

Chapter 2

Role of Chemokines in Shaping Macrophage Activity in AMD

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Abstract Age-related macular degeneration (AMD) is a multifactorial disorder that affects millions of individuals worldwide. While the advent of anti-VEGF therapy has allowed for effective treatment of neovascular ‘wet’ AMD, no treatments are available to mitigate the more prevalent ‘dry’ forms of the disease. A role for inflammatory processes in the progression of AMD has emerged over a period of many years, particularly the characterisation of leukocyte infiltrates in AMD-affected eyes, as well as in animal models. This review focuses on the burgeoning understanding of chemokines in the retina, and their potential role in shaping the recruitment and activation of macrophages in AMD. Understanding the mechanisms which promote macrophage activity in the degenerating retina may be key to controlling the potentially devastating consequences of inflammation in diseases such as AMD.

Keywords Retinal degenerations · Age-related macular degeneration (AMD) · Inflammation · Macrophages · Microglia · Chemokines

2.1 Introduction

Age-related macular degeneration (AMD) affects millions of individuals worldwide, and is the leading cause of blindness in the industrialised world (Ambati et al. 2003a). AMD is a multifactorial disorder, involving complex interaction between environmental and genetic factors. Evidence for a role of inflammation

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in progression AMD has been accruing over a period of many years, particularly through the observations of leukocyte infiltrates within AMD-affected eyes (Penfold et al. 2001; Forrester 2003).

2.2 Macrophage Recruitment in AMD

The involvement of inflammatory processes in the histopathology of AMD was first noted almost 100 years ago (Hegner 1916), and several histological studies since have established the presence of aggregations of choroidal leukocyte infiltrates in association with disciform macular lesions (Hegner 1916; Paul 1927; Green and Key 1977).

Those early observations were confirmed and extended in a number of electron microscopical investigations which demonstrated the involvement of a number of inflammatory cells—including macrophages, lymphocytes, and mast cells—in RPE atrophy, and breakdown of Bruch's membrane (Penfold et al. 1984, 1985). Macrophages and other leukocytes have also been described in excised neovascular membranes (Lopez et al. 1991; Gehrs et al. 1992; Seregard et al. 1994). Ultrastructural studies also identified a close relationship between macrophages and the formation of choroidal neovascular membranes in wet AMD (Penfold et al. 1987). Multinucleated giant cells—which may form through union of multiple macrophages or microglia (Dickson 1986)—have also been found to correlate spatially with regions of breakdown in Bruch's membrane and with CNV (choroidal neovascularisation) (Penfold et al. 1985). Chronic involvement of macrophages and giant cells has also been shown in atrophic AMD lesions, and on the expanding edges (Penfold et al. 1987; Cherepanoff et al. 2009). Other investigations have shown changes in parenchymal microglia in association with early AMD, including increased MHC-II expression and morphological changes suggestive of activation (Penfold et al. 1997). In advanced AMD, activated amoeboid microglia infiltrate the ONL and subretinal space in the degenerating outer retina, where they are associated with neovascular structures (Combadiere et al. 2007), and appear to have a role in the phagocytosis of photoreceptor debris (Gupta et al. 2003; Combadiere et al. 2007).

2.3 Role of Chemokines

First discovered in 1987 (Walz et al. 1987; Yoshimura et al. 1987), chemokines are a large, growing family comprising more than 50 molecules interacting with at least 20 chemokine receptors, that play an important role in the chemotactic guidance of leukocyte migration and activation (Moser and Loetscher 2001; Bajetto et al. 2002). Chemokines are small molecules grouped according to the relative position of their first N-terminal cysteine residues, comprising C (γ chemokines), CC (β chemokines), CXC (α chemokines), and CX3C (δ chemokines) families (Loetscher et al.

2000; Murphy et al. 2000; Zlotnik and Yoshie 2000; Bajetto et al. 2002). These may be expressed by endothelial cells, resident macrophages (including microglia), as well as infiltrating leukocytes (Crane and Liversidge 2008). Chemokines exert their biological activity through binding cell surface chemokine receptors, which are part of the superfamily of seven transmembrane domain receptors that signal through coupled heterotrimeric G-proteins, consisting of C, CC, CXC, CX3C receptor subclasses (Bajetto et al. 2002). Many of these receptors show a degree of redundancy, as multiple chemokines may bind several receptors; although interactions are mainly restricted to within particular subclasses (Bajetto et al. 2002). Chemokine expression typically generates chemical ligand gradients, which serve as directional cues for guidance of leukocytes bearing the appropriate chemokine receptors to sites of injury, and are also thought to aid in extravasation of leukocytes (Luster 1998).

The expression of chemokines in the guidance and activation of macrophages has garnered considerable interest in AMD. Retinas from human donors show increased expression of both α (Cxcl1, Cxcl1) and β (Ccl2) chemokine genes in 'wet' and 'dry' AMD (Newman et al. 2012), while elevated levels of Ccl2 protein—a potent chemoattractant for monocytes (Matsushima et al. 1989; Yoshimura et al. 1989)—have been detected in aqueous humour samples taken from patients in advanced stages of AMD (Jonas et al. 2010; Kramer et al. 2011). Additionally, elevation in Ccl2 is evident within atrophic 'dry' AMD lesions and is accompanied by influxes of monocytes expressing Ccr2 (Sennlaub et al. 2013), which is the receptor for Ccl2 signalling (Yoshimura and Leonard 1990).

A direct role of chemokines has been elucidated with animal models of AMD (Patel and Chan 2008). Investigations using laser-induced CNV in mice have focused on the role of β chemokine signalling in neovascular AMD. Ablation of Ccl2 using target gene knockout has been shown to inhibit the infiltration of macrophages and results in reduced lesion size following laser-induced CNV compared to controls (Luhmann et al. 2009). Moreover, a mouse knockout of the receptor Ccr2 exhibits decreased macrophage recruitment and vastly reduced neovascularisation following experimental laser-induced CNV (Tsutsumi et al. 2003). In models of atrophic 'dry' AMD which utilise bright light as a damaging stimulus (Marc et al. 2008; Rutar et al. 2010), the suppression of Ccl2 using either ablation or siRNA-mediated knockdown reduces macrophage recruitment and the extent of cell death (Rutar et al. 2012; Sennlaub et al. 2013). Conversely, other studies suggest that a degree of β chemokine signalling may be necessary for the maintenance retinal homeostasis, and prevention of AMD. An investigation in aged, dual Ccl2/Ccr2 knockout mice showed retinal features similar to AMD including formation of lipofuscin, drusen, photoreceptor degeneration, and neovascularisation (Ambati et al. 2003b), although the AMD-like phenotype in this model has been questioned (Luhmann et al. 2009). Ccl2/Ccr2 knockout results in the accumulation of hypertrophied subretinal macrophages, possibly because of impaired monocyte trafficking (Luhmann et al. 2009).

The only δ chemokine receptor characterised, Cx3cr1, has also been implicated in maintenance of homeostasis and genesis of AMD-like pathology. Cx3cr1 is a chemokine receptor found on microglia, macrophages, astrocytes, and T-cells (Patel

and Chan 2008), whose ligand chemokine Cx3cl1 is constitutively expressed on many cell types in the retina, and together are thought to mediate the trafficking of microglia and macrophages in the clearance of extracellular deposits (Fong et al. 1998; Silverman et al. 2003). Targeted knockout of Cx3cr1 in light-stressed mice induces progressive degeneration of photoreceptors in correlation with an accumulation of engorged subretinal microglia/macrophages and other AMD-like features (Combadiere et al. 2007). Moreover, ablation of Cx3cr1 is associated with an increase in lesion size following experimental neovascularisation (Combadiere et al. 2007).

2.4 Summary

Over a period of many years, the role of inflammation in AMD has gradually emerged as an important factor underpinning its pathogenesis. This is exemplified by traditional histological examinations and electron microscopy identifying macrophage/microglial infiltration in AMD-affected eyes, and more recently through investigations utilising animal models. The expression of chemokine-related genes is prodigious in all forms of AMD pathology, and animal models of both ‘dry’ and ‘wet’ AMD indicate that chemokine expression modulates both the recruitment and activation of macrophages, as well as the extent of retinal degeneration. Reducing inflammation by altering macrophage activity in retina may prove an important therapeutic tool in ameliorating degeneration in AMD.

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