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The Role of Inflammation in Age-Related Macular Degeneration: Updates and Possible Therapeutic Approaches

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Abstract: Age-related macular degeneration (AMD) is a common retinal disease characterized by complex pathogenesis and extremely heterogeneous characteristics. Both in "dry" and "wet" AMD forms, the inflammation has a central role to promote the degenerative process and to stimulate the onset of complications. AMD is characterized by several proinflammatory stimuli, cells and mediators involved, and metabolic pathways. Nowadays, inflammatory biomarkers may be unveiled and analyzed by means of several techniques, including laboratory approaches, histology, immunohistochemistry, and noninvasive multimodal retinal imaging. These methodologies allowed to perform remarkable steps forward for understanding the role of inflammation in AMD pathogenesis, also offering new opportunities to optimize the diagnostic workup of the patients and to develop new treatments. The main goal of the present paper is to provide an updated scenario of the current knowledge regarding the role of inflammation in "dry" and "wet" AMD and to discuss new possible therapeutic strategies.

Key Words: age-related macular degeneration, geographic atrophy, inflammation, macular neovascularization

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Age-related macular degeneration (AMD) is a leading cause of visual impairment in developed countries. Its prevalence is expected to remarkably increase in the next future, reaching up to 288 million by the year 2040.[1] AMD is characterized by different stages and possible neovascular or atrophic complications. Dry AMD accounts approximatively for 85%–90% of cases, whereas wet AMD for 10%–15% of cases.[2] The term "dry AMD" includes all the stages of the diseases characterized by the absence of the neovascular complication, from the early form up to the onset to the more severe geographic atrophy (GA). Dry AMD shows progressive retinal pigmented epithelium (RPE) dysfunction, photoreceptor loss, and retinal degeneration.[2] On the other side, wet AMD is characterized by the onset of macular neovascularization and exudation.[2] Looking at the overall AMD pathogenesis, inflammation covers a primary role for the onset and progression both of wet and dry forms. This would mean that, other than considering vascular endothelial growth factor (VEGF) the primary therapeutic target, many other factors should be considered, both in terms of pathogenic mechanisms and therapeutic implications. Indeed, inflammation is rapid and powerful response to an imminent danger, which can be stimulated by a variety of factors, including increased toxicity, proinflammatory mediators, and intracellular components released in the extracellular space secondary to cellular degeneration.

The main goal of the present paper is to provide an updated overview of the pathogenic contribution of inflammation in AMD and the possible therapeutic perspectives.

METHODS

We used key words to explore all English language human subject articles in the MEDLINE library, considering an interval between January 1980 and April 2022. The key words included the following: AMD, inflammation, exudation, atrophy, VEGF, intravitreal injections, emerging treatment. All the references were carefully examined by 2 expert researchers (F.B., A.A.), who collated and arranged all the relevant information for the present study.

The Role of Inflammation in Dry AMD

Although inflammation has been more investigated in the wet form of AMD, it undoubtedly takes a major role also in dry AMD. The first step of dry AMD is the accelerated progression of aging signs, such as the accumulation of lipofuscin and toxic debris below the RPE, known as drusen.[2] The progressive dysfunction of RPE cells is responsible for a toxic microenvironment leading to increased oxidative stress, altered lipid metabolism, and accumulation of toxic products of the visual cycle.[3,4] The chronicity of these phenomena is responsible for the onset and progression of a proinflammatory microenvironment, acting as a further source of RPE and photoreceptors cells damage. RPE cells act against the increased oxidative stress towards the upregulation of antioxidative mechanisms and the promotion of autophagy for removing cellular components.[5,6] Unfortunately, the oxidative burden associated with progressive RPE impairment make these compensatory mechanisms insufficient to compensate prooxidative and proinflammatory cascades. The occurrence of a wide proinflammatory activity is supported by

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the evidence of increased levels of several cytokines and proinflammatory mediators found both in humans and in animal models of AMD. Interleukin (IL)-1 has been found to contribute to the progressive loss of photoreceptors.[7] Similarly, IL-17 has been found highly concentrated in AMD patients,[8,9] promoting the recruitment and activity of macrophages.[10] In AMD eyes complicated by GA, the levels of IL-6, tumor necrosis factor (TNF) receptor 2, and C-reactive protein have been found significantly higher than healthy controls; their levels also correlated with the progression rate of GA.[11] In 2018, dosed the level of proinflammatory cytokines in the aqueous sample of dry AMD patients, reporting significantly higher levels of C-X-C motif chemokine ligand 5 (CXCL5), C-C motif chemokine ligand 11 (CCL11), CCL24, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, CCL2, CCL13, macrophage migration inhibitory factor (MIF), CCL19, CCL17, transforming growth factor beta 2 (TGF-β2), and TGF-β3, although the statistical correction highlighted only CXCL5, CXCL6, and MIG/CXCL9 as effectively relevant.[12] The increased level of these cytokines allowed to advance the hypothesis also of T-cell lymphocytes-mediated phenomena in the pathogenesis of AMD.

If ILs and cytokines are important proinflammatory factors in dry AMD, culminating in the activation of the inflammasome complex,[13] even growing evidence highlighted a major role of the complement system activation. Complement system physiologically promotes the clearance of apoptotic cells by opsonizing the cells for removal via phagocytosis, being activated by 3 different pathways.[14] Proteomic, histological, and biochemical analyses reported complement factors and related proteins as major components of drusen.[15,16] Furthermore, immunohistochemical analysis of enucleated donor eyes highlighted a massive complement factors accumulation in the outer retina.[17,18] Many studies showed many signs of abnormal complement system activation in AMD. C3d, C3a, Ba, Bb, and C5a complement factors have been found elevated in plasma of patients with AMD.[19,20]

Complement system dysfunction in dry AMD and GA has been further supported by the evidence of genetic variants characterizing AMD patients, associated with its abnormal upregulation. In particular, previous studies identified complement factor H (CFH), complement factor I (CFI), complement component 3 (C3), and complement component 9 (C9) variants promoting the alternative complement pathway activation.[21–23] The high importance of complement system activation in the pathogenesis of AMD and GA stimulated companies in developing therapies targeting complement factors. Although an approved treatment for GA is still missing, many clinical trials are ongoing attempting to interfere with the abnormal complement system activation and other proinflammatory mediators.

The imaging counterpart of proinflammatory activity in AMD is represented by the optical coherence tomography (OCT)-detected hyperreflective foci (HF). HF are defined as small, discrete, well-circumscribed, hyperreflective dots, detected both within retinal layers and choroid. The current hypotheses regarding the interpretation of HF include aggregates of inflammatory cells and activated microglia, lipid extravasation, and RPE migration phenomena.[24–26] Although representing a poorly specific OCT sign, described almost in all retinal diseases, increased HF number is a very sensitive sign of disease activity and progression. Their number significantly correlated with disease severity and progression rate, both considering dry AMD stages and GA (Fig. 1).[27,28] Indeed, HF evaluation is overall considered a valuable assessment in the diagnostic workup of AMD patients.

The Role of Inflammation in Wet AMD

Most of the current literature assessed the role of inflammation in the pathogenesis of wet AMD.

Like dry AMD and GA, local inflammation is responsible of the degeneration of RPE and photoreceptors outer segment. In this context, several proinflammatory mediators have been associated with the pathogenesis of wet AMD, including IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, TNF-α, and interferon-γ.[29] Some of these molecules act as proangiogenic factors. IL-1β is promoted by progressively increasing retinal damage, and it was associated both with proinflammatory and angiogenetic activities.[30,31] Other than being a potent proinflammatory cytokine, IL-6 stimulates the signal transducer and activator of transcription-3 (STAT3), which has been associated with the development and growth of murine norovirus (MNV).[32] Furthermore, IL-17 has been found to promote angiogenesis via CXCL8 and CCL2 mediators.[33] TNF-α is a potent proangiogenic factor, causing the upregulation of VEGF production through the reactive oxygen species-dependent β-catenin activation pathway.[34] Another mediator showing a big proangiogenic activity is the TGF-β, although the current literature shows contradictory results. Indeed, from one side high-levels of TGF-β have been associated with the promotion of MNV development through the Smad2/3-VEGF/TNF-α signaling pathway.[35,36] On the other side, other studies associated the depletion of TGF-β with the onset of the neovascular complication. The absence of this mediator was associated with gliotic degeneration of retinal glia driving the neuroinflammatory contribution to the onset and progression of MNV.[37,38] Other proinflammatory and proangiogenic mediators including monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, and MIP-1β, obtained from aqueous and vitreous samples, significantly associated with wet AMD.[39,40] A recent meta-analysis analyzed the main studies focused on this topic, highlighting MCP-1, MIG, TGF-β, and VEGF as the only mediators whose higher levels reached enough statistical relevance.[41] Interestingly, the aqueous levels of MCP-1, MIP-1β, and VEGF measured before intravitreal treatments, and the levels of IL-6, MCP-1, and MIP-1β obtained after anti-VEGF injections resulted significantly associated with the risk of macular atrophy.[42]

In addition, several inflammatory cytotypes may contribute to the neovascular complication. Many cytokines including IL-1β, IL-12, IL-23, interferon-γ, and TNF-α can induce the promotion and activation of classically activated macrophages (M1) disclosing a major proinflammatory activity.[43] On the other hand, alternatively activated macrophages (M2) are anti-inflammatory cells, facilitating tissue repairing and angiogenesis.[43] However, the conversion from M1 to M2, and vice versa, can be driven by changes of the microenvironment.[44] Hence, it was hypothesized that the degeneration-induced inflammation might act as a stimulus for M1 migration within the retina; then, the

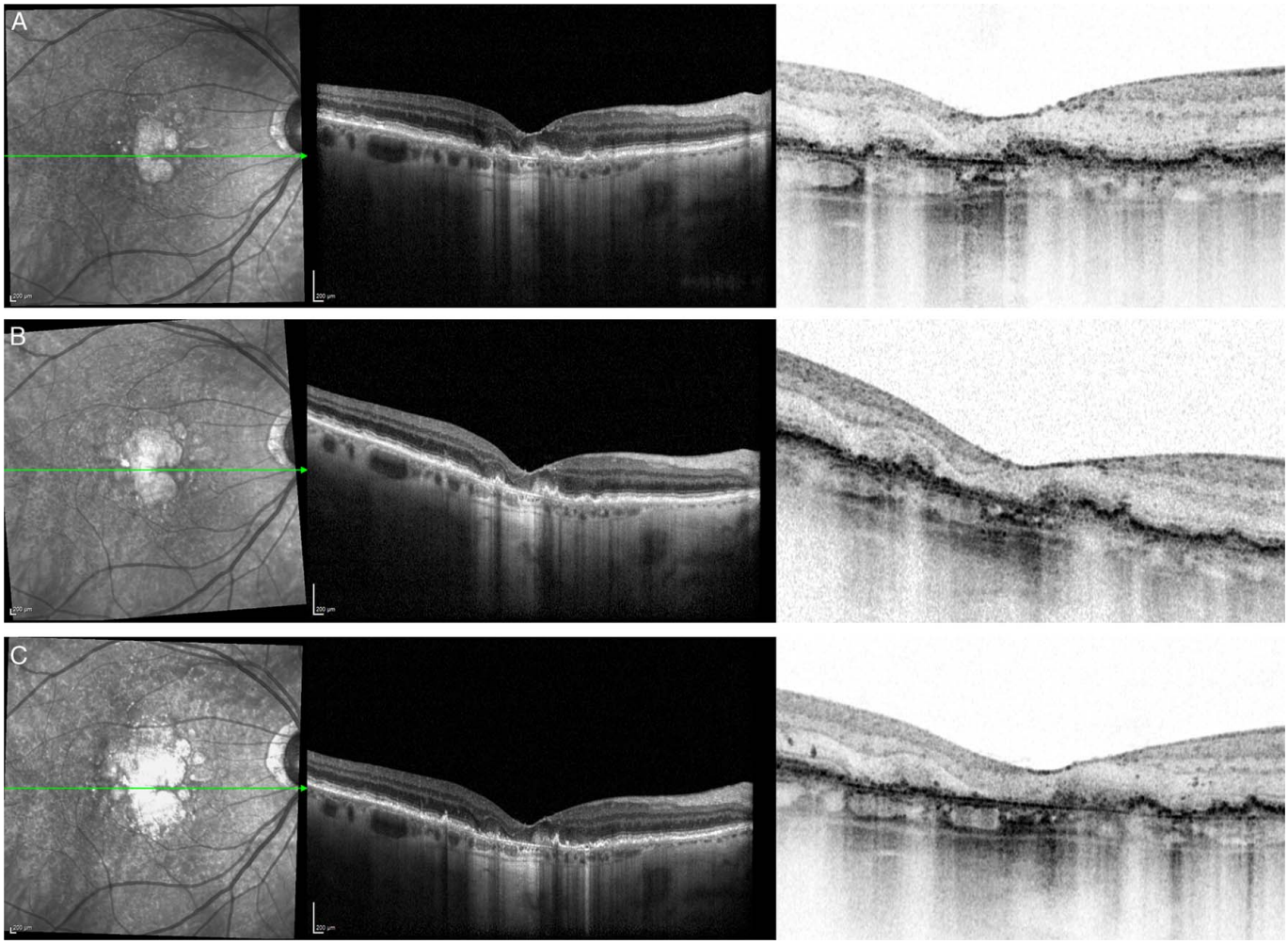

FIGURE 1. Hyperreflective foci (HF) in dry age-related macular degeneration. The presence of these hyperreflective discrete dots, detected on structural optical coherence tomography (A), characterizes both the outer retina and the choroid, and are better highlighted by inverted white-black color optical coherence tomography. The number of HF is associated with the severity of geographic atrophy expansion. HF are mainly localized at the borders of the atrophy, where the degenerative and proinflammatory mechanisms go on (B, C).

Rho-associated kinase signaling profile characterizing AMD might promote the conversion from M1 to M2, thus creating the proangiogenic condition for developing the neovascular complication.[45] In this context, the invasion of the Bruch membrane by macrophages is essential for activating the neovascular processes. M2 macrophages polarization is also stimulated by chitinase-3-like-1, which can also promote the release of VEGF.[46] If this hypothesis somehow described a linear process passing through the M1-M2 conversion and driving MNV development, other investigations suggested an opposing activity of M1 and M2 cytotypes. Indeed, Zhou et al[47] advanced the hypothesis that M1 macrophages have a major role in suppressing MNV development, working against the proangiogenic promotion of M2 macrophages. Animal models also revealed a proangiogenic role of other cytotypes, including dendritic cells and neutrophils.[48,49] On the other side, the reduction of Th1 cells and CXCR3+CD4+T lymphocytes has been associated with dysregulation of VEGF metabolism and increased angiogenic activity.[50]

In addition, the activation of the membrane attack complex (MAC) has been found involved in the angiogenetic process. Indeed, MAC activation may induce the downregulation of VEGF modulators, together with the release of mediators and cytokines stimulating the neovascular process.[51,52]

Also in wet AMD, the OCT-based evaluation of HF has been found clinically relevant for monitoring MNV activity and for evaluating treatment outcome (Fig. 2).[53–56] Furthermore, it has been shown that HF may precede the onset of MNV, thus further supporting the role of a proinflammatory microenvironment favoring the beginning of angiogenetic processes and then representing a potential predictive biomarker of wet AMD complication.[57]

The Other Side of the Medal: The Proinflammatory Role of Intravitreal Injections

Although sterile inflammation and endophthalmitis represent rare complications,[58] intravitreal procedures may act as a potential proinflammatory stimulus for many reasons. First, the surgical procedure itself is a source of transient inflammation. Moreover, patients may be predisposed to proinflammatory activity because of the presence of autoantibodies against drugs, the previous history of ocular or systemic inflammation, and other factors.[59]

A potentially relevant proinflammatory role is carried out by anti-VEGF molecules. The immunogenicity profile of anti-VEGF drugs depends on many factors, including the size of the molecule, the concentration, and the biochemical profile.

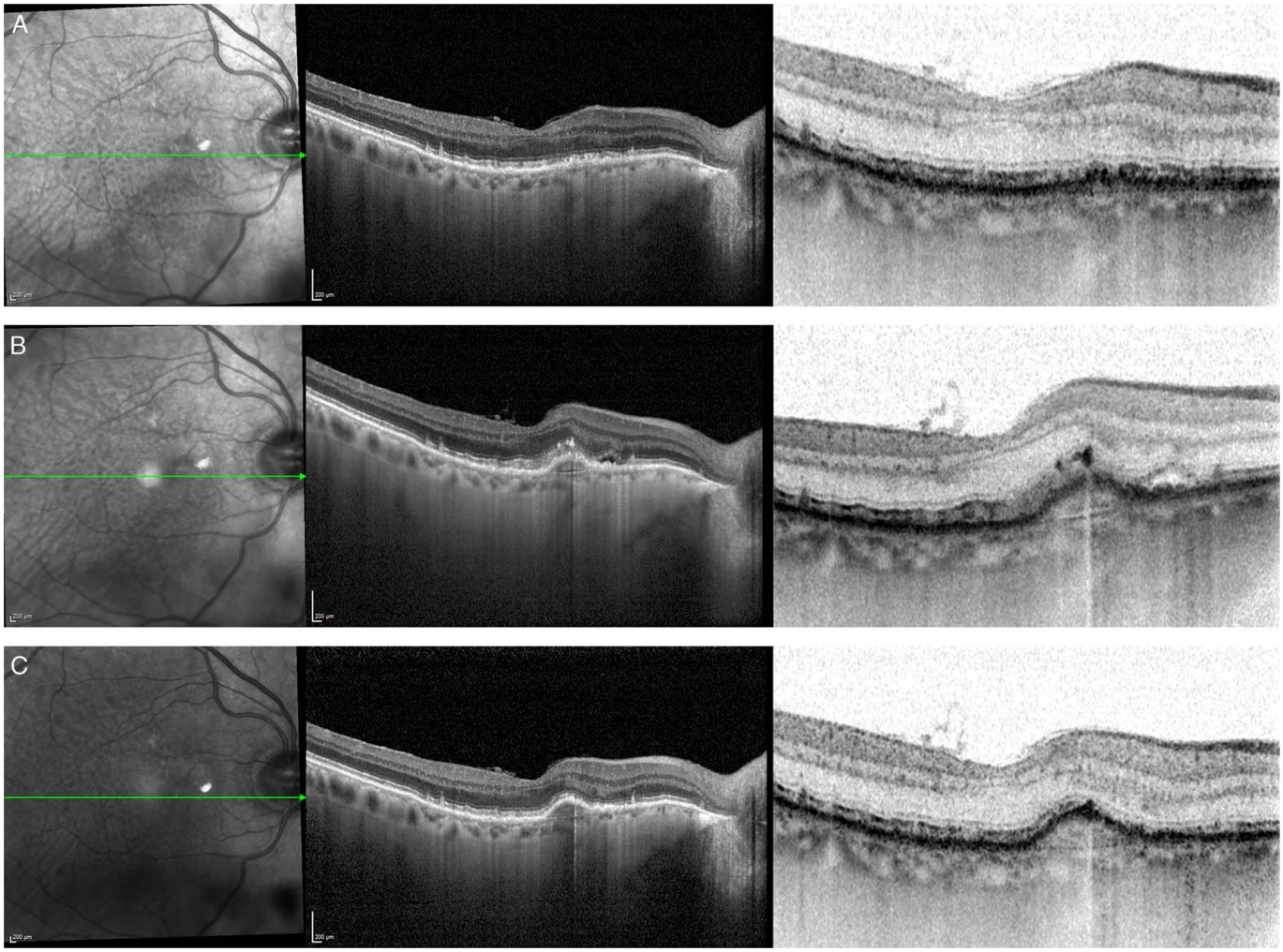

FIGURE 2. Hyperreflective foci (HF) in wet age-related macular degeneration. HF can be detected already in intermediate age-related macular degeneration stage (A), better highlighted by inverted white-black color optical coherence tomography. HF number increases as the neovascularization appears, with clear phenomena of pigment migration within outer retinal layers (B). Their number correlates with murine norovirus activity, resulting in reduction as the lesion is managed by intravitreal antivascular endothelial growth factor injections and the exudation results reabsorbed (C).

The most used anti-VEGF molecules are bevacizumab (Avastin, Hoffmann-La Roche), ranibizumab (Lucentis, Novartis Pharmaceuticals Canada Inc), aflibercept (Eylea, BAYER Pharma AG, Germany), and brolucizumab (Beovu, Novartis Pharmaceuticals Canada Inc). The pharmacologic features of these molecules are shown in Table 1. Based on previous reports, the overall incidence of intraocular inflammation (IOI) for older anti-VEGF molecules is between 0% and 5% of cases, with severe complications representing extremely rare eventualities.[60–62] For this reason, bevacizumab, ranibizumab, and aflibercept are universally considered safe drugs. The situation is quite different for the newly introduced brolucizumab, whose reported incidence of IOI resulted much higher that older molecules (4.4% vs 0.3% of aflibercept in HAWK/HARRIER studies;[63] 11% in SHIFT study;[64] other reports between 4% and 12%),[65,66] with remarkably high risk of retinal occlusive vasculitis complication.[67] The emerged disconnect between clinical trials and real-world reports of IOI (overall 2% vs 10% of cases) made necessary an alert from the American Society of Retina Specialists, which advanced the hypothesis of autoimmune pathogenesis.[68] Indeed, the lack of the fragment crystallizable (Fc) region makes brolucizumab not able to activate the complement system or participate in antibody-dependent cell-mediated cytotoxicity processes.[69] Post-hoc analyses of HAWK/HARRIER studies provided the following IOI incidences: 4.6% observed incidence of definite/probable drug-related events within the spectrum of IOI, retinal vasculitis, and/or vascular occlusion; 3.3% observed incidence of definite/probable IOI+retinal vasculitis; 2.1% observed incidence of definite/probable IOI+retinal vasculitis+retinal vascular occlusion.[70] The more recent Intelligent Research in Sight (IRIS) Registry and Komodo Healthcare Map provided an overall incidence of brolucizumab-related IOI of 2.4%, identifying previous history of IOI or retinal occlusive events, and female gender as statistically significant risk factors of inflammatory complications following brolucizumab injections.[71] Other studies tried to ascertain the reason of the higher incidence of IOI related with brolucizumab, reporting the presence of pretreatment antidrug antibody, found to be 35%–52% in naive patients treated with brolucizumab versus to pretreatment antidrug antibody below 5% found for ranibizumab and aflibercept drugs.[59,72] These findings allowed to advance Type III or IV hypersensitivity reaction as likely pathogenic mechanisms occurring in brolucizumab-

TABLE 1. Main Anti-VEGF Intravitreal Molecules for the Treatment of Neovascular AMD

Molecule	Name	Company	Format	Mechanism of Action	Molecular Weight (kDa)	Clinical Dose (mg)	Phase 3 Clinical Trials AMD
Bevacizumab	Avastin	Hoffmann-La Roche	IgG1 antibody	Anti-VEGF-A	147	0.5	NA; off-label
Ranibizumab	Lucentis	Novartis Pharmaceuticals Canada Inc	Fab fragment	Anti-VEGF-A	48	0.4	MARINA, ANCHOR
Aflibercept	Eylea	BAYER Pharma AG, Germany	VEGFR1/2-Fc fusion protein	Anti-VEGF-A/PIGF/ VEGF-B	97-115	1.0	VIEW 1, VIEW 2
Brolucizumab	Beovu	Novartis Pharmaceuticals Canada Inc	scFv	Anti-VEGF-A	26	6.0	HAWK, HARRIER
Faricimab	Vabysmo	Roche/Genentech	Bispecific monoclonal antibody	Anti-VEGF-A/Ang-2	149	6.0	TENAYA, LUCERNE

AMD indicates age-related macular degeneration; Ang-2, angiopoietin-2; Fab, fragment antigen-binding; Fc, fragment crystallizable; IgG, immunoglobulin G; PIGF, placental growth factor; scFv, single-chain variable fragment; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

treated patients, although other still-unknown causes might be also possible.[59,72] An international committee of experts developed the Avoiding Brolucizumab Related Adverse Event by Scrutinizing Available Evidence (A BRAVE-SAVE) recommendation to optimize patients' selection and management in brolucizumab setting, focusing the attention on the careful evaluation of proinflammatory risk and the deep monitoring of the patients.[72] From this point of view, the development of new diagnostic procedures dedicated on tracing the proinflammatory profile of AMD patients will be useful to reduce the risk of IOI and to improve treatment customization.

Therapeutic Considerations

Overall considering all the aspects discussed in the present survey, AMD pathogenetic mechanisms are more complex than a mere proangiogenic activity. VEGF undoubtedly represents a major pathogenic factor in AMD, although inflammation might represent a new relevant therapeutic target. In this section, we discuss only new therapeutic approaches showing a role against proinflammatory phenomena. The rationale of anti-inflammatory agents in AMD has been explored starting from the administration of topical treatments, nonsteroidal anti-inflammatory drugs, whose usage in combination with anti-VEGF injections has been slightly associated with adjunctive benefits, although the level of evidence was not high enough to draw definite conclusions.[73] The same low level of evidence concerned the usage of corticosteroids.[74,75]

After the conduction of Age-Related Eye Disease Study (AREDS) and AREDS2 clinical trials, the nutraceutical approach for reducing oxidative stress and proinflammatory activity in AMD has been established as a useful approach to reduce the degenerative burden.[76,77] The current AREDS2 formulation based on vitamin C, vitamin E, cupric acid, zinc oxide, lutein+zeaxanthin, and omega-3 long chain polyunsaturated fatty acids represents the only therapeutic option for dry AMD and a valid support for wet AMD, although the level of evidence regarding the impact on disease progression is still low.[78] It is worth of notice that the role of long-chain polyunsaturated fatty acids in AMD pathogenesis is quite controversial. Indeed, from one side many studies, including AREDS and AREDS2 reports, suggested a protective role of these molecules, being considered important modulators of inflammation.[79] On the other side, the unsaturated structure of long chain polyunsaturated fatty acids is susceptible to oxidative degradation by lipid peroxidation, which is an important promoter of oxidative stress, cellular damage, and inflammation.[79] For all these reasons, further studies should be focused on the deep assessment of the involvement of each long chain polyunsaturated fatty acid in the pathogenesis of AMD, either considering the potential protective or negative role.

For wet AMD, promising perspectives came from combined VEGF and angiopoietin system blockage, thus reducing vascular permeability, inflammation, and angiogenesis.[80] Angiopoietin system consists in angiopoietin-1 (Ang-1) factor, binding Tie-2 receptor and acting to decrease vascular leakage, and in angiopoietin-2 (Ang-2), representing an Ang-1 antagonist increasing leakage, inflammation, and angiogenesis.[80] Faricimab (Roche/Genentech, San Francisco, CA) is a bispecific monoclonal anti-

body targeting both VEGF-A and Ang-2 factor,[81] recently approved for the treatment of neovascular AMD. Endoglin is a transmembrane glycoprotein working as a coreceptor for several ligands of the TGF-β family, showing both proangiogenic and proinflammatory activities.[82] A therapeutic approach has been tested by using DE-122 anti-endoglin antibody (Carotuximab; Santen, Kita-Ku, Osaka, and TRACON Pharmaceuticals, San Diego, CA); however, the trial has been discontinued for the lack of consistent results. The enhancement of Tie-2 activity might represent another therapeutic target, which is now under investigation by using AXT-107 (AsclepiX Therapeutics, Jersey City, NJ), a VEGF receptor-2 inhibitor and a potential stimulator of Tie-2 activity (NCT04746963).

Since many papers have already been dedicated to the deep description of complement system inhibitors, we would only mention the central role of this class of molecules as a promising new therapeutic option for AMD, especially looking at GA.[83–87] The current molecules designed as complement system inhibitors include: eculizumab (immunoglobulin G antibody blocking complement factor C5; NCT00935883), lampalizumab (antigen-binding fragment of humanized monoclonal antibody blocking complement factor D; NCT02247479, NCT02247531), avacincaptad pegol (Zimura; anti-C5 aptamer; NCT02686658, NCT04435366), sirolimus (mammalian target of rapamycin inhibitor; NCT00766649), pegcetacoplan (complement factor C3 inhibitor; NCT02503332, NCT03525600, NCT0355613), and Tedisolumab (complement factor C5 inhibitor; NCT01527500). The complement system targets of each drug are shown in Figure 3. It is worth of notice that eculizumab and lampalizumab failed to reach the primary targets of the clinical trials.

The integrin system represents an important promoter of proinflammatory and proangiogenic activities, involved in several retinal diseases including AMD and representing a potential therapeutic target.[88] The current anti-integrin molecules tested in clinical trials include: risuteganib (Luminate, Allegro Ophthalmics, CA; NCT03626636), THR-687 (Oxurion, Leuven, Belgium; NCT05063734), SF-0166 (SciFluor Life Science, MA; NCT02914639), volociximab (Ophthotech Corporation, NY, now Iveric Bio; NCT00782093), JSM-6427 (Takeda Pharmaceutical Company, Tokyo, Japan; NCT00536016). It is worth of notice that, although showing promising results, most of integrin inhibitors are still in early stages of investigations, thus requiring further studies to draw definite conclusions regarding their role as a new treatment for AMD. Furthermore, many other integrin inhibitor molecules are still in preclinical stages of investigation, including SB-267268 (GlaxoSmithKline),[89] AXT-107 (AsclepiX Therapeutics, NJ),[90] JNJ-26076713 (Johnson & Johnson Pharmaceutical, PA),[91] cilengitide (Merck-Serono, Germany),[92] and lebecetin.[93]

Apurinic/apyrimidinic endonuclease/redox effector factor-1 (APE/REF-1) is a ubiquitously expressed predominant apurinic/apyrimidinic endonuclease, involved in cellular homeostasis, oxidative stress regulation and repairing functions.[94] It was demonstrated a role of APE/REF-1 redox activity in promoting retinal damage and neovascularization in AMD, thus offering the basis for novel antiangiogenic therapies.[95,96] MRZ-99030 is a novel molecule able to not disturb with protein–protein interactions between amyloid-β monomers but interfering with the formation of toxic amyloidogenic fibrillar aggregates.[97] Amyloid-β is a primary constituent of drusen, whose accumulations can trigger proinflammatory and proangiogenic activities.[98] Also in this case, MRZ-99030 might offer a new way to interfere with the degenerative microenvironment characterizing both dry and wet AMD. Celecoxib inhibits cyclooxygenase-2, an enzyme promoting the biosynthesis of proinflammatory prostaglandins. The celecoxib-induced reduction of prostaglandin levels has been associated with significant reduction of pro-degenerative and proangiogenic mechanisms,[99] and could be the object of a clinical trial focused on AMD.

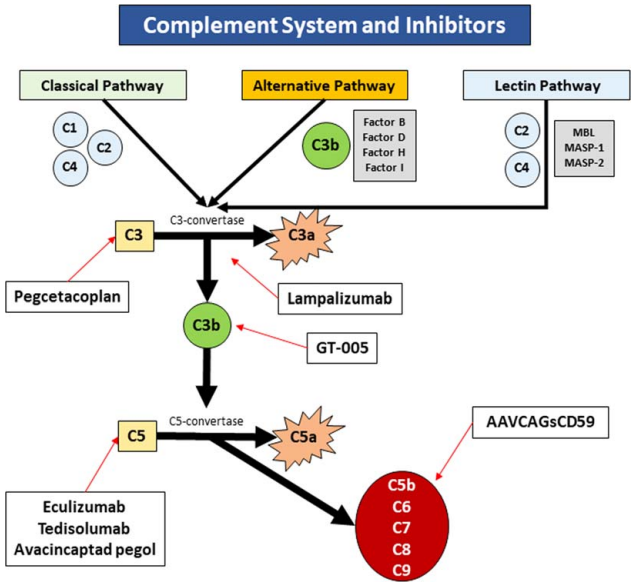

FIGURE 3. Complement system pathways and targets of the new experimental drugs. The complement system consists of 3 different pathways, all converging on the production of the active forms of C3 and C5 (C3a and C5a, respectively) acting as proinflammatory mediators. The residual parts are fundamental for inducing further proinflammatory mechanisms. C3b stimulates the splicing of C5 by C5-convertase enzyme, whereas C5b takes part of the membrane attack complex, together with C6, C7, C8, and C9. The experimental drugs under investigations may target different factors involved in the complement system network.

Adeno-associated viral (AAV) vectors represent another technology to carry therapeutic molecules for inhibiting inflammation. AAVCAGsCD59 has been developed to reduce the formation of MAC, which represents an important step for the subsequent activities of the complement system (NCT03144999, NCT03585556). GT005 is an AAV encoding complement factor I, designed to downregulate the alternative complement system pathway (NCT03846193). Furthermore, AAV might be used to enhance the expression of complement factor H, thus promoting the inhibition of complement factor C3.[100]

Another interesting therapeutic frontier is represented by systemic hydroxyl-terminated polyamidoamine dendrimer-triamcinolone acetonide conjugate (D-TA) nanotherapy targeting the suppression of choroidal inflammation and neovascularization process in AMD.[101]

AMD and Inflammation: Unmet Needs

Although the current scientific evidence agrees in considering inflammation as a major pathogenic component

both of dry and wet AMD, several aspects remain unsolved. First of all, a good part of the current knowledge came from animal models of AMD and neovascularization. Although providing useful insights, it should be considered that the mechanisms and mediators involved in AMD pathogenesis and progression might be different. In induced murine models of neovascularization, the technique adopted, either laser, surgery, or genetic approaches, follows different metabolic pathways with respect to what happens in the human AMD retina, thus making assumable that none of these models can fully reproduce the pathogenic scenario of AMD-related MNV.[102] In addition, murine models are characterized by the absence of the macula, which is known to be characterized by retinal cytotypes with higher level of specialization, with respect to the rest of the retina. Further immunohistochemical findings coming from human donors are then warranted to better trace the proinflammatory mechanisms underlying AMD pathogenesis. With respect to aqueous and vitreous sampling, it should be considered the lack of standardized procedures and the potentially high variability of the samples. Furthermore, the biochemical profiles of AMD-related mediators might vary among different stages of the disease, thus making possible to hypothesize that many other molecules are involved in AMD pathogenesis, over than those described in the present survey. Moreover, the relationship between intravitreal treatments and IOI should be better addressed, deeply investigating the causes of this complication and considering new diagnostic paradigms to improve the safety of the treatments. In addition, several potential new therapies are currently under investigation, considering multitarget approaches also focused on proinflammatory pathways. However, considering the importance of inflammation in AMD, further diagnostic modalities are dedicated to obtaining a precise proinflammatory profile of the patient, and further powerful anti-inflammatory approaches are warranted to improve the clinical management of AMD patients and to improve the outcome of the disease.

CONCLUSIONS

In conclusion, AMD shows a very intricate pathogenesis involving inflammation as a major cause of retinal damage and disease progression. Proinflammatory pathways involve several mediators, which might be potential targets of future treatments. The future development of deep patients' inflammation profile assessments and new multitarget therapeutic approaches will help customize the treatment strategy, improve the safety, and optimize the morphofunctional outcome.

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