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A brief discussion on lipid activated nuclear receptors and their potential role in regulating microglia in age-related macular degeneration (AMD)

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Abstract

Age-related macular degeneration (AMD) is the leading cause of legal blindness and visual impairment in individuals over 60 years of age in the Western World. A common morphological denominator in all forms of AMD is the accumulation of microglia within the sub-retinal space, which is believed to be a contributing factor to AMD progression. However, the signaling pathway and molecular players regulating microglial recruitment have not been completely identified. Multiple *in-vitro* and *in-vivo* studies, to date, have highlighted the contributions of nuclear receptor ligands in the treatment of inflammation related disorders such as atherosclerosis and Alzheimer's disease. Given that inflammation and the immune response play a vital role in the initiation and progression of AMD, in this brief review we will highlight some of these studies with a particular focus on the lipid activated "adopted orphan" nuclear receptors, the liver x receptors (LXRs) and the peroxisome proliferator-activated receptors (PPARs). The results of these studies strongly support the rationale that treatment with LXR and PPAR ligands may ameliorate microglial activation in the sub-retinal space and ultimately slow down or reverse the progression of AMD.

XX. 1 Introduction

Age-related macular degeneration (AMD) is one of the leading causes of progressive blindness in the elderly (Coleman et al. 2008). Clinically, AMD progresses from early to intermediate stages of the disease and subsequently to the two major advanced forms, namely, geographic atrophy (GA) or "late dry" and neovascular or "wet" AMD. The pathogenesis of early AMD involves the accumulation of lipid- and protein-rich extracellular deposits called drusen under the retinal pigment epithelial cells (RPE). The progression to the late dry form involves RPE dystrophy with a loss of photoreceptors in the central macula and subsequent blindness. Wet AMD, which affects approximately 10% of the AMD patients, is characterized by development of abnormal choroidal neovascularization (CNV) under the retina, which leads to scarring in the macular region. Currently there are no treatments available for dry AMD, but anti-angiogenic approaches targeting vascular endothelial growth factor (VEGF) are available for wet AMD patients with some success.

Therefore there is an immediate need to identify new targets and develop alternate therapeutic approaches to help people afflicted with this disease.

XX. 2 Microglial cells accumulate within the retina and subretinal space of AMD patients

Retinal microglia represent a population of macrophages, which constantly survey their microenvironment, responding to cellular damage by increasing their phagocytic activity (Karlstetter and Langmann 2014). Multiple reports have corroborated the role of inflammation and microglial cells in the pathogenesis of the early and late forms of AMD (Patel and Chan 2008). The sub-retinal space, the interface between the RPE and the outer segments of photoreceptors, in particular, is a region of great interest in studies of inflammation in AMD. Under normal conditions, retinal microglia are excluded from the outer retina, due to the presence of immunosuppressive factors secreted by the RPE (Zamiri et al. 2007). As such, RPE cells play an important role in immunomodulation of the outer retina, regulating RPE-microglial interactions through expression of cytokine receptors, production and secretion of inflammatory cytokines and adhesion molecules, and regulation of the tight-junction integrity (Holtkamp et al. 2001; Streilein et al. 2002). In advanced age, following light-induced photoreceptor injury, and in late AMD, an influx of microglia to outer retina has been observed, followed by their accumulation within the sub-retinal space (Ng and Streilein 2001; Gupta et al. 2003). In support of this, evaluation of retinal samples from the *Cx3cr1*^{-/-} mice [chemokine (c-x3-c motif) receptor 1, important in microglial migration], has revealed the accumulation of subretinal microglia associated with drusen-like deposits, RPE structural alterations, and CNV formation (Combadiere et al. 2007; Tuo et al. 2007). It is clear, that a better understanding of RPE-microglial cell interactions is imperative in accurately explaining the inflammatory etiology of AMD and ultimately developing new therapeutic targets.

XX.3 Overview of lipid-activated nuclear receptors

Nuclear receptors are the largest superfamily of transcription factors in the human genome. There is increasing evidence of their involvement in metabolic regulation of immune cells. This mini-review will focus on the role of the liver x receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) in shaping the metabolic and immune functions of microglial cells and macrophages, since these receptors have been most extensively studied in diseases, which share common pathogenic pathways with AMD, including atherosclerosis, metabolic syndrome and Alzheimer's disease.

LXRs are critical regulators of cholesterol homeostasis, glucose homeostasis, detoxification of bile acids, immunity, and neurological functions (Apfel et al. 1994). Their activating ligands include endogenous oxidized and hydroxylated cholesterol derivatives (22(R)-hydroxycholesterol and 24(S)-hydroxycholesterol) and synthetic agonists (GW3965 and TO901317) (Lehmann et al. 1997; Viennois et al. 2011). Although the two isoforms, LXR α (NR1H3) and LXR β (NR1H2) show significant similarities in their DNA binding domain and ligand binding domains, their tissue expression patterns are different (Jakobsson et al.

2012). LXR α is predominantly expressed in metabolically active tissues, while LXR β is ubiquitously expressed (Laffitte et al. 2001).

PPARs were originally discovered as receptors that induce the proliferation of peroxisomes in *Xenopus* (Dreyer et al. 1992). Three isoforms have been identified (Berger and Moller 2002). PPAR α (NR1C1) regulates fatty acid oxidation and is highly expressed in tissues which perform substantial mitochondrial and peroxisomal β -oxidation such as brown adipose tissue, liver, kidney and heart (Kliwer et al. 1994). PPAR β/δ (NR1C2) has a ubiquitous expression pattern and plays a more general role in the activation of oxidative metabolism (Escher et al. 2001). PPAR γ (NR1C3) plays a major role in the activation of adipocyte differentiation and is expressed in adipose tissue (Tontonoz et al. 1994). A broad range of endogenous molecules can act as agonists for the PPARs. These include a variety of unsaturated fatty acids, branched chain fatty acids, oxidized fatty acids eicosanoids, phospholipids and serotonin metabolites (Schupp and Lazar 2010). A number of synthetic ligands have also been identified for the different isoforms of PPARs (Grygiel-Gorniak 2014). PPAR α ligands include fenofibrate, clofibrate and gemfibrozil; PPAR β/δ ligands include GW0742, GW501516 and GW9578; PPAR γ ligands include rosiglitazone, pioglitazone, troglitazone, ciglitazone, farglitazar, S26948 and INT131.

XX. 4 LXRs and PPARs regulate inflammation

In addition to their role in reverse cholesterol transport, LXRs are important regulators of inflammatory gene expression and innate immunity. Regulation of inflammation by LXRs can be highlighted by reviewing previous studies demonstrating that LXR activation downregulates the expression of pro-inflammatory molecules, such as inducible nitric oxide synthase (iNOS), IL-6, IL-1 β , cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1 (MCP-1), prostaglandin E2, and matrix metalloproteinase-9 (MMP-9) in cultured macrophages, and primary isolated microglia and astrocytes in response to lipopolysaccharide (LPS) stimulation or bacterial infection (Castrillo and Tontonoz 2004; Rigamonti et al. 2008). LXR agonists can also attenuate inflammation through suppression of NF- κ B DNA-binding activity, by blocking the degradation of I κ B- α in LPS-stimulated microglia (Zhang-Gandhi and Drew 2007). Similarly, GW3965 attenuates LPS-induced inflammation in primary rat Kupffer cells through repression of tumor necrosis factor- α (TNF- α) and prostaglandin E2 (Wang et al. 2009). Another LXR agonist, T0901317, has been shown to downregulate interferon- γ (IFN- γ), TNF- α and IL-2 secretion by Th1 lymphocytes (Liu et al. 2012). Finally, LXR agonists have been shown to attenuate inflammatory responses *in vivo*, in experimental autoimmune encephalomyelitis, and irritant and allergic contact dermatitis models (Hindinger et al. 2006; Cui et al. 2011).

PPARs, also important in maintaining lipid homeostasis through regulation of fatty acid metabolism, have been shown to be molecular mediators of inflammatory pathways. For example, PPAR β/δ -dependent repression of NF- κ B/AP1 transcription represents a major mechanism of attenuating inflammation by PPAR β/δ agonists (Schnegg and Robbins 2011). PPAR γ activation leads to protection against atherosclerosis through reduced expression of inflammatory markers such as TNF- α and MMP-9 in both macrophages and artery wall tissue samples (Chawla et al. 2001a). While, loss of PPAR γ bone marrow expression was

associated with a significant increase in atherosclerotic lesion development. It is important to note, that an alternative explanation of the anti-inflammatory and atheroprotective effects of PPAR γ has been proposed and this involves its ability of PPAR to crosstalk and induce LXR α expression. This in turn can lead to induction of cholesterol efflux as well as attenuation of expression of pro-inflammatory molecules in macrophages (Chawla et al. 2001b). The ability of LXRs and PPARs to repress expression of pro-inflammatory cytokines provides us with a likely therapeutic target to attenuate inflammation and their harmful downstream effects.

XX.5 Rationale for studying the therapeutic potential of nuclear receptors in AMD

Morphological examinations of retinas from AMD patients have revealed the accumulation and retention of activated microglia within the outer nuclear layer as well as the sub-retinal space (Gupta et al. 2003). The presence of these immune cells in the outer retina may contribute to the initiation of AMD pathology. The convergence of these morphological studies of AMD tissue, and investigations of nuclear receptor regulation of inflammation in other diseases that share common pathogenic pathways with AMD, advocate the notion that reversal of age-related accumulation and influx of activated microglia modulated by nuclear receptors is a viable path to pursue to ameliorate the progression of AMD. Cellular targets for prevention and/or reversal of microglial influx may include the RPE, since RPE cells are critical in maintaining the immunosuppressive state and are contributors to local cytokine production and secretion. While the microglial cells, which in their activated state have been shown to be associated with drusenoid deposits as well as CNV lesions, would be potential targets for reversal of immune cell influx, serving as a therapeutic avenue for the treatment of both forms of late AMD. Most recently direct evidence for the use of LXR agonists in late AMD comes from studies, which have demonstrated that treatment with LXR agonists in an eye drop formulation is effective in reducing the severity of CNV lesions in an experimental model of wet AMD (Sene et al. 2013). Though throughout this review we have focused on the potential benefit of targeting nuclear receptors in decreasing inflammation, it is not trivial to note that, additionally, these ligands may also slow down AMD progression by regulating cholesterol and lipid homeostasis.

XX.6 Conclusions

In the healthy retina, microglia are excluded from the sub-retinal space. However, changes in the sub-retinal microenvironment and RPE due to aging results in invasion of the sub-retinal space by these immune cells, where they can tip the balance to a “pathological state” and contribute to the progression of AMD. Given the potential that activation of LXRs and PPARs can lead to a downregulation of pro-inflammatory signals, targeting these nuclear receptors appear to provide an important therapeutic opportunity to tip the balance back again to a homeostatic state and hopefully either delay the onset of AMD or slow down its progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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