Chapter 9
The NLRP3 Inflammasome and its Role in Age-Related Macular Degeneration

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Abstract Age related macular degeneration (AMD) is the most common cause of blindness among people of 65 years and older in developed countries (Klein and Klein, Invest Ophthalmol Vis Sci 54:7395–7401, 2013). Recent advances in dry AMD research points towards an important role of the inflammatory response in the development of the disease. The presence of inflammatory cells, antibodies, complement factors and pro-inflammatory cytokines in AMD retinas and drusen indicates that the immune system could be an important driving force in dry AMD. The NLRP3 inflammasome has been proposed as an integrator of process associated with AMD and the induction of inflammation. Herein we summarize the most recent studies that attempt to understand the role of the NLRP3 inflammasome in AMD.

Keywords Blindness · Complement system proteins · Cytokines · Immune system · Immunity · Inflammasomes · Inflammation · Macular degeneration

9.1 Introduction

Using genome wide association studies, variations in the complement factor H (CFH) have been associated with AMD (Narayanan et al. 2007; Shastry 2007). Complement proteins are also found in drusen (Mullins et al. 2000). These studies

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provided a potential link between inflammatory processes and the development of AMD. Over the years the hypothesis that sterile inflammation plays a key role in the development of AMD has taken center stage (Camelo 2014). As a result, researchers have focused on the NLRP3 inflammasome signaling pathway.

When activated, the NLRP3 inflammasome forms a large cytoplasmic complex (Stutz et al. 2009). The Nod-like receptor family, pyrin domain containing 3 (NLRP3) is an intracellular receptor that responds to wide range of pathogen associated molecular patterns (PAMPS) and danger associated molecules (DAMPS) such as extracellular ATP (Cassel et al. 2009). It has a ligand binding leucine-rich repeat domain (LRR), a nucleotide binding and oligomerization domain (NATCH), and a pyrin domain (PYD). Upon engagement by a ligand, the NLRP3 receptor associates with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) through a PYD-PYD interaction. The recruited ASC, in turn, recruits the pro-caspase-1 via its caspase activation and recruitment domain (CARD) (Srinivasula et al. 2002). This CARD-CARD interaction activates caspase-1, which can then process the pro-forms of the interleukins 1 beta (IL-1β) and 18 (IL-18). These proteolytically activated cytokines are then secreted to initiate a pro-inflammatory response.

The expression of NLRP3 and the transcription of both IL-1β and IL-18 are regulated by transcription factor NF-kB. Signaling pathways such as those initiated by the Toll-like Receptor 4 (TLR-4) can activate NF-κB and induce the expression of NLRP3 and its signaling components (Bauernfeind et al. 2009). A secondary signal sensed by the NLRP3 receptor is responsible for the assembly of the inflammasome multi-protein complex.

9.2   Activation of the NLRP3 Inflammasome in AMD

The NLRP3 inflammasome has been found to be present in samples from AMD patients (Kaneko et al. 2011). Several compounds associated with AMD have been shown to activate the inflammasome. The reactive aldehyde 4-hydroxynonenal (4-HNE) was demonstrated to activate the inflammasome *in vitro* (Kauppinen et al. 2012). The addition of 4-HNE to ARPE-19 cells (a human RPE like cell line) caused the secretion of IL-1β, thus suggesting a potential link between oxidative stress and activation of the inflammasome. Proteins modified by carboxyethylpyrrole (CEP), an oxidation production of docosahexanoic acid, have been discovered within drusen from patients with AMD (Crabb et al. 2002). The CEP adducts have been shown to induce the activation of the inflammasome (Doyle et al. 2012) and activate macrophages (Cruz-Guilloty et al. 2014) when delivered *in vivo*. Another molecule associated with AMD that was demonstrated to induce the activation of the inflammasome is the pyridinium bisretinoid A2E (Anderson et al. 2013). A2E is a byproduct of the condensation of all trans-retinal that accumulates within the RPE cells with aging. The internalization of A2E can induce the secretion of IL-1β

in ARPE-19 cells. Anderson et al. also demonstrated that in ABCA4 knock-out mice there are increased levels of IL-1β that correlate with increase in A2E accumulation.

Amyloid beta (Aβ) protein is seen in drusen (Johnson et al. 2002). One of the effects of Aβ in the RPE is the induction of senescence. Further more, Aβ induces the secretion of matrix metalloproteinase 9 and the destabilization of the tight junctions between the RPE cells (Cao et al. 2013), suggesting that Aβ can induce the breakdown of the retina-blood-barrier known to occur in AMD. In another report, Liu and co-workers demonstrated that intravitreal injection of Aβ in rats resulted in the induction of IL-1β, IL-18, and MIP-3α (CCL20) (Liu et al. 2013). This group also reported an increase in all the components of the NLRP3 inflammasome not only in the RPE/choroid layer but also within the neural retina. Their results suggest that cells other than microglia can be a source of inflammasome activation.

One mechanism proposed to activate the inflammasome in AMD is the destabilization of the lysosomes. Destabilization of lysosomes in ARPE-19 cells resulted in activation of caspase-1 and release of IL-1β (Tseng et al. 2013). Cell death induced by the lysosomal destabilization was abrogated by the inhibition of caspase-1, the key enzyme in the process of pyroptosis (Fernandes-Alnemri et al. 2007; Miao et al. 2011). Similarly, defects in autophagy in the RPE may stimulate inflammation (Kaarniranta et al. 2013) by activating the inflammasome.

Inflammasome activation can be stimulated in RPE cells when co-cultured with activated microglia (Ma et al. 2009). When transplanted sub-retinally, activated microglia promotes neovascularization and RPE disorganization. These results suggest that migration of microglia into the subretinal space contributes to AMD by provoking inflammation and dysplasia of the RPE.

9.3   Cytokines Induced by the NLRP3 Inflammasome and Their Role in AMD

The cytokine IL-1β is a potent pro-inflammatory cytokine. As one of the cytokines processed by the NLRP3 inflammasome, the role of IL-1β on AMD has become of great importance to AMD research. A potential role for IL-1β in AMD was highlighted by Marneros et al. who showed that VEGF-A, a molecule associated with the development of neovascular AMD, can induce the secretion of IL-1β (Marneros 2013). The knock-down of either NLRP3 or IL-1R decreased the neovascular lesions characteristically observed in mice that over-express VEGF-A. Of note, when IL-18 was knocked down in this model, a modest increase in the neovascular lesions was observed.

Once activated by caspase-1, IL-18 is secreted from the cell. Extracellular IL-18 can bind to either its cognate receptor IL-18R or to the IL-18 binding protein (IL-18BP). Upon binding to IL-18R, a signaling pathway involving the activation of the protein MyD88 leads to the expression of other cytokines such as VEGF, IL-6 and TNF-α (Dinarello et al. 2013).

The function of IL-18 in the development of AMD remains unclear. In 2012 Doyle et al. reported that deletion of the NRLP3 followed by laser injury of the retina leads to an increase in neovascularization when compared with eyes that expressed this receptor (Doyle et al. 2012). This group also reported that drusen isolated from AMD eyes increase IL-1β secretion from peripheral blood mononuclear cells obtained from healthy human donors. In a follow up study, they demonstrated that pro-IL-18 induces the swelling of RPE cells leading to cell death (Doyle et al. 2014). Injecting the active form of the IL-18 into the mouse retina did not cause damage to the tissue, however. In agreement with their original findings, they found that injection of IL-18 either alone or in combination with anti-VEGF therapy reduced neovascularization in the laser-induced CNV mouse model. Their results point towards a protective role of IL-18 in wet AMD.

Conflicting data regarding the protective role of IL-18 on AMD has emerged from different labs. Researchers reported in 2011 that there is a decreased expression of the enzyme DICER in donated eyes from patients with AMD. In the same article, Kaneko and colleagues demonstrated that decrease of this enzyme is sufficient to induce RPE damage due to the accumulation of the *Alu* RNA (Kaneko et al. 2011). In follow up studies, this group demonstrated that the *Alu* induced RPE toxicity was dependent on the expression of the NLRP3 inflammasome components such as caspase-1 and PYCARD (Tarallo et al. 2012). To test the IL-18 protective role hypothesis, this group injected IL-18 in mice lacking caspases-1 and found that it induced an RPE damage similar to the accumulation of *Alu* RNA.

9.4   Targeting the NLRP3 Signaling Pathway

The purinergic receptor P2X7 was shown to modulate the activity of the NLRP3 inflammasome in *Alu*-induced AMD model (Kerur et al. 2013). Mice lacking the expression of P2X7 or NF-κB were protected from the RPE damaged induced by the *Alu* RNA. Another proposed target for the treatment of AMD is the signaling molecule MyD88. The inhibition of MyD88 with an inhibitor peptide protected mice from the degeneration induced by *Alu* RNA (Tarallo et al. 2012). One potential advantage of targeting MyD88 is that it is important for both the induction of NLRP3 expression and as a signaling component of the IL-18 receptor.

Although conflicting evidence regarding the function of IL-18 in AMD remains to be resolved, this cytokine presents another potential target for therapy. It is likely that increased expression of IL-18 exacerbates the inflammatory response in early AMD and in geographic atrophy. While Campbell et al. (Campbell et al. 2014; Doyle et al. 2014) have suggested injecting purified IL-18 as a treatment for wet AMD, this protein is a potent inflammatory cytokine with significant potential side effects relative to current inhibitors of VEGF signaling.

The extracellular-signal-regulated kinase 1/2 (ERK1/2) has been implicated in AMD. Inhibition of ERK by the specific inhibitor PD98059 protected the RPE of mice treated with *Alu* RNA (Dridi et al. 2012). No protection was observed when

mice receive inhibitors of either p38 or JNK. Interestingly, the route of administration utilized in this study was systemic which protected their retinas without adverse side-effects. Their results suggest that ERK 1/2 could be a potential target in AMD.

The rate limiting step of the inflammasome signaling is the activation of caspase-1, which makes it a therapeutic target for the treatment of AMD. Mice lacking caspase-1 are viable and develop normally (Kuida et al. 1995). Furthermore, several CARD only proteins (COPS) have been identified as negative regulators of the inflammasome signaling suggesting that it is plausible to inhibit its activity under certain situations (Le and Harton 2013). The induction of some of these COPS, or their exogenous over expression within the retina via gene therapy is an alternative that deserves further investigation.

9.5   Conclusion

Even though patients affected by AMD do not usually succumb to complete blindness, their visual impairment significantly affects their quality of life. Current treatment for wet AMD is based on the monthly injection of biological agents like ranibizumab that block VEGF signaling thus halting the growth of new blood vessels. Unfortunately there is no treatment available for dry AMD. Being a multifactorial disease there are different animal models of the disease that recapitulate certain aspects of the disease (Fletcher et al. 2014). The consensus among experts in the field points towards an active role of NLRP3 signaling in both dry and wet AMD (Campbell and Doyle 2013). By studying different animal and cellular models of AMD and human specimens from donor patients it has been possible to identify several important molecules associated with the NLRP3 inflammasome signaling pathway that could be targeted as a therapy. With the development of novel animal models of AMD, especially those with a defined macular region, developing an effective treatment for geographic atrophy becomes more likely.

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