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The Pathogenesis of Neovascular Age-Related Macular Degeneration and its Immune-Based Therapies

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Abstract

Age-related macular degeneration (AMD) is one of the leading causes of irreversible central vision loss in elderly individuals, further affecting their ability to do everyday tasks, from reading to even recognizing faces, and thus the need for comprehensional understanding of the disease is important for the discovery of treatments that can help in maintaining their visual acuity, studies have shown that a lots of factors play a role in the disease's pathogenesis from the structural changes and cellular dysfunction seen in aging, the role of inflammatory mediators especially the role of polymorphic complement H factor (CFH) gene to some controversial finding of the macrophages role in the disease progression, studies have come so far where immune-based therapies by using monoclonal antibodies against vascular endothelial growth factors (VEGF) which play a significant role in the disease's pathogenesis, with promising finding in the majority of patients.

Introduction

The eyes are the photosensitive organs responsible for vision, receiving light through the cornea, then the light is focused by the lens on the retina, which contains specialized cells responsible for photoreception, encoding various patterns of the image to be transmitted to the brain via the optic nerve (Gartner et al., 2015).

The fovea is the specialized regions of the retina which is responsible for discrimination of details and colored vision, accounting for the extremely precise visual acuity, it's found at the central part of the macula, and composed entirely of cone cells (Gartner et al., 2015).

Lots of diseases can affect the retina one of which is age-related macular degeneration (AMD) being the leading cause of irreversible central vision loss in developed countries, especially in individuals over the age of 50s (Gartner et al., 2015).

Clinically, there are mainly two types of AMD: the dry (non-exudative) and the wet (exudative), where the former (non-neovascular) type is characterized by extracellular deposition of yellow, polymorphous debris consisting mainly of amyloid-ß, ApoE, complement proteins and retinal metabolites known as drusen, while the latter (neovascular) type is characterized by the formation

of leaky subretinal choroidal neovascularization, where these blood vessels extend from the choriocapillaris (Luthert, 2011).

In fact, the neovascular form of AMD and geographic atrophy (also known as end-stage dry AMD or atrophic AMD) are advanced forms of dry AMD (Luthert, 2011).

Where it has been shown that patients with small drusen deposits are asymptomatic or they may have mild symptoms, but the expansion and coalescence of the deposits are hallmarks of AMD progression, in which the RPE cells can no longer support photoreceptor function owing to spontaneous degeneration of both (Luthert, 2011).

Considering that neovascular AMD is responsible for the vast majority of vision loss caused by AMD, the aim of this study is to highlight the immunological role in AMD pathogenesis and its immune-based therapy.

Materials and Methods

Animal models have been used with specific genes have been knocked out, to see their role in the pathogenesis of the disease, with the study of histopathological changes by the light microscope and the electron micrograph.

Results

One of the most significant recent advances in ophthalmology has been seen in the use of ranibizumab therapy in neovascular AMD, where a study involving 430 participants taking 0.3mg of ranibizumab has shown vision maintenance in 95% of patients with the loss of fewer than 15 letters in 12 months (Tashkin et al., 2008).

Vision improving also has been seen in another study where 716 participants were given 0.5mg of ranibizumab, and 34% of them gained 15 letters or more in 12 months (Tashkin et al., 2008).

Discussion

AMD is a complex disease with demographic and environmental factors (age, gender, diet, high bod-mass index, hypertension, and smoking), as well as the involvement of multiple polymorphic genes (Michalska-Małecka, Kabiesz, Nowak, & piewak, 2015).

The polymorphic genes associated with AMD including mainly those encoding CFH (complement factor H), high-temperature requirement A serine peptidase 1 (HTRA1), age-related maculopathy susceptibility 2 (ARMS-2) to the ApoE genotype involvement (Ambati, Atkinson, & Gelfand, 2013).

Retinal changes can be seen with aging, but with the wet form of AMD these changes are exacerbated, involving the deposition of drusen, BM thickening and in advance, photoreceptor degeneration may occur following the invasion of choroidal blood vessels through the BM into the retina (Luthert, 2011).

Generally, at early stages of AMD small, spread out deposits of (hard) drusen can be seen at this stage, where many patients show no symptoms, with time the accumulation of deposits leads to the formation of a large cluster with poorly defined edges called (soft drusen) (Luthert, 2011).

Also, the accumulation of abnormal basal laminar deposit like material as well as lipids deposition in BM causing it to become thicker, which reduce the fluid net flow causing the detachment of the RPE, as well as reducing the clearance of waste and delivery of nutrients to the most external layer of the retina (Luthert, 2011).

On the other hand, there is marked thinning of elastin forming the innermost layer of BM in patients AMD, where it has been shown that elastin degradation products have a pro-inflammatory effect as well as provoking the angiogenesis of choriocapillaris (Luthert, 2011).

It suggested that mutations in tissue inhibitor of metalloproteinases-3 (TIMP-3) increase the elastin degradation, where it appears that TIMP-3 bind preferentially to elastin (Luthert, 2011).

In angiogenesis lots of factors have been seen to affect the extension of highly preamble vessels from the choriocapillaris into the outer retina, as the response of RPE to the hypoxic environment by the releasing of (VEGF-A), to the linkage disequilibrium seen ARMS-2/Htra1 genes (Luthert, 2011).

Age-related maculopathy susceptibility 2 (ARMS-2) gene is found on the 10q26 region showing a strong association with high-temperature requirement factor A1 (Htra1) gene, where specific single nucleotide polymorphism (SNPs) in this region is strongly associated with AMD (Luthert, 2011).

The function of Htra1 is believed to be though binding transforming growth factor-beta family member, inhibiting its angiogenesis effect, while the ARMS-2 function is not clearly demonstrated yet (Luthert, 2011).

With all these changes existing there is also a decrease in the RPE function, it could be their existence in an environment with a high risk of oxidative causing their dysfunction, where failure of lysosome and autophagy-mediated digestion of cellular components is seen, which probably cause the local drusen biosynthesis (Ambati & Fowler, 2012).

As well as, the presence of macrophages, inflammatory mediators, complement factors and even auto-antibodies against the protein adduct carboxyethylpyrrole (CEP), all as components of drusen was an early pointer to the importance of inflammation in AMD (Luthert, 2011).

The overall immunological role in the pathogenesis of neovascular AMD

Normally, the retina has a degree of the immune privilege conferred by the intact blood-retinal barrier, the expression of endogenous immunosuppressive factors and the absence of functional intraocular lymphatics (Ambati et al., 2013).

However, the presence of specific foreign or endogenous inflammatory signals evokes innate immune responses, where the resident inflammatory cells of the retina are the microglia, working

in a similar way to tissue macrophages, and are found in proximity to retinal blood vessels in the inner layers of the neural retina (Ambati et al., 2013).

The subretinal migration of microglia is necessary to eliminate the by-product however, they also play a role in the neovascularization, and thus the impairment of microglial migration into or out of the subretinal space promotes the death of photoreceptor cells, as well as studies have shown that immune cells do have dual opposing roles in preventing and promoting the disease (Ambati et al., 2013).

The use of aged mice models with impaired immune cell trafficking, such as those with CC-chemokine ligand 2 (CCL2), CC-chemokine receptor 2 (CCR2) or CX3C-chemokine receptor 1 (CX3CR1) deficiency, results in animals having AMD-like features, supporting to the idea that immune cell trafficking to sites of local tissue damage is essential for retinal health (Ambati & Fowler, 2012).

Even genetic studies also support the role where impaired repair of damaged tissue in AMD seen in polymorphisms of the CX3CR1 gene, associated with macrophage migratory defects (Ambati et al., 2013).

On the other hand, the breakdown of the blood-retinal barrier with the recruitment of macrophages and dendritic cells from the underlying choroid or from the system circulation, where macrophages together with RPE cells are the major source of pro-angiogenic factor (VEGF-A) (Luthert, 2011).

The overall point is although a functioning retinal immune system is crucial for visual homeostasis, still the overactivation of specific immune processes owing to sustained tissue stress in the ageing retina which may cause the development of AMD (Ambati & Fowler, 2012).

Lots of immune pathways are involved in AMD, from the uncontrolled activation of the complement system to the presence of auto-antibodies.

Complement relation with developing AMD

The overactivation of the alternative complement system is the most well-established and widely accepted as contributing to AMD (Luthert, 2011).

According to the complement hypothesis, the inability of appropriate inhibition of the alternative pathway results in retinal tissue damage driving the AMD pathology, it has been shown that approximately 50% of the heritability of AMD could be accounted for by SNP in an exon encoding complement factor H (CFH) (Ambati et al., 2013).

The risk variant of CFH (402His) does not regulate the alternative pathway of complement activation as efficiently as the main allele (Tyr402) does, where it binds with lower affinity to numerous constituents of the damaged retina, decreasing its inhibitory effect of CFH on the complement pathway, which results in a greater degree of complement activation (Ambati et al., 2013).

Inflammasome activation

The inflammasome is a cytosolic protein complex that is activated by foreign or endogenous danger signals, composed of three proteins: a sensor protein (NOD-like receptor mainly NLRP3), an adaptor protein and the enzyme caspase-1 (Ambati et al., 2013).

The activation of the inflammasome occurs when some proteins bind to its sensor part, it has been found that carboxyethylpyrrole-adducted proteins which accumulate in the retina with aging, as well as some drusen components: amyloid- ß and the complement component C1q all can activate the NLRP3 inflammasome in macrophages (Ambati et al., 2013).

This eventually activates the caspase-1 enzyme that cleavage the precursor form of IL-1 ß to its biologically active form to be released with the release of IL-18 (Ambati et al., 2013).

IL-1 ß is an inflammatory mediator that recruits leukocytes, two different types of macrophages are been involved, M1 (pro-inflammatory) and M2 (pro-angiogenic), as well as the potent

cytotoxic effect of IL18 and IL-1ß on the RPE disrupting the blood-retinal barrier (Ambati et al., 2013).

Toll-like receptor (TLR) signaling

The activation TLR by the binding to damage-associated-molecular patterns (DAMPs) results in rapid and vigorous inflammatory responses, where retinal cells express multiple TLR family members, most of which are expressed by RPE cells where studies have shown that the TLRs activation broadly contribute to AMD pathologies, but the full range of agonists and outcomes is unknown yet (Ambati et al., 2013).

It has been suggested that the activation of TLR3 suppresses the development of choroidal neovascularization, while the activation of TLR2 by its ligands (carboxyethylpyrrole-adducted proteins) promotes choroidal neovascularization, thus TLR2 has the major role in the pathogenesis of neovascular AMD (Ambati et al., 2013).

Adaptive immunity

B lymphocytes and T lymphocytes do not have a direct defined role in mediating angiogenesis or tissue damage in AMD, but there is evidence of retinal antigen presentation and indirect autoantibody-mediated retinal degeneration, which could be through the complement activation (Ambati et al., 2013).

Immune-based therapy for neovascular AMD

The understanding of the immune system role in AMD is important for making the most effective therapy possible, where the most effective treatment mainly used are the anti-VEGF antibodies given as intravitreal injection, mainly including ranibizumab, bevacizumab, and aflibercept (Michalska-Małecka et al., 2014).

The use of anti-VEGF drugs to treat neovascular AMD has significantly changed the prognosis of the disease and have led to significant improvements in visual acuity.

Ranibizumab is a recombinant monoclonal immunoglobulin Fab fragment that neutralizes all active forms of VEGF-A (Tashkin et al., 2008).

Bevacizumab is a whole recombinant monoclonal immunoglobulin G that binds and inhibits all VEGF isoforms (Michalska-Małecka et al., 2014).

Aflibercept is a recombinant fusion protein made up of portions of the extracellular domains of human VEGF receptors (VEGFR) 1 and 2 fused to the Fc portion of the human IgG, serving as a soluble receptor to VEGF-A and VEGF-B, having longer duration of action compering to the previous two (Michalska-Małecka et al., 2014).

The general concept is to decrease the extracellular availability of VEGF which can arrest choroidal angiogenesis and reduce vascular permeability for a limited period of time, and thus decreasing the accumulation of subretinal fluid and the accompanying macrophages infiltration (Ambati et al., 2013).

Even though vision remains stable or improves in approximately 80% of the patients, 20% of treated patients continue to lose vision despite treatment, it has been suggested that genetic variants may also explain part of the treatment outcome variability (Tashkin et al., 2008).

The development of new agents for wet AMD has focused on both improving efficacy and extending the duration of action in comparison with the commonly used anti-VEGF drugs ranibizumab and bevacizumab (Michalska-Małecka et al., 2014).

Conclusion

The current understanding of the AMD pathogenesis is still imprecise, as in the conflict of the role of the macrophages seen in animal models with knocked out chemokines receptors to the undergoing genetic studies, but on the other hand, a lots of inflammatory mediators have been known, the most important one is VEGF-A being responsible for the angiogenesis in the wet type, where drugs targeting it shows significant improvement in majority of patients.

Future work

Studies are working on developing more effective anti-VEGF drugs, as well as working on new potential targets for drugs such as IL-1β, IL-18 and inflammasome components, some other recent undergoing trials have started to use human embryonic stem cell (hESC) - derived RPE transplants to treat AMD patients with subfoveal CNVs.

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