ starts years if not decades prior to memory failure and cognitive decline (10). Given the fact that Aβ acts as a strong DAMP, it seems likely that the interval between early Aβ accumulation and later hallmarks of disease progression such as tau pathology and brain atrophy are influenced, if not driven by, innate immune responses. Concerning such an important role of the innate immune system, one has to consider that Aβ may just represent the initial but not exclusive immune stimulus in the AD brain. In case of neuronal demise, cellular debris will accumulate and this debris is usually an equal or even stronger immune inducing DAMP. A prominent example of mediators that would follow and initial round of activation is the accumulation of chromogranin A, radical oxygen species and further inflammatory products such as MRP14, complement factors and cytokines, all of which can sustain or even further fuel such an inflammatory reaction in the brain.

One of the canonical pathways of this innate immune response evoked by Aβ is the activation of the NOD-like receptor (NLR)

family, pyrin domain containing 3 (NLRP3) inflammasome that became a focus of intense research. NLRP3 inflammasome activation results from TLR ligation and concomitant uptake of Aβ in models of AD. Activation of NLRP3 leads to the generation of interleukin-1β (IL-1β) and interleukin 18 (IL-18), which are being cleaved by caspase-1 from their inactive precursors and subsequently.

NLRP INFLAMMASOME ACTIVATION *IN VITRO*

Activation of the NLRP3 inflammasome by fibrillar Aβ has been described first by Halle et al. in 2008. In this experiment, a 5 μM concentration of Aβ was able to induce IL-1β production in a NLRP3 and ASC-dependent manner (7). Incubation with the caspase-1 inhibitor z-YVAD was effective in blocking the observed IL-1β production. NLRP3 activation was characterized by ASC speck formation in an immune-activated microglial cell line and required a dual signal to become effective: the phagocytic uptake of Aβ and cathepsin B release after lysosomal disruption. NLRP3 inflammasome formation and subsequent activation of caspase-1 cleavage capacity was instrumental for Aβ-induced nitric oxide production and TNF-α release. Likewise, genetic caspase-1 deficiency blocked the release of several chemokines including CCL2, CCL4 and CXCL2. Together, these data suggest that NLRP3 inflammasome activation represents an important and initiating factor in the inflammatory signaling cascade evoked by fibrillar Aβ.

Soluble Aβ (sAβ)-induces NLRP3 inflammasome activation, however it requires the presence of the surface receptor CD36 (12). Interestingly, sAβ was at least as immune stimulatory as compared to fibrillar Aβ with respect to IL-1β production. Absence of CD36 blocked approximately 50% of sAβ stimulated IL-1β release. While Aβ is not the only relevant immunostimulant in the AD brain, it seems to require the presence of the full assembled inflammasome (15). Other possible innate immune factors, such as Chromogranin A, do not require the NLRP3 inflammasome and may stimulate IL-1β production independently in microglia. A similar mechanism may be in play, when astrocytes generate IL-1β after phagocytosis of Aβ. Interestingly, the latter phenomenon also depends on the presence of ASC (3). However, as astrocytes *per se* are not fully equipped with all the proteins needed to assemble a NLRP3 inflammasome, one may speculate whether ASC specks, which are being released from pyroptotic microglia, can also be taken up by astrocytes (6).

In various models of inflammation, ASC speck formation has now become a measure of inflammasome activity and are used as a readout for NLRP3 inflammasome inhibition by drugs such as the fenamate class of non-steroidal anti-inflammatory drugs (5).

The NLRP3 inflammasome may, however, not be the only inflammasome, which contributes to the pathogenesis of AD. Recently, Kaushal et al. described the involvement of NLRP1 inflammasome activation in neurons. In these experiments, serum deprivation induced NLRP1-dependent caspase-1 activity and ASC speck formation, which resulted in caspase-6 activation and an increase in the Aβ42/total Aβ ratio (11). Further data, showing "neuronal pyroptosis" of Aβ exposed neurons in a NLRP1-dependent and caspase-1-mediated manner may point to a vicious cycle, by which NLRP1 is causing neurodegeneration in response to increased Aβ production (14). Together, these data suggest that in addition to detrimental changes caused by microglial NLRP3 activation, NLRP1 inflammasome activity in neurons may contribute to Aβ pathology and neuronal demise.

NLRP3 INFLAMMASOME EXPRESSION AND ACTIVATION *IN VIVO*

Injection of Aβ in the murine striatum, a brain region belonging to the basal ganglia resulted in an increase of F4/80 microglia and macrophage activation (7). Interestingly, myeloid cell recruitment was blocked in animals deficient for ASC and caspase-1 knockout animals. Likewise genetic IL-1 receptor or MyD88 deficient mice did not show substantial immune reaction in this experimental paradigm, suggesting that the acute activation of an inflammatory myeloid reaction by Aβ requires a multi-step-signaling event, that includes MyD88 as well as the assembly of the NLRP3 inflammasome.

In an AD-related experimental approach, NLRP3 knockout animals were crossed into APP/PS1 transgenic animals and analyzed for neuropathological changes, behavioral symptoms and microglial function in the neocortex and in the hippocampus (8). Aged APP/PS1 transgenic mice displayed a proinflammatory phenotype which was characterized by immune-activated microglial cells, showing process retraction and volume increases of soma-near branches and the cell soma itself. Brains from these mice showed higher levels of pro-inflammatory mediators. On a histological level, microglial cells were found to surround Aβ plaques without any signs of phagocytic uptake and thus appeared to be rather paralyzed than phagocytically active. In strong contrast, APP/PS1dE9 animals, which were genetically deficient for NLRP3 were sending processes into the Aβ deposit, which were clearly filled with Aβ immunopositive material and revealed a twofold increase in phagocytic clearance capacity in a quantitative FACS/methoxy xo4-based *in vivo* assay. Likewise APP/PS1xNLRP3 knockout animals showed a twofold increase in insulin-degrading enzyme (IDE) expression. This is of particular interest as IDE is one of few enzymes which is able to degrade extracellular Aβ. The observed increase may bear functional relevance as a paper by Leissring et al. had previously demonstrated that an IDE increase of similar magnitude strongly limited cerebral Aβ accumulation in a single APP transgenic mouse model (16). The combined effect of the increased IDE production and phagocytic Aβ clearance reduced the cerebral Aβ load substantially, even at late life. Since immunohistochemistry found NLRP3 exclusively expressed in microglial cells, it has been concluded that the observed changes were entirely due to NLRP3 inflammasome modulation in these cells. More important than the removal of Aβ was the finding that innate immune inhibition would protect from APP/PS1 induced spine loss in apical cortical neurons. Both, NLRP3- and caspase-1 knockout animals showed complete protection when crossed into the APP/PS1 model. At the same time, analysis of hippocampal long-term potentiation (LTP), an electrophysiological measure of memory formation and consolidation, showed a marked reduction in APP/PS1 mice. Knockout of NLRP3 completely normalized LTP, suggesting that innate immune factors downstream of the NLRP3 signaling pathway, are responsible for LTP suppression instead of Aβ itself. One possible NLRP3-dependent factor here is IL-1β itself, since IL-1β suppression of LTP has shown previously in wild-type mouse hippocampa slices and genetic knockout of NLRP3

completely prevents IL-1β production. Functional protection, however, was not restricted to slice culture physiology: Testing the mice at and advanced age showed that NLRP3- and caspase-1 knockout would prevent hippocampal memory failure such as spatial navigation memory and also object recognition.

Using a similar mouse model, Shi et al. treated animals with the antimalarial drug artemisinin, showing that this treatment results in inhibition of NFkB and presumably the NLRP3 inflammasome (13). However, the blots provided just show a reduction of the p20 band of the caspase-1 cleavage product. In the absence of a complete analysis including the pro-caspase-1 levels and the cleaved product of caspase-1 on the very same blot, no final conclusion is possible. Any other analysis of functional caspase-1 modification is missing. Using the herbizide, *N*,*N*′-dimethyl-4,4′-bipyridinium dichloride (paraquat) as a mitochondrial toxin, which is known to induce oxidative stress, Chen et al. found increased levels of caspase-1 and IL-1β in brain of wild type and APP/PS1 transgenic mice (2), suggesting that those were due to NLRP3 inflammasome activation. This finding was associated with spatial memory dysfunction and an increase in Aβ plaque deposition. Comparing paraquat-mediated effects in animals overexpressing the antioxidant defense enzyme peroxiredoxin 3, decreased caspase-1 and IL-1β levels compared to wildtype levels, suggesting that the possible NRLP3 activation is partly mediated by oxidative stress. Further evidence for a neuroprotective role of NLRP3 inflammasome inhibition, in this case by fenamate NSAIDs, comes from a model of acute intracerebroventricular injection of Aβ1-42, which is characterized by deficits of novel object recognition. Such data, however, have to be interpreted with caution, since the drugs used possess a wide spectrum of different anti-inflammatory properties, which can, in an *in vivo* setting, not always being precisely distinguished. Thus, in the case of fenamate NSAIDs, the blocking of prostanoid production may also account for some of the beneficial effects observed (5).

EVIDENCE FOR HUMAN INFLAMMASOME ACTIVITY

In 2012, Cribbs et al. undertook a systematic study of the expression of genes related to the immune system in healthy aging and in AD cases in a region- and gender-specific manner (4). Detection of mRNA levels of ASC and NLRP3 did not detect any changes—either in samples from healthy aged donors or of those derived from AD patients. In contrast, gene expression of caspase-1 seemed to be upregulated in several brain regions in both types of samples. Together these changes suggested that during healthy aging as well as during AD pathogenesis, the mRNA levels of those proteins that are required for inflammasome formation and activation are present. However, when analyzing protein levels, cleaved caspase-1 was found exclusively elevated in AD patients brain samples derived from frontal cortex or hippocampus, two brain regions that are widely affected by the disease process (8). Of note, similar differences were observed when comparing brain samples from mild cognitive impairment (MCI) patients or cases of early AD onset with age-matched non-demented controls. Thus, while even during aging, innate immune cells of the brain are well equipped for inflammasome formation on an mRNA level, it seems to require a certain inducing agent or pathological sequelae to finally trigger the cascade of events which ultimately results in the generation of proinflammatory cytokines.

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