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Immuno-modulatory Effect of IFN-gamma in AMD and its Role as a Possible Target for Therapy

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Abstract

Age-related macular degeneration (AMD) is a neurodegenerative disease characterized by retinal cell atrophy, and/or choroidal neovascularization in the macula and constitutes the most common cause of blindness among the elderly in industrialized countries. The management of AMD is constrained by our insufficient knowledge of its underlying mechanisms. Recent studies point towards an emerging involvement of interferon-gamma (IFN-γ), a soluble cytokine associated with innate and adaptive immunity. IFN-γ promotes proinflammatory responses by activating proinflammatory cytokines and chemokines, thereby recruiting immune cells such as macrophages and T cells. On the other hand, IFN-γ modulates inflammatory response by upregulating anti-inflammatory factors or inhibiting development of immune cells related to autoimmune response. The complex role of IFN-γ in AMD pathogenesis is intriguing and worth further investigation in terms of therapeutic development.

Keywords

Age-related macular degeneration; Interferon gamma; Cytokines; Pro-inflammatory modulation; RPE; Drusen; STAT1; CXCL11

Introduction

Age-related macular degeneration (AMD) is the most common cause of blindness among the elderly in industrialized countries [1]. AMD is characterized by retinal pigment epithelium (RPE) dysfunction and sub-RPE drusen formation in the early stage. With time, it may progress to retinal cell atrophy, and/or choroidal neovascularization in the macula. Recent studies incorporating genetic and epidemiological data have made a credible argument for chronic inflammatory events playing a central role in the pathogenesis and development of AMD. Interferon-gamma (IFN-γ), a soluble cytokine associated with innate and adaptive

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immunity, is considered to be a pro-inflammatory factor. Recent studies point towards an emerging relationship between IFN-γ and mechanisms underlying the pathogenesis of AMD. Along with other pro-inflammatory factors such as IL-1 and TNF-α, IFN-γ functions synergistically to activate inflammatory components, including the complement cascade and recruit immune cells such as macrophages, microglia, NK and T cells [2–5]. In AMD eyes, these immune cells are present in areas surrounding the outer retina and drusen deposits [6–8], they can induce direct damage to photoreceptors [8,9], potentially leading to vision loss. Yet, the interaction of pathways activated by IFN-γ is complex and not fully understood. Also, the role of IFN-γ as a possible therapy target is still unclear. Herein, we will review the literature on IFN-γ in the outer retina with focus on its role as a potential target for therapy for chronic inflammatory diseases of the eye.

Is IFN-γ a Possible Target for Treatment of AMD?

In our previous studies, we found that constituents of drusen such as amyloid beta and advanced glycation endproducts (AGE) are capable of activating the IFN-γ pathway [10,11]. AGE not only upregulated IFN-γ but also several of its downstream effectors including RSAD2, STAT1, CXCL10, and CXCL11 (Figures 1, 2 and3). Indeed, in postmortem human eyes, we found increased accumulation of RSAD2, CXCL10, and CXCL11 to be associated with the presence of drusen deposit [12]. Others have shown that *in vitro* stimulation of cultured RPE cells with IFN-γ led to polarized complement factor H (CFH) secretion predominantly localized to the apical surface [13–15]. This localization has been proposed to form a CFH gradient that could maintain retinal homeostasis and suppress a proinflammatory environment surrounding the photoreceptors. CFH is also a chemoattractant for monocytes [16]. In addition, when co-cultured with activated T cells, RPE cells produce an apical gradient of increased CCL7, CXCL9, CXCL10, and CXCL11 through T cell derived IFN-γ [5].

IFN-γ may be involved in AMD pathogenesis through macrophage polarization. Depending on the different microenvironment, macrophages can polarize into specific phenotypes, such as M1 or M2 macrophages [17]. The M2 subtype is predominantly pro-angiogenic, facilitating tissue repair and tends to increase during the normal aging process [18]. In contrast, the M1 subtype is predominantly proinflammatory and there is a pathological shift towards M1 subtype with the development of AMD. INF-γ can selectively promote polarization into M1 subtype [19]. M1 macrophages promote pathological inflammation through the secretion of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α [20,21]. IL-1β is a strong proinflammatory factor. Along with IFN-γ, it synergistically increases the expression and secretion of IL-6, a potent inflammatory factor involved in the autoimmune and inflammatory disorders, in RPE cells [2]. Stimulation of human cells with IFN-γ potentiates IL-1β release and production [22].

Antibodies designed to block IFN-γ activity have been effective in the treatment of chronic inflammatory disorders such as rheumatoid arthritis and Crohn’s disease [23,24]. Antagonizing the IFN-γ pathway has been investigated in the context of AMD. Interferons are separated into three subtypes (type 1, 2, and 3) and differentiation between subtypes is based on the receptor through which they signal [25]. Type 1 interferon comprises of large

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sub-categories in humans including IFN-α, β, ε, κ and ω [26]. INF-γ is the only member of the type 2 subclass [25]. More recently, IFN-λ has been discovered and it is currently the sole member of the type 3 subclass [27]. Type 1 interferon including IFN-α and β have anti-proliferative and anti-angiogenic effects and has an antagonistic role to IFN-γ [28–32]. In the 1990s, IFN-α and β were used in the treatment of AMD [33–35]. It was found that IFN-α has minimal long-term therapeutic benefit and this was postulated to be due to the generation of anti-IFN-α antibodies as a result of treatment [36,37]. The utility of IFN-β was found to be more promising as it promotes proliferation and repair of damaged RPE and regression of CNV in monkey AMD models. More studies on the effectiveness of IFN-β have been published in literature [38–41]. Taken together, these studies indicate the importance of IFNs in AMD pathogenesis.

However, the use of IFN-γ as a therapeutic target can be complicated since in lower concentrations, IFN-γ shifts from being a proinflammatory factor to a more anti-inflammatory one [42,43]. At low levels, IFN-γ impedes homing of naïve T cells and Th2 cells to target organ [44]. Th2 cells induce fibrosis thereby counterbalancing the destructive effects of Th1 cells, which promote apoptosis [45,46]. Thus in the pathology of AMD, blocking IFN-γ may reduce the protective effects of Th2 and consequently aggravating the destructive function of Th1 cells [20,47].

Is there any Beneficial Role of IFN-γ in Terms of Protective/Anti-inflammatory Effect?

The role of IFN-γ is complex, since IFN-γ is associated with both protective and destructive inflammatory processes. IFN-γ is classically considered as a pro-inflammatory factor, yet in recent years, multiple studies have found IFN-γ to mediate an immune-modulatory and protective function. For example, in human endothelial cells IFN-γ inhibits the angiogenic activity of VEGF through activation of STAT1 pathway [48], down-regulating VEGF mRNA in a dosage-dependent manner [49]. This may help to inhibit excess angiogenesis process in wet AMD. Interestingly, another study suggests that IFN-γ is able to mediate VEGF upregulation in RPE cells through the PI-3K/Akt/mTOR/p70 S6 kinase pathway, and is independent of STAT1 [50]. Therefore, IFN-γ associated STAT1 activation may be beneficial. Another piece of evidence comes from the study of IFN-γ up-regulating CFH expression in RPE cells [15]. CFH can keep the complement cascade in check and prevent tissue injury from excessive complement activation [51]. CFH is transcriptionally upregulated by STAT1, but oxidative stress, one of the most important risk factors for AMD, can disrupt this process by acetylating FOXO3, which competes with STAT1 for binding to the CFH promoter [15,52]. It is also known that STAT1-deficient mouse are highly susceptible to autoimmune disorders [53] and given that AMD may be considered an autoimmune disease [54,55], preserving STAT1 activation by IFN-γ may be important in mitigating AMD progression. Furthermore, IFN-γ can tilt the balance toward STAT1 by deactivating STAT3. STAT1 and STAT3 are negative regulators of each other and activate distinctly different downstream pathways [56]. STAT1 plays a key role in inhibiting angiogenesis, while STAT3 induces the production of VEGF directly or indirectly through hypoxia-inducible factor 1α in tumor cells [57-61]. INF-γ deactivates STAT3 by promoting

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STAT3 dephosphorylation [62]. Topical IFN-γ is being investigated for as a means of treatment for macular edema in uveitis (http://clinicaltrials.gov/show/NCT00943982).

IFN-γ further down-regulates the VEGF pathway through the up-regulation IL-1RA [42]. IL-1RA inhibits IL-1 receptor bindings to IL-1, and thus performs an anti-inflammatory function [42]. IL-1β is strongly implicated in the pathogenesis of chronic inflammatory diseases [63]. Indeed, human RPE cells treated with amyloid beta strongly upregulated IL-1β [10]. Aberrant auto-upregulation of IL- 1β leads to excessive inflammation and promotes angiogenesis through upregulation of VEGF [64]. IL-1β is capable of inducing reactive oxygen species (ROS) in RPE cells [65] and ROS triggers the release of IL-8, which recruits pro-inflammatory cells such as macrophages [10,65,66]. With macrophages present in drusen deposits of AMD eyes, it plays a key role in promoting neovascular proliferation [67,68]. In AMD models IL-1RA have been shown to be effective in reducing the degree of CNV formation, likely through the inhibition of IL-1 pathways [69].

IFN-γ may also play a beneficial role by regulating Th17 cells. Th17 cells have been characterized as a subclass of T cells and implicated in numerous autoimmune disorders including diabetes, autoimmune encephalomyelitis, autoimmune uveitis, and thyroiditis [43]. Recently Th17 cell specific cytokines, IL-17 and IL-22 are found to be elevated in serum of AMD patients, further implicating Th17 cells in the pathogenesis of AMD [70]. The upregulation of IL-17 is believed to be mediated by complement activation product C5a [70]. In AMD patients, C5a is elevated in serum and may be associated with AMD at risk gene variant, which regulates complement activation [51,71]. IFN-γ inhibits T cell differentiation into Th17 and in murine models of Th1 related autoimmune disorders, knocking out IFN-γ results in a more severe disease process. This worsening is believed to be mediated through Th17 cell [72–74].

Conclusion

In conclusion, IFN-γ plays an intriguing role in the pathogenesis of AMD. Certainly, several lines of evidence suggest that inhibition of IFN-γ may prevent inflammation-mediated responses that contribute to the progression of AMD. However, given the evidence suggesting its involvement in anti-inflammatory and neuroprotective mechanisms in a number of murine autoimmune disease models [31], it is still debatable whether therapeutic inhibition of IFN-γ pathways would help counteract the progression of AMD. Further characterization of the IFN-γ mediated immunomodulatory pathways that are involved in the pathogenesis of AMD is necessary.

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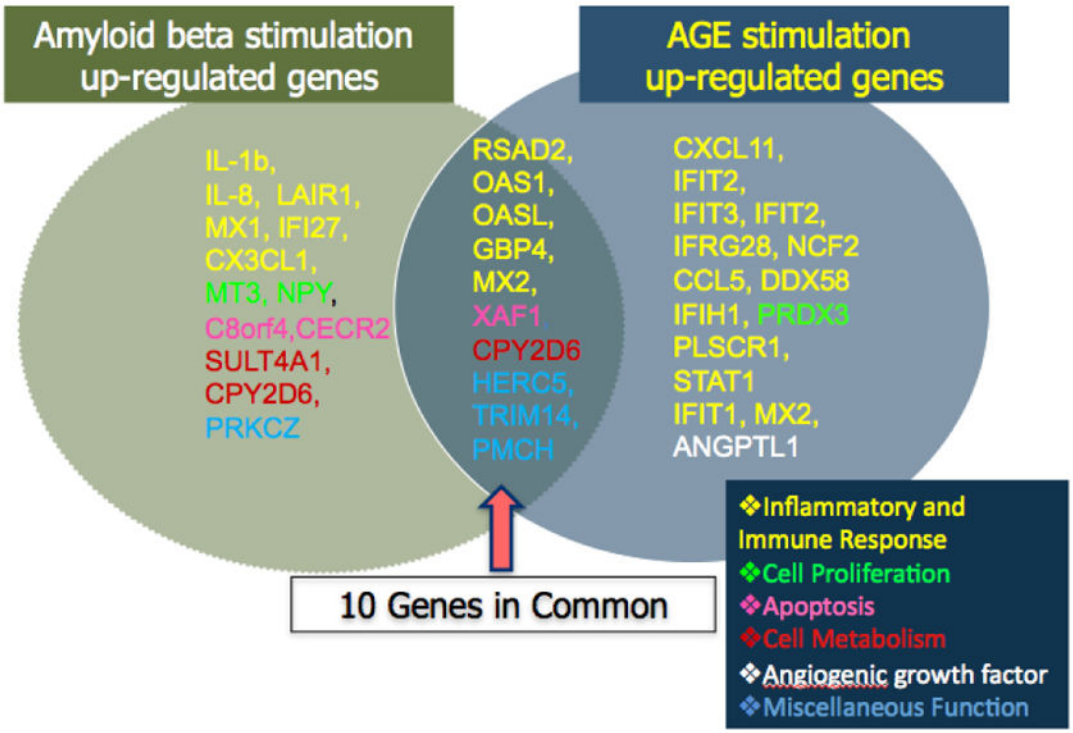

Figure 1.

Summary diagram of differentially expressed gene results obtained from a microarray study of human RPE cell response to *in vitro* stimulation with amyloid beta (0.3 μM, left oval) or advanced glycation endproducts (AGE, 10 μg/mL, right oval). Amyloid beta and AGE are two known components of drusen, and results suggest that both induce proinflammatory responses, including IFN-γ signaling.

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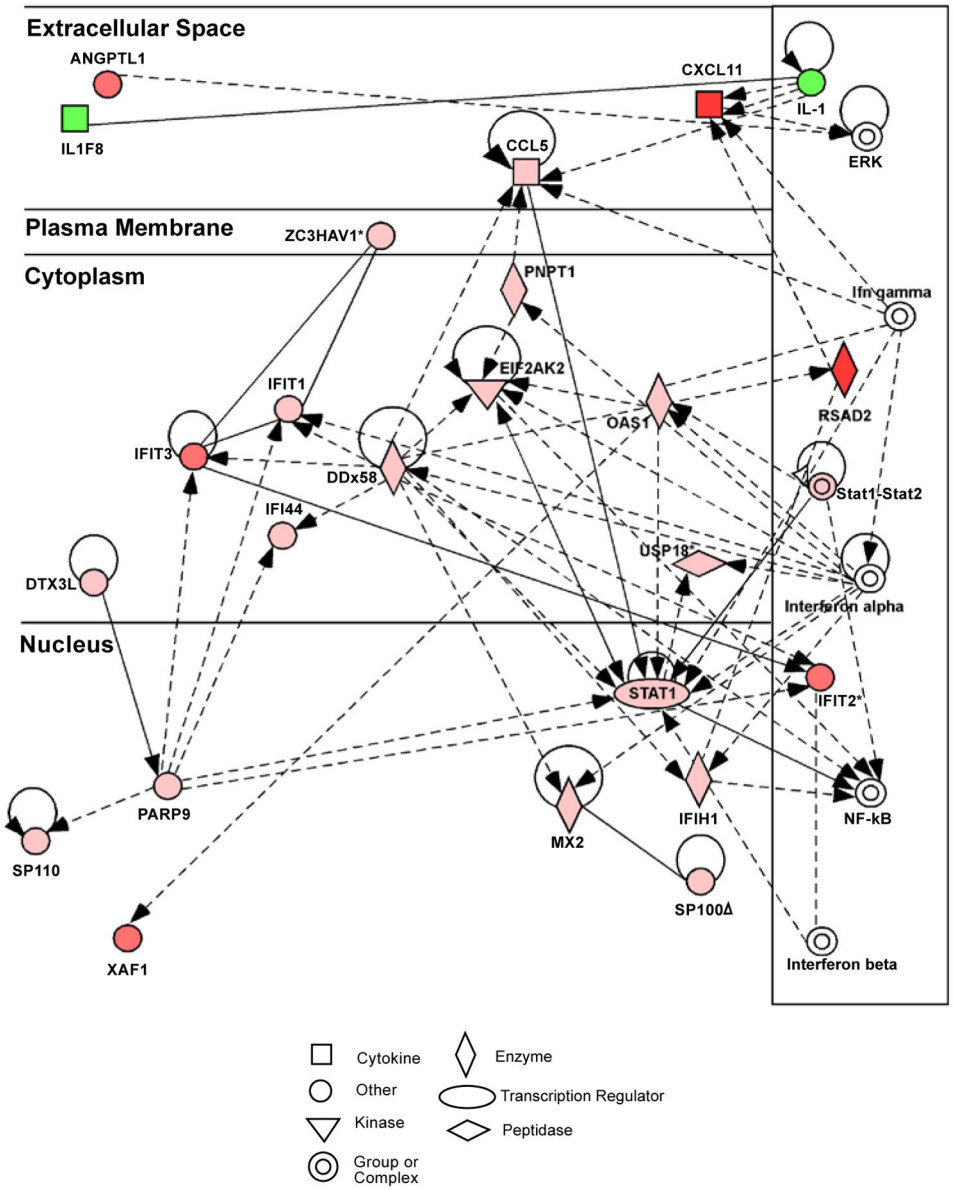

Figure 2.

Molecular network generated by Ingenuity Pathway Analysis (IPA) of highly significant gene changes in human RPE cells after *in vitro* stimulation with AGE (10 μg/mL). Colored symbols represent genes that were significantly highly upregulated (red) with decreasing relative levels indicated by lighter shades (pink and light pink) or downregulated (green) in our data set [11]. The white entries are molecules from the Ingenuity database, inserted to connect all relevant molecules in a single network. Solid lines indicate known direct physical relationships between molecules, while dashed lines indicate known indirect functional relationships. Note the chemokine, CXCL11, and RSAD2 (viperin) are shown to be highly upregulated in this network, and were also associated with drusen in postmortem donor eyes [12]. The top two functionalities identified by Ingenuity for this molecular network are

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“Interferon Signaling,” “Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses”.

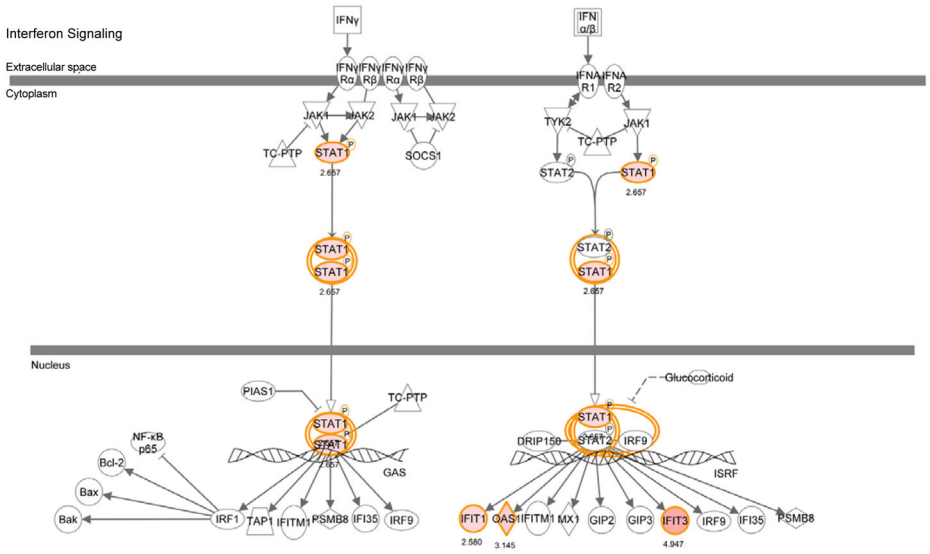

Figure 3.

The Interferon Signaling Pathway identified by Ingenuity software. This is one of the canonical pathways that contain statistically significantly more genes than expected by chance in the group of 41-up and 18-down regulated RPE genes in response to AGE stimulation in vitro. Red symbols represent the genes from our stimulation study, while the white symbols represent genes inserted by Ingenuity to connect relevant molecules into a single pathway.

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