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Crystallins and neuroinflammation: the glial side of the story

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Abstract

Background—There is an abundance of evidence to support the association of damaging neuroinflammation and neurodegeneration across a multitude of diseases. One of the links between these pathological phenomena is the role of chaperone proteins as both neuroprotective and immune-regulatory agents.

Scope of Review—Chaperone proteins are highly expressed at sites of neuroinflammation both in glial cells and in the injured neurons that initiate the immune response. For this reason, the use of chaperones as treatment for various diseases associated with neuroinflammation is a highly active area of investigation. This review explores the various ways that the small heat shock protein chaperones, α-crystallins, can affect glial cell function with a specific focus on their implication in the inflammatory response associated with neurodegenerative disorders, and their potential as therapeutic treatment.

Major Conclusions—Although the mechanisms are still under investigation, a clear link has now been established between alpha-crystallins and neuroinflammation, especially through their roles in microglial and macroglial cells. Interestingly, similar to inflammation in itself, crystallins can have a beneficial or detrimental impact on the CNS based on the context and duration of the condition.

General Significance—Overall this review points out the novel roles that chaperones such as alpha-crystallins can play outside of the classical protein folding pathways, and their potential in the development of new therapies for the treatment of neuroinflammatory/neurodegenerative diseases.

Keywords

crystallins; neuroinflammation; microglia; astrocytes; central nervous system

Introduction

Neuroinflammation is a relatively new field of study focusing on the function and regulation of the immune system in the very unique environment of the long-lived neuronal cells of the

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central nervous system (CNS). CNS-resident glial cells develop and migrate to their appropriate environments early in embryogenesis, the first demonstration of the critical role these cells play in the central nervous system development and homeostasis. These cells are part of the innate immune response, designed to respond rapidly to any identified threat to the system ranging from foreign pathogens to increased cell death in the course of normal aging, and as such, constitutes a beneficial, pro-survival network aimed at maintaining the health of the highly sensitive neuronal tissue. Unfortunately, the efficacy of neuroinflammation balances precariously as if on the edge of a knife. Chronic insults such as from unresolved infection or injury, changes in metabolism, or increased protein aggregation can tip the balance of neuroinflammation from beneficial to detrimental (overview in Figure 1).

The cellular stress response is key to our understanding of both neuronal cell death and glial cell activation. As the cells' first line of defense, chaperone proteins and pathways upregulated in response to stress are important for determining not only the fate of individual cells, but of the tissue overall. One class of chaperones, the heat shock proteins, (HSPs), are characterized for their role in promoting proper protein folding and preventing aggregation. HSPs encompass a wide range of sizes and structures, permitting diversity in their clients and partners to better respond to a variety of insults. One family of HSPs is the ATP-independent small HSPs, characterized by their small monomeric size, ability to form elaborate oligomeric structures, and high variability in sequence amongst the family members. These small HSPs were originally identified as co-chaperones to the more recognized ATP-dependent chaperones including Hsp70s and Hsp90s. Due to this designation, independent functions for small HSPs have only relatively recently begun to be investigated. Closer analysis of these "co-chaperones" demonstrated that they have functions entirely of their own and appear to be more prevalent and integral to cellular homeostasis than originally thought. Two of these small HSPs, αA- and αB-crystallin were originally thought to reside only in the ocular lens, but upon discovering a broader tissue expression for these chaperones, an explosion of data has occurred demonstrating their function in the normal stress response as well as key players in several disease states.

Here, we review the role of the molecular chaperones, αA- and αB-crystallin, in regulating inflammation in the CNS. We highlight recent advancements in our understanding of the function of α-crystallins in glial cells, the potential role of α-crystallins in extracellular signaling throughout the glial cell network, and the effect of α-crystallins on glial cell activation. This review not only points out the anti-inflammatory properties of α-crystallin chaperones, but also how these properties could be used to treat inflammatory diseases.

1. Expression of α-crystallins in glial cells

1.1 α-Crystallins in Microglia

Microglial cells are bone marrow-derived cells that resides in the CNS, which are considered to present two distinct states, the resting and the activated state[1]. In the absence of pathogens or extracellular signals, microglia are in a resting state characterized by small cellular bodies and several, thin, and extended processes. The resting microglial cells are surveyor cells, constantly sampling their environment in order to locate any injury or

exogenous pathogens and non-self antigens. When the microglial cells detect an insult, they undergo a process termed activation in which they switch from their resting form into an amoeboid form. During activation, the cell bodies expand and the processes shrink and are retracted. Expression of various receptors and enzymes changes, as does the expression of cytokines and other immune response-associated molecules. Activated microglia migrate toward the area of insult and once there, secrete pro-inflammatory cytokines in order to mount an appropriate immune response. If the insult persists, the microglia can undergo another transformation and become phagocytic, removing apoptotic cells and cell debris as well as pathogens. Through phagocytosis, microglia mediate the immune response in the brain.

Both αA- and αB-crystallin have been shown to play a role in microglial cells. αB-crystallin is upregulated in microglial cells in the brains of Alzheimer's disease patients compared to non-demented controls[2–5]. The increased expression of αB-crystallin was not detected in all activated microglia, but is found in microglia surrounding amyloid plaques suggesting an involvement of αB-crystallin in recognizing and phagocytosing extracellular amyloid deposits. More recently, increased expression of αB-crystallin was shown in the microglia in a model of Parkinson's disease[6]. Unlike αB-crystallin, expression of αA-crystallin is more limited and as such, no data regarding its expression in microglia in the brain has been reported; however it has been shown to have an important role in microglial activation, an aspect that will be developed later in this review. Additionally, while their level of expression and specific role in microglia remains unclear, αA- and αB-crystallins are highly expressed by other glial cells of the retina under various disease conditions.

The microglial expression of αB-crystallin in the Parkinson's disease model coincided with increased protein aggregates in the microglia suggesting that αB-crystallin may be upregulated in order to resolve protein aggregation and prevent further protein misfolding[6, 7]. Like many other molecular chaperones, α-crystallins are upregulated in response to stresses such as oxidative stress, changes in metabolic state, and viral and bacterial infection in order to regulate protein folding and prevent aggregation[8, 9]. In addition to their role in maintaining protein homeostasis, α-crystallins also regulate apoptosis by binding pro-apoptotic proteins to prevent cell death from occurring until the situation is resolved or the stress response pathways are overwhelmed and the cell undergoes programmed cell death[10, 11]. One proposed hypothesis for the increased expression of αB-crystallin in microglial cells in the brain is that they may be part of the normal stress response to regulate aberrant protein aggregation, and may prevent apoptosis in the face of the increased phagocytosis and protein aggregate load[12]. Alternatively, another hypothesis is that the switch from the resting state to the activated state may induce a stress response as gene expression changes, processes are retracted, and intercellular communication increases and thus, α-crystallins are upregulated to promote a smooth transition between the resting and activated states. Indeed, since it has been shown that αB-crystallin can interact with several cytoskeletal proteins, increased expression in microglial cells may play a role in process retraction and migration of microglia to the site of injury[13–15]. The role of α-crystallins in microglial cells is only in the preliminary stages of investigation, but the functions and mechanisms associated with these chaperones elucidated from other cell types offer many exciting possibilities moving forward.

1.2 α-Crystallins in Macroglial cells

Aside from the microglial cells, the central nervous system contains several other non-neuronal cells playing more supportive roles for the entire nervous tissue.

1.2.1 Astrocytes—Similar to microglia, astrocytes, which are neuroectodermal in origin, are also known to exist in two primary states, resting and reactive[16]. However, astrocytes and microglia primarily serve very distinct functions in normal neural tissue, as astrocytes are more directly involved in neuronal support and homeostasis regulation. The resting state of astrocytes is characterized by their role in synapse homeostasis, maintaining ion and neurotransmitter levels, and bridging the neuronal and vascular networks for metabolic support. Unlike microglia, astrocytes display a variety of morphologies, from the classic star-like appearance with several, long processes to that of the radial glial characterized by two main processes. The morphology of the astrocytes is highly dependent on where in the CNS the astrocytes are located. In response to pathogens or injury, astrocytes can also transition to a reactive state in which the cell body expands, the processes retract, and proinflammatory and reactive specific genes are highly expressed. Reactive astrocytes can in some cases migrate to the area of injury to provide immune support. More generally the astrocytes already residing at the damage site can react and form a glial scar, and aid with neuronal recovery after resolution of the insult.

Increased levels of expression of α-crystallins in astrocytes have been reported in several models of disease-associated neuroinflammation. Indeed it has been shown in reactive astrogliosis associated with Alzheimer's disease[3, 5], Parkinson's disease[5, 6], Alexander disease[17], prion disease[18], tauopathies[7], multiple sclerosis (MS)[19, 20], stroke[21], traumatic injury[22], and retinopathy as a complication of diabetes[23, 24]. Analogous to microglia, α-crystallin expression increases during or following activation of astrocytes suggesting a role for the chaperones in regulating the function of reactive astrocytes in response to stress including pro-inflammatory signals. Expression of α-crystallins, as well as other heat shock chaperones, can be induced in astrocytes by cytokines including TNF-α, TGF-β2, and several members of the interleukin family[25–27]. Induced expression by pro-inflammatory cytokines, in conjunction with their upregulation in diabetic retinopathy and MS suggests an important role for α-crystallins in the ongoing activation of astrocytes during chronic, pathological neuroinflammation.

As α-crystallin chaperones can regulate apoptosis by binding pro-apoptotic proteins, one hypothesis for the function of increased α-crystallin expression in astrocytes is to prevent apoptosis. Indeed, overexpression of either αA- or αB-crystallin in cultured astrocytes prevented cell death induced by ceramide and staurosporine[28] or H2O2[29]. This anti-apoptotic function has been linked to the p38 MAPK signaling cascade, the activation of which has been shown to result from inflammation. Interestingly, prevention of apoptosis in astrocytes by increased α-crystallins may be dependent on its phosphorylation state, an observation consistent with previous findings that phosphorylation of α-crystallins take place in response to various types of stress and regulates their chaperone activity[30–33].

One interesting role of αB-crystallin in astrocytes is exemplified by the pathology of Alexander disease. In this disease, mutations in glial fibrillary acidic protein (GFAP), an

intermediate filament upregulated in reactive astrocytes, cause the protein to become insoluble and aggregate in the cytoplasm of astrocytes resulting in cell death. αB-crystallin is upregulated in this disease[17] and localizes to the GFAP inclusions[34]. Overexpression of αB-crystallin in cultured astrocytes with GFAP inclusions results in resolubilization of the GFAP and enhanced cell survival[34]. These data support a role for αB-crystallin in cytoskeletal organization within reactive astrocytes to prevent aggregation. Although these GFAP inclusions are specific to this disease, αB-crystallin may still function to organize the cytoskeletal network to promote efficient activation and migration of the glial cells in other disease conditions.

The chaperone functions proposed for α-crystallins in astrocytes have all been described in non-glial cell types, but α-crystallins may also play a role in mechanisms specific to glial cells. For example, in cultured astrocytes lacking αB-crystallin, expression of pro-inflammatory molecules was significantly increased compared to controls[35]. Consistent with this observation, astrocytes from αB-crystallin deficient mice are highly activated even in absence of stress, though it is not associated with neuronal cell loss[35]. These data suggest that αB-crystallin may play a role outside its normal chaperone function that regulates astrogliosis and activation through a novel pathway. In a recent study, Shao et al. used a mouse model of aging that lacks the dopamine receptor, DRD2[35]. Correlative with aging and the progressive impairment of cognitive and motor skills is the downregulation of DRD2 in specific regions of the brain such as the substantia nigra. Astrocytes lacking DRD2 are actually hyper-responsive, becoming more highly activated in response to stimulus. Loss of DRD2 in both cultured astrocytes and animal models significantly decreased expression of αB-crystallin at the same time, while increasing the expression of pro-inflammatory molecules. Furthermore, overexpression of αB-crystallin in the *Drd2-null* astrocytes reversed the effects of the loss of DRD2. These novel findings suggest that astrocytes may play an integral role in the aging brain and that modulation of their role in neuroinflammation, specifically through pathways involving αB-crystallin, may slow the progression of cognitive and motor function impairment associated with both normal aging and age-onset neurodegenerative disease.

1.2.2 Specialized macroglial cells: Oligodendrocytes and Müller cells—

Oligodendrocytes have the specific function, distinct from those of astrocytes and microglia, of producing myelin, which makes up the myelin sheath protecting the axons of neurons in the CNS. Oligodendrocytes have several processes that connect to the myelin segments on axons and are responsible for secreting both myelin and its various binding partners. Recurring loss of the myelin sheath, such as is the case in MS, leads to increased neurodegeneration and accumulation of immune cells such as infiltrating lymphocytes, macrophages, and activated microglia and astrocytes. In fact, αB-crystallin was identified not only as a binding partner of myelin, but also an important antigen leading to the increased inflammation and phagocytosis of myelin sheaths[20]. In active lesions, αB-crystallin expression is significantly increased in both oligodendrocytes and astrocytes and may be involved in the stress response in these cells, though the role of αB-crystallin specifically in oligodendrocytes has not been elucidated[19]. In addition to its impact in MS, αB-crystallin is also involved in oligodendrocyte survival as a result of ischemia of the optic

nerve. In experimental anterior ischemic optic neuropathy, oligodendrocytes undergo apoptosis within the first week and there is progressive demyelination within two weeks. During these first weeks, αB-crystallin is upregulated in the optic nerve, correlative with increased microglial activation, suggesting activation of an inflammatory and stress response at the optic nerve[36]. Additionally, intravenous or intravitreal injections of purified αB-crystallin led to a decrease in microglial activation in response to experimental ischemia as well as an increase in oligodendrocyte survival[36]. Whether the effect on oligodendrocyte survival is a direct effect of αB-crystallin interaction with the oligodendrocytes or an indirect result of the chaperone's regulation of the local inflammatory environment is still unknown. In this model, administration of αB-crystallin did not increase ganglion cell survival, but other reports using optic nerve injury models to investigate α-crystallin function in neuroprotection provide contradicting evidence. Addition of α-crystallin promoted axon survival and regeneration in an optic nerve axotomy model[37] and prevented ganglion cell death in an optic nerve crush model[38]. All of these reports, however, highlight the role of α-crystallin as an anti-inflammatory agent in the immune response to optic nerve injury.

The retina contains specialized radial glia called Müller glia, with a characteristic morphology, extending processes among the various retinal layers. Müller glia play an important role in the retina as the architectural support for the neurons and in regulation of neuronal homeostasis, including recycling neurotransmitters, maintaining the ionic environment, and cleaning neuronal debris after injury. Like the other glial cells, Müller glia are activated in response to disease and injury. Interestingly, α-crystallins are increased in the Müller glia in both rodent models of diabetes[23] as well as in diabetic human donor tissues (our unpublished data). These data suggest that similar to other glial cells, α-crystallins may play a role in survival or activation of these specialized glia.

Despite the increased expression of α-crystallins in all types of glial cells, reported data suggest that α-crystallins may have distinct roles in each cell type (overview in Figure 2). As each cell type may respond differently to stress and has an individual role in the inflammatory response, it is understandable that α-crystallins function differently, even among glial cells. Furthermore, various types of neuroinflammatory insults may also trigger distinct cell signaling pathways, which then direct α-crystallin to specific functions. Therefore, the function of α-crystallin in astrocytes in response to amyloid aggregates in the brain may be very different from the function of α-crystallin in astrocytes in reactive MS lesions and much work is needed to elucidate how these chaperones can be targeted therapeutically in each specific disease state.

2. Regulation of Glial Cells function by α-Crystallins

2.1 The role of α-crystallins in microglial activation

The α-crystallin chaperones affect microglial and astrocytic activation, most often in an anti-inflammatory manner resulting in decreased cytokine production and tempering of the innate immune response. However, as discussed in the first part of this review, α-crystallin expression can be upregulated in activated glial cells in response to various conditions and may therefore also be involved in regulating cytokine production from within the glial cells.

In any given condition, only a fraction or sub-population of the glial cell population tends to express α-crystallin, a population often localized to a specific region near or at the injury site. Moreover, a growing number of reports have described the ability of exogenously applied α-crystallin to control glial cell activation and attenuate the immune response. One of the first reports to show that α-crystallins played a role in microglial activation incubated cultured primary microglia cells from newborn rats with purified α-crystallin and examined the levels of cytokine production[39]. Interestingly, this report showed that both αA- and αB-crystallin independently increased production of TNFα and iNOS in non-activated microglia suggesting a role for regulation of the innate immune response by extracellular α-crystallins. Of note, this initial report suggested that α-crystallins are promoting inflammation by inducing expression of pro-inflammatory cytokines. Interestingly, a report ten years later observed a similar effect of α-crystallins, but noticed that increased cytokine production only occurred in non-activated microglia and not in microglia already activated by injury or natural triggers (lipopolysaccharides)[40]. Several more recent reports showed that mice lacking α-crystallins showed increased basal inflammation, while additional reports determined that treatment of mouse models of inflammation and treatment of cultured cells with α-crystallins results in a decrease in overall cytokine production and glial cell activation[36, 38, 40–43]. Thus, the vast majority of studies suggest that α-crystallins are anti-inflammatory signals and play a role in attenuating the immune response during disease, though they may also contribute to the initial microglial activation.

As described previously, glial cell activation primarily occurs through signals originating from the extracellular environment directing glia to respond to pathogens or neuronal injury in the CNS. How, then, could an intracellular molecular chaperone known for its role in protein folding and regulation of apoptosis, not only make it out into the extracellular space, but also then bind to glial cells to cause an anti-inflammatory signaling cascade? Several theories have been investigated as to how α-crystallins get into the extracellular space, and once there, what role they may have, especially in the context of inflammatory diseases. This will be the focus of the next part of this review.

2.2 Exosome-mediated Secretion of α-Crystallins

Intercellular signaling is a critical process in the immune response, guiding activation, cytokine secretion, phagocytosis, and attenuation. One of the key pathways involved in these processes is the exosome-mediated pathway, in which target molecules to be secreted are encapsulated in membrane-bound vesicles called exosomes, often including a sampling of the cytoplasm as well. Exosomes themselves are then collected in larger membrane-bound vesicles, which can then fuse with the plasma membrane, spilling their contents into the extracellular space. Once in the extracellular space, these exosomes can interact with the plasma membrane of neighboring cells, and through an as yet not well-understood process, be taken up into the cell where they can affect signaling pathways by releasing their content. This pathway is clearly important in cellular communication between inflammatory cells, as several types of glial cells have been shown to secrete exosomes, thus perhaps coordinating an immune response among several different cell types[44].

Several members of the HSP family of chaperones have been shown to be present in exosomes and actively secreted into the extracellular space by a wide variety of cell types including macrophages and B lymphocytes[45–47]. Among them, αB-crystallin, specifically, has been shown to be secreted by exosomes. In two independent studies, researchers showed that another important non-neuronal cell type of the retina, retinal pigment epithelial (RPE) cells, can secrete αB-crystallin via exosomes, both under basal conditions as well as in response to oxidative stress, in which secretion is exacerbated[48, 49]. These data suggest that secretion of α-crystallins may be a normal aspect of the stress response. Interestingly, in polarized RPE cells, αB-crystallin was preferentially secreted from the apical side, or the interface of the RPE with the photoreceptor cells. Further analysis showed that under stress conditions, exosomes carrying αB-crystallin were taken up by both photoreceptors and neighboring RPE cells suggesting that secretion of αB-crystallin is stress-dependent and possibly neuroprotective[49].

More recently, a study has shown that secretion of αB-crystallin from astrocytes also occurs via an exosome-mediated pathway[50]. These tumor-derived astrocytes are a model for glioblastoma multiforme, an aggressive and common form of human brain cancer in which inflammation drives progression of the disease. In this study, the authors showed that both expression and secretion of αB-crystallin was intensified when cells were treated with TNF-α or IL-1β, mimicking the pathophysiological state. This study also demonstrated for the first time that glial cells can secrete αB-crystallin in response to pro-inflammatory cytokines and suggests that αB-crystallin may play a role in the normal intercellular signaling pathways between different types of inflammatory cells or between inflammatory cells and surrounding neurons. Given its role as an anti-inflammatory molecule, one could speculate that secretion of αB-crystallin is part of a biofeedback network between different types of cells involved in the inflammatory response to prevent chronic, damaging neuroinflammation. Contrarily, exosomes loaded with αB-crystallin from glial cells may be targeted for uptake by neurons and may thus play more of a neuroprotective role by directly promoting survival of neurons. Though these two hypotheses are not mutually exclusive, further investigation is needed to determine the impact of exosome-mediated secretion of α-crystallins on both glial and neuronal cells.

2.3 The role of α-crystallins as DAMPs

The local innate immune response is propagated on the recognition of “non-self” and “altered self” patterns, also called “danger signals,” by molecules and receptors expressed by glial and neuronal cells[51]. These molecules and receptors are called pattern recognition receptors (PRRs). PRRs can be either soluble or membrane-bound and the outcome is based on the type of ligand and receptor that interact. There are several classes of receptors expressed on glial cells responsible for recognizing both self and non-self molecules, and each receptor can bind multiple ligands suggesting a large number of outcomes that could result. In order to make better sense of the complexity of possible interactions, combinations of ligands and receptors have been classified into groups termed “molecular patterns” and designated by the initial immune-activating signal. For example, pathogenic-associated molecular patterns (PAMPs), in which a foreign or microbial invader is the cause of the immune response, are the network of cytokines, ligands, and receptors involved in

responding to foreign pathogens. A less well-characterized class are the danger-associated molecular patterns (DAMPs) in which dying cells express certain “altered self” signals which promote clearance of dead and dying cells to maintain tissue homeostasis. The best-characterized DAMP signal is phosphatidyl-serine, which is expressed on the plasma membrane of apoptotic cells, but other signals include HMGB-1, nucleic acids, sugars, and oxidized LPLs[52].

Many types of neurodegenerative diseases display associated neuroinflammation suggesting that apoptotic neurons may signal neighboring glial cells and may do this through the expression or release of DAMPs. As HSP chaperones are upregulated in response to stress and often localize to the site of the insult, they are logical candidates as DAMPs. Several HSPs, including Hsp60 and Hsp70 are expressed in injured axons, and can be released by cells undergoing necrosis or apoptosis [53–56]. A growing body of evidence suggests that HSPs can bind to Toll-like receptors (TLRs), one class of PRRs that recognize other known DAMPs and are integral to glial cell signaling and cytokine production[57–59]. Interestingly, both αA- and αB-crystallin are expressed by retinal neurons under stress condition[23, 60, 61], and have also been shown to bind TLR4 and TLR2 suggesting that α-crystallins may also regulate glial cells as a result of release from dying neurons[62–64]. Therefore, another function for the extracelllular localization of α-crystallins is its potential involvement in such signaling from neurons to the glial cells to promote clearance of cell debris.

Interestingly, a very new theory suggests that some HSPs belong to a new class of immune signals termed resolution-associated molecular patterns (RAMPs)[65, 66]. These immunoregulatory chaperones are involved in restoring immunological homeostasis and thus may be important targets for treating chronic neuroinflammation during disease.

2.4 Amyloid Fibrils and α-crystallins

Several neurodegenerative diseases associated with neuroinflammation including Alzheimer’s disease, Parkinson’s disease, and tauopathies are characterized by the deposition of amyloid plaques in the extracellular space. Several types of amyloid fibrils have been shown to trigger the innate immune response suggesting that the accumulation of amyloidogenic protein into aggregates may represent a novel DAMP[67–74]. Although the mechanism driving secretion of amyloid fibrils is still highly debated, one of the hypotheses is that their presence in the extracellular space is a neuroprotective event resulting in these toxic species being secreted by neurons, and inducing glial cell activation and clearance of the fibrils by phagocytosis. One of the major innate immune pathways triggered by amyloid fibrils is the activation of the inflammasome, which can be activated by a variety of signals including ATP, bacterial toxins, and various crystals[75, 76]. Therefore, amyloid fibrils seem to be another endogenous ‘danger’ signal. Further investigation are underway to establish whether the ability of amyloid fibrils to trigger an immune response is beneficial or harmful, what other pathways or receptors are targeted by these aggregates, and whether these plaques will be useful therapeutic targets in preventing chronic neuroinflammation.

As molecular chaperones, one of the primary functions of αA- and αB-crystallin is to prevent protein misfolding by binding to exposed hydrophobic regions and preventing

aggregation[77]. Both αA- and αB-crystallin can prevent amyloid fibril formation by various proteins including amyloid-β, β2-microglobulin, and α-synuclein[78–83]. In disease conditions, α-crystallins can be found localized to these extracellular aggregates including amyloid-β plaques and neurofibrillary tangles composed of tau in Alzheimer's disease[3, 84]. It is possible that this localization of α-crystallins to the extracellular amyloid plaques would have a critical impact on the associated glial cell activation but this remains to be clearly established. While studies have shown that fibrils of purified amyloidogenic protein are sufficient for glial cell activation in vitro, the presence of α-crystallins in amyloid aggregates in vivo may contribute to the glial response, perhaps as a negative regulator[85]. Recent studies have shown a correlation between the chaperone activity of αB-crystallin and its immune-regulatory ability, specifically its ability to bind proinflammatory proteins and reduce aggregation[86]. Additionally, a short region of the chaperone, aa73–92, was shown to be sufficient to reduce paralysis and ameliorate neuroinflammation in an EAE model [70]. Of all the peptides tested, only the peptides that exhibited chaperone activity were able to reduce inflammation, suggesting that the immune-regulatory activity of α-crystallins may be intricately connected to the chaperone's ability to bind client proteins.

Interestingly, short peptides analogous to those used in the previous study to demonstrate that chaperone activity was critical for therapeutic function were also shown in an independent study to form amyloid fibrils[87]. In fact, Tanaka and colleagues demonstrated that the chaperone activity of this short peptide of αA-crystallin was dependent on its ability to form amyloid fibrils. Following this discovery, several hexapeptides of αB-crystallin were shown to not only form amyloid fibrils, but in doing so were able to bind and precipitate pro-inflammatory proteins[70]. This new data correlated with previous data that amyloid-β fibrils were able to precipitate similar plasma proteins[88]. Therefore, amyloid fibrils are endogenous regulators of the innate immune response and this ability of proteins, including α-crystallins, to form amyloid fibrils can be utilized when designing therapies to reduce inflammation.

2.5 α-Crystallins as auto-antigens

A major finding that brought the role of α-crystallins in immune regulation into light, was the study performed by van Noort and colleagues in 1995 showing that αB-crystallin was enriched in the myelin in the brain of patients with MS and was a potent antigen for T cells from both healthy and MS patients[20]. Since then, the role of αB-crystallin as an autoantigen in inflammatory diseases has been under intense investigation. One of the confounding parts of the data on human T cell activation by αB-crystallin is that T cells from both healthy and MS patients react to αB-crystallin as an antigen suggesting this response is not exclusively disease-associated. Several studies have been reported investigating autoantibodies against αB-crystallin[89–93]. Unfortunately, these reports have failed to conclusively demonstrate if antibodies against αB-crystallin are increased in patients with a wide range of chronic disease conditions including diabetes, cancer, neuropathies, and cardiomyopathies. These cumulative data suggest that αB-crystallin can indeed induce an adaptive immune response, and to this end, immunodominant epitopes within α-crystallins have been identified that are recognized by antibodies from patients[94]. At first glance, these data appears to be somewhat in contradiction with the anti-

inflammatory role of crystallins depicted in the previous sections of this review but could reflect the impact of unresolved long-lasting inflammation.

One hypothesis put forth to resolve this question is based on the very definitions of the innate and adaptive immune responses. The innate immune response is the first response to pathogens or insult and as such is critical in the activation of the adaptive response. If the innate immune response is sufficient to efficiently resolve the situation, recruitment and activation of the adaptive immune network is unnecessary. However, in cases where the innate network is overwhelmed by the infection or injury, a secondary and more specific response is needed leading to activation of the adaptive immune response. The sum of the reported studies suggest that αB-crystallin is a critical regulator of the innate immune response, but its accumulation could also play a pivotal role in determining when and if an adaptive immune response should be mounted. As the glial cells accumulate at the site of infection or injury in the CNS, so do α-crystallin proteins[20, 91, 95, 96]. Thus the accumulation of autoantibodies against α-crystallins could result from the increased local concentration and increased risk that α-crystallins are taken up by antigen-presenting cells and used to signal T cell activation. This hypothesis has led to suggest that α-crystallins circulating antibodies could be a specific marker of an overwhelmed immune response, and thus a potentially valuable marker in measuring chronic advert neuroinflammation.

3. Treatment of inflammatory disease with α-crystallins

As with all disease-associated research, the primary goal is to identify molecules and pathways that are good therapeutic targets in order to prevent or slow disease pathology and the correlated symptoms. In the case of autoimmune and inflammation-associated diseases, the targets are unfortunately more challenging. The human immune response has evolved as a pro-survival mechanism and a way to fight disease and infection; treating an inflammatory disease is thus not as simple as preventing an immune response. Additionally, the immune response is a collection of inter-connected networks of pro- and anti-inflammatory signals with an array of receptors that can be utilized for both purposes. +Treating these diseases will not be as easy as targeting a single pathway or receptor as its role in inflammation must be understood in the context of all the other receptor-mediated pathways that make up the collective response.

The discovery that α-crystallins are negative regulators of neuroinflammation offers a real and novel approach for treating diseases associated with inflammation. Several studies have used the administration of α-crystallins to treat the symptoms of inflammatory disease with promising results. Addition of α-crystallins has been shown to decrease inflammation in models of MS, bacterial infection, stroke, spinal cord injury, optic nerve injury, ischemic optic neuropathy, and COPD[36–38, 41–43, 97–100]. Administration of α-crystallins not only prevents damaging inflammation, but also prevents neurodegeneration in these models, leading to a reduction in symptoms. In addition, since α-crystallins are endogenous regulators, present in many tissues of the body and already function as chaperones, these chaperones are part of the glial and neuronal cells' normal stress response and, as such, would minimize activation of off-target pathways.

One of the challenges to using proteins therapeutically is the purification and folding of those proteins to ensure their function once administered. Several recent reports have shown that peptides of α-crystallin can be just as functional as the fully folded protein[70, 86, 87, 101–105]. Use of the mini-chaperone peptides engineered by Sharma and colleagues or the amyloidogenic hexapeptides characterized by Steinman and colleagues offer a more controlled and more affordable alternative to fully folded proteins. These peptides can perform similar chaperone activity to the whole protein and can interact with glial cells to attenuate inflammation. These peptides can be easily synthesized in high concentrations with no contaminating factors and are much less susceptible to be targeted by function-altering post-translational modifications. The major phosphorylation sites affecting αB-crystallin chaperone function [106, 107] are indeed outside of the most promising peptide sequence aa73–92 [102]. Furthermore, while the fully folded protein can act as an antigen to activate the adaptive immune response, these peptides can be engineered so as to not contain the epitopes necessary for recognition by antibodies, thus decreasing the risks of activation of the adaptive response. Finally, peptides such as these can be loaded into nanoparticles or micro-vesicles for delivery to specific tissues [99]. Use of a ‘vehicle’ to deliver drugs and small molecules to their target tissues is becoming more common and peptides of α-crystallin are certainly amenable to conjugation with various vehicles to target delivery.

All in all, though much more work needs to be done to determine the mechanisms of α-crystallin function in immune regulation and neuroprotection, the most current data strongly suggest that α-crystallins may be a novel target in treating inflammatory diseases and dampening the effects of chronic neuroinflammation.

4. Concluding Remarks

Though still not well understood, a link between neuroinflammation and the chaperones α-crystallins has clearly been established over the last two decades. The role of α-crystallins in regulation of the immune response has evolved from identification of the chaperone as an antigen in MS models, through its initial characterization as a proinflammatory molecule, to our current understanding of the chaperone as an antiinflammatory agent. Preliminary investigations in mice have even begun to report the use of the chaperone as treatment for inflammatory diseases. While the exact nature of the role of α-crystallins in the immune response is being pursued, this raises the question of the role of other small heat shock proteins in the innate immune response. Though not discussed here, Hsp27 and Hsp22 have also been shown to function in immune regulation. These studies have shed light on new roles for chaperones outside of the classical protein folding pathways and offer intriguing opportunities for the identification of novel immune-regulatory pathways endogenous to the CNS.

Though our initial understanding of the immune response as a beneficial network still holds true, we are beginning to understand how a good system can be corrupted over time and promote disease pathology rather than prevent it. As the number and type of diseases associated with detrimental inflammation grows, our basic understanding of the pro- and anti-inflammatory networks must also expand to include novel pathways and to connect the known pathways propagated by many different cells simultaneously. Although attaining this

understanding will be considerably challenging, the benefits will be far-reaching, promoting multi-discipline approaches to therapeutic research across a wide variety of diseases classically thought of as distinct and independent.

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Highlights

- → Alpha-crystallins are expressed during neuroinflammation
- → Alpha-crystallins are expressed by microglia and macroglial cells
- → Alpha-crystallins are intrinsically involved in neuroinflammation regulation
- → The exact role of alpha-crystallins in neuroinflammation is complex and still requires a lot of attention

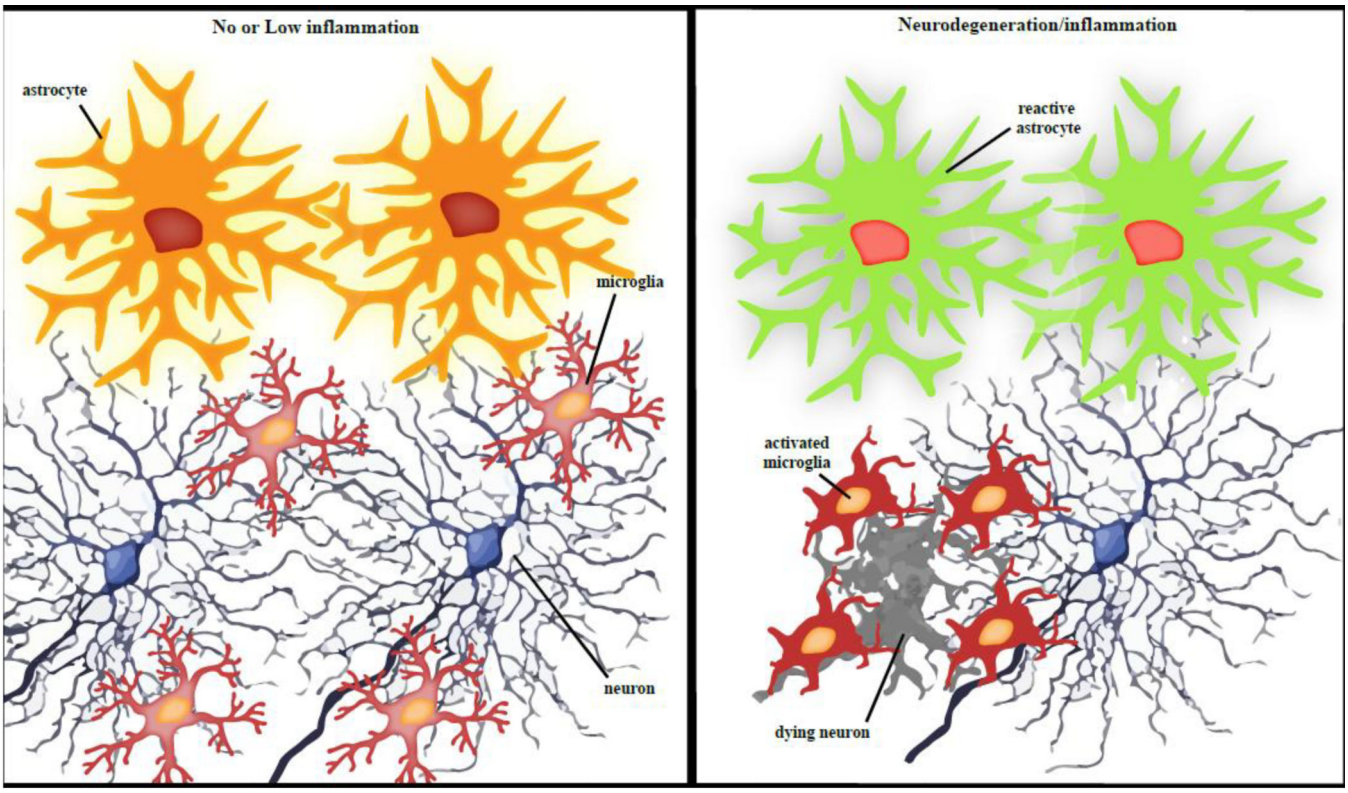

Figure 1. Overview of the neuroglial status in the CNS under normal or pathological conditions

Schematic of the interaction of microglia and macroglial cells with neurons and the changes induced by neurodegeneration/neuroinflammation conditions which include neuronal cell death as well as astroglia reactivity and microglial activation.

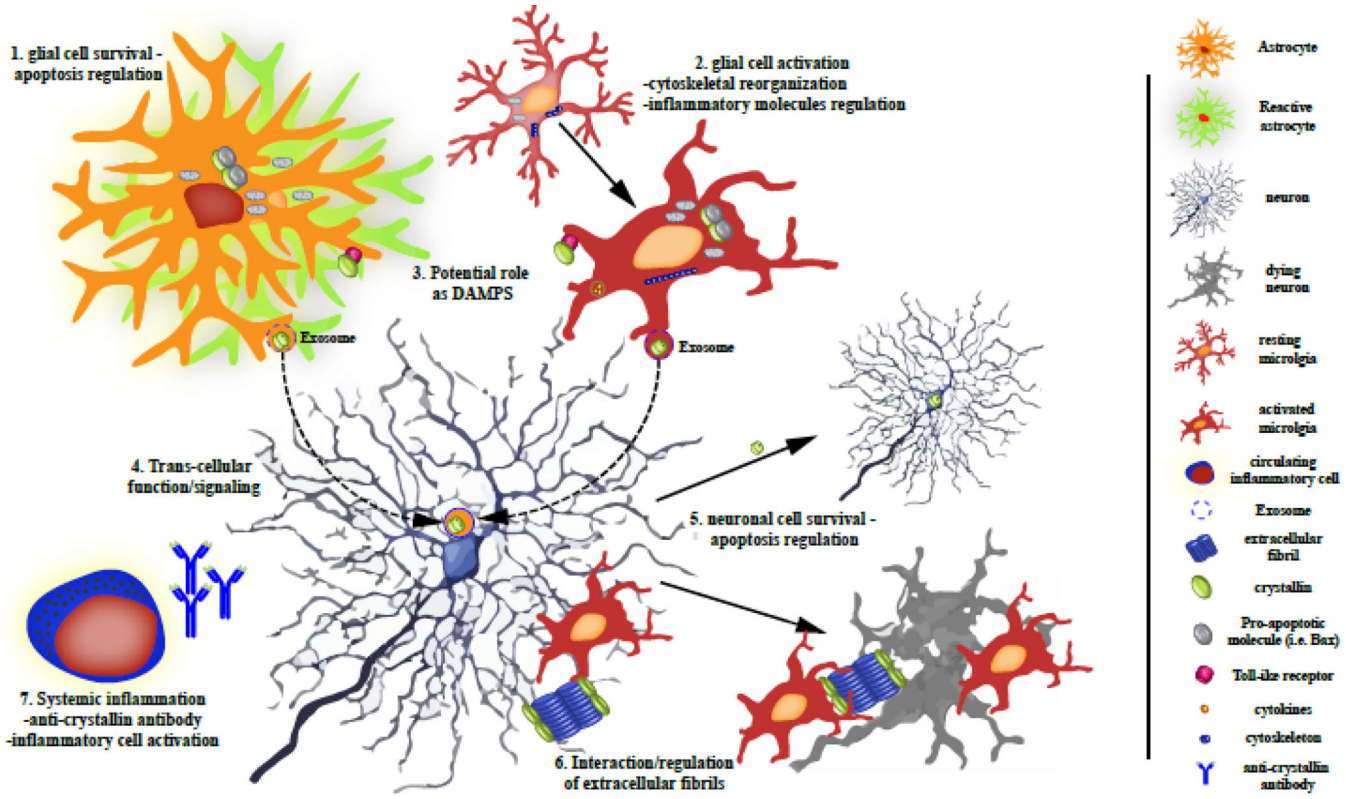

Figure 2. Overview of the different roles that crystallin proteins can play in the CNS, especially during neuroinflammation

Schematic representation of the various roles and implications that crystallin proteins can have in neuronal survival, CNS tissue homeostasis, and glial cell function.

Table 1

microglial and macroglial cells display upregulation of αB-crystallin in a variety of diseases and conditions.

	Upregulation of αB-crystallin	Reference
Microglia	Alzheimer's disease	2 – 5
	Parkinson's disease	6
Astrocytes	Alzheimer's disease	3, 5
	Parkinson's disease	5, 6
	Alexander disease	17
	Diabetic Retinopathy	23, 24
	Multiple Sclerosis	19, 20
	tauopathies	7
	prion disease	18
	traumatic brain injury	22
	stroke	21
	activation by cytokines	25 – 27
Oligodendrocytes	Multiple Sclerosis	19, 20
Müller glia	Diabetic Retinopathy	23 (rodent)
		unpublished (human)