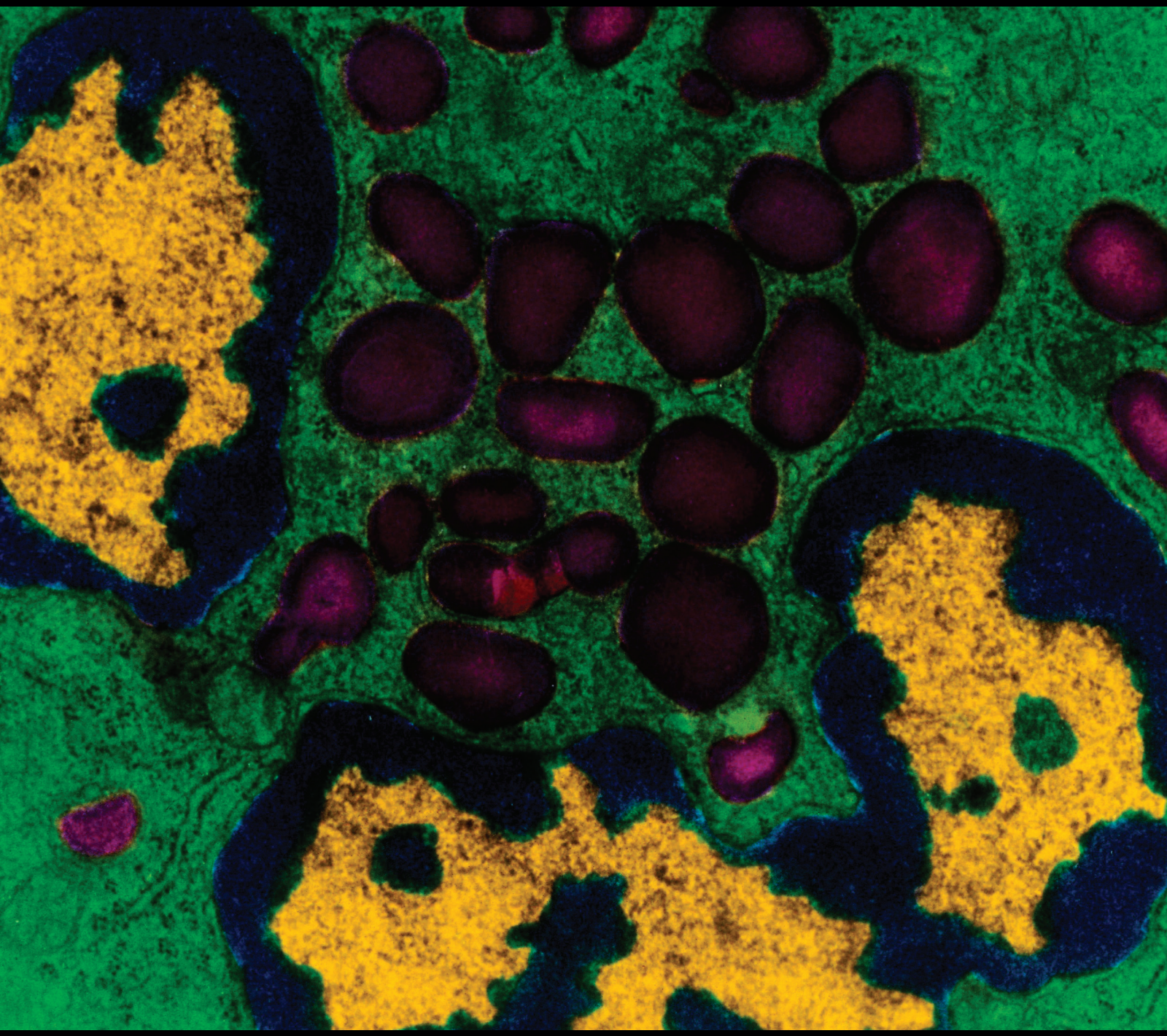
Mediators of Inflammation

Intravitreal Inflammation: From Benchside to Bedside 2013

Guest Editors: Mario R. Romano and John Christoforidis



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Editorial Board

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Editorial

Intravitreal Inflammation: From Benchside to Bedside 2013

Mario R. Romano[1,2] and John Christoforidis[3]

[1] *Department of Ophthalmology, Humanitas Research Center, Rozzano, 20089 Milan, Italy*
[2] *Department of Neuroscience, University of Naples Federico II, 80131 Naples, Italy*
[3] *Department of Ophthalmology, College of Medicine, The University of Arizona, Tucson, AZ 85711, USA*

Correspondence should be addressed to Mario R. Romano; mario.romano.md@gmail.com

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The aim of this special issue is to describe the role of inflammation in biological mechanisms of long-known diseases, providing an up-to-date viewpoint for therapeutic approaches. The authors provide a comprehensive overview, with original and review articles, on the implications of inflammation in proliferative vitreous retinopathy, on diabetic retinopathy, and on the potential iatrogenic damage induced by sustained intravitreal anti-VEGF therapy.

Despite the copious literature, the exact mechanism of idiopathic epiretinal membranes formation (iERM) is still unclear. M. Joshi et al. showed the importance of glial cells and hyalocytes in its pathogenesis. Such cellular constituents of iERMs in concert with cytokines, growth factors (TGF and NGF), and anomalous posterior vitreous detachment are responsible for the prognosis and potential growth of epiretinal traction. The proliferative vitreoretinopathy (PVR) is recognized as a common secondary severe inflammatory complication of retinal detachment. C. Azzolini et al. reported two possible pathways (VEGF-A, Otx2, p53, p63, and Otx1 and Otx3) of PVR. These genetic pathways could represent a novel target of therapy of PVR progression. PVR is an even more common complication in patients with open-globe injury. F. Morescalchi et al. describe the role of immune cells into the vitreous gel in penetrating ocular injury. The cells stimulate the production of growth factors and cytokines, in particular PDGFR-α, which seem to be crucial in the development of PVR and, therefore, considered another potential therapeutic target. In a prospective, nonrandomized, observational study, C. Costagliola et al. showed that tear fluid collection is a useful and noninvasive method for the finding of proliferative diabetic retinopathy. The authors reported that TNF-alpha concentrations increase in tears according to the severity of proliferative and nonproliferative diabetic retinopathy.

F. Parmeggiani et al. demonstrated the evidence of a relationship between AMD-risk genotypes, immunoinflammatory endophenotypes, and the networks of acquired or epigenetic factors.

The authors reported that proinflammatory effects secondary to chronic inflammation and heterogeneous types of oxidative stress induce degenerative damage to the photoreceptors and outer retinal-blood barrier. In particular, the carriers of CFH, ARMS2/HRTA1, and C2/CFB genotypes demonstrated high odd ratio values. A score, calculating a risk including genetic findings, could be useful to identify individual risk, in order to provide preventive treatments.

The intraviteral inflammation can be also secondary to iatrogenic treatment as intravitreal anti-VEGF therapy or vitreoretinal surgery.

S. Agrawal et al. compared the sterile inflammation among the different intravitreal anti-VEGF drugs currently used for neovascular diseases. The acute intraocular inflammation is more frequently associated with bevacizumab likely due to the less strict purification procedure of the drug. In most cases, the inflammation solves spontaneously and vision returns to baseline. A history of previous inflammation does not increase the risk with following intravitreal injections.

Previous vitreoretinal surgery can also induce intravitreal inflammation responsible for cytotoxic macular oedema. V. Romano et al. reported that intravitreal inflammation may increase after pars plana vitrectomy due to tractional

phenomena at vitreomacular interfaces or due to a vasogenic damage. The oedema is characterized by fluid accumulation in the parenchymatous retinal cells (intracellular oedema) or by extracellular fluid accumulation due to a blood-retinal barrier damage (extracellular oedema).

The treatment of vitreous inflammation is still a challenging issue; however, there are multiple methods of systemic treatment including T-cell inhibitors/calcineurin, antimetabolites, corticosteroids, alkylating agents, inhibitors, and biologic agents. A. Jiang et al. showed that these drugs can be used either alone or together in order to control vitreous inflammation due to variety of etiologies, either infectious or noninfectious. A. Russo et al. demonstrated that the topical nonsteroidal anti-inflammatory drugs (NSAIDs) allow for greater drug penetration into the vitreous. Topical NSAIDs reduce the exudation secondary to age-related macular degeneration or diabetic maculopathy. NSAIDs are also considered a favorable adjunct together with anti-VEGF that could potentially reduce the number of anti-VEGF injections. Finally, the chronicity of certain inflammatory conditions limits the efficacy of locally administered drugs. J. Wang et al. reported the last updates on implantable devices and particulate delivery systems such as nanoparticles, microparticles, and liposomes. These topics are actually considered the research focus of biomedical engineering, pharmacology, and molecular biology.

We wish that this special issue will provide helpful information to recognize the clinical features of vitreous inflammation, to understand the mechanism beyond, and to identify the latest treatments in the disease in which the vitreous is involved.

Mario R. Romano
John Christoforidis

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Review Article

Mechanism of Inflammation in Age-Related Macular Degeneration: An Up-to-Date on Genetic Landmarks

Francesco Parmeggiani,[1] Francesco S. Sorrentino,[1] Mario R. Romano,[2,3] Ciro Costagliola,[3] Francesco Semeraro,[4] Carlo Incorvaia,[1] Sergio D'Angelo,[1] Paolo Perri,[1] Katia De Nadai,[1,5] Elia Bonomo Roversi,[6] Paola Franceschelli,[6] Adolfo Sebastiani,[1] and Michele Rubini[6]

[1] *Department of Biomedical and Specialty Surgical Sciences, Ophthalmology Unit, University of Ferrara, Corso Giovecca 203, 44121 Ferrara, Italy*
[2] *Department of Ophthalmology, Istituto Clinico Humanitas, Milan, Italy*
[3] *Department of Health Sciences, University of Molise, Campobasso, Italy*
[4] *Department of Ophthalmology, University of Brescia, Brescia, Italy*
[5] *Center for Retinitis Pigmentosa of Veneto Region, ULSS 15 Alta Padovana, Camposampiero, Italy*
[6] *Department of Biomedical and Specialty Surgical Sciences, Medical Genetic Unit, University of Ferrara, Ferrara, Italy*

Correspondence should be addressed to Francesco Parmeggiani; francesco.parmeggiani@unife.it

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Age-related macular degeneration (AMD) is the most common cause of irreversible visual impairment among people over 50 years of age, accounting for up to 50% of all cases of legal blindness in Western countries. Although the aging represents the main determinant of AMD, it must be considered a multifaceted disease caused by interactions among environmental risk factors and genetic backgrounds. Mounting evidence and/or arguments document the crucial role of inflammation and immune-mediated processes in the pathogenesis of AMD. Proinflammatory effects secondary to chronic inflammation (e.g., alternative complement activation) and heterogeneous types of oxidative stress (e.g., impaired cholesterol homeostasis) can result in degenerative damages at the level of crucial macular structures, that is photoreceptors, retinal pigment epithelium, and Bruch's membrane. In the most recent years, the association of AMD with genes, directly or indirectly, involved in immunoinflammatory pathways is increasingly becoming an essential core for AMD knowledge. Starting from the key basic-research notions detectable at the root of AMD pathogenesis, the present up-to-date paper reviews the best-known and/or the most attractive genetic findings linked to the mechanisms of inflammation of this complex disease.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss in elderly populations in developed countries, and 30–50 million people are affected worldwide. In the United States, it has been estimated that the prevalence of AMD is 13.4% among persons aged 60 years and older [1, 2]. AMD affects all retinal layers of the macula, the structure responsible for the central vision involving, in different degenerative patterns, photoreceptors, retinal pigment epithelium (RPE), and Bruch's membrane. AMD is primary characterized by the development of drusen, pathological extracellular deposits between RPE and Bruch's membrane mainly containing glycolipids, proteins, and cellular debris. At the level of macular area, the presence of few small hard drusen can be considered as a part of the normal tissue aging. On the other hand, AMD consists of numerous and large soft drusen, RPE dystrophy, macular pigmentary changes, and/or thickening of Bruch's membrane [3–8]. Advanced AMD can manifest as either dry form or wet one. In dry AMD, geographic atrophy with RPE and photoreceptors degenerative changes occurs. Conversely, wet AMD is characterized by the presence of choroidal neovascularization (CNV) with exudative and

hemorrhagic phenomena leading to fibrotic scar formation [8–11].

Susceptibility to develop AMD is influenced by a number of genetic and environmental factors [12]. AMD is in fact considered a multifactorial disease, caused by the interplay between multiple acquired factors and polygenic background. Recent epidemiological data have identified numerous risk factors as potential modulators of AMD; aging, cigarette smoking, previous cataract surgery, and family history of AMD show strong associations with the disease, whereas higher body mass index, history of cardiovascular disease, hypertension, and higher plasma fibrinogen result in moderate AMD's risk [13]. Although family history is a well-known risk factor for AMD, it is not a routine practice to alert those with a family history to the increased risk and advise them of the relevance of recognizing early symptoms. An accurate quantification of risk associated with a family history of AMD has been reported in the UK population [14]. This study confirmed that family history is a strong risk element for AMD, highlighting the importance of genetic factors in the pathogenesis of AMD. Individuals with an affected sibling or an affected parent were reported to have a 12-fold increase in the odds of disease. Odds ratio (ORs) adjusted for age and smoking were even higher: 23% of siblings resulted to be affected by CNV or geographic atrophy with an OR of 10.8, which increased to 16.1 adjusting it for patient's age. Similar figure was previously reported in two large population-based studies, the Rotterdam Eye Study [15] and the Beaver Dam Eye Study [16]. The first reported an OR of 14.3, showing that the risk of AMD increased to 19.8 if smoking was taken into account. The latter study resulted in an OR of 10.3, which probably was underestimated, as the study relied on at least one sibling being free of disease at baseline and the sibships where all siblings were affected by AMD were excluded; when only randomly selected sibships were included, the OR was 26. Other studies reported family history of AMD being associated with lower ORs, but that could be attributed to the inclusion of cases with less severe disease [17–20]. A recent UK study has documented that the onset of disease was earlier in cases who had AMD affected siblings than in those without. This is typical for a complex disease with a substantial genetic component, for which the onset of disease is at a younger age in those with a family history [14].

Numerous genomic regions and a variety of candidate genes have also been seen to impact AMD susceptibility. Although strong associations between genetic factors and AMD have been illustrated, it is likely that a significant part of the heritability of AMD cannot be explained through current known mechanisms [21–23]. A number of genetic variants have been associated with AMD, and a recent genome-wide association study (GWAS) has provided significant ($P < 5 \times 10^{-8}$) evidence of nineteen AMD susceptibility loci [24]. Meta-analyses of GWASs for AMD estimated that currently identified loci account for approximately 60–70% of the inherited predisposition to clinically significant AMD forms [24–27]. The hugely mounting number of scientific reports regarding AMD-related gene variants counteracts the chance to get any unequivocal interpretation of these correlative data. In this complex scenario, which has built up more than ever in the last five years, several genes seem to be the most attractive in playing remarkable roles in different steps of AMD pathogenesis [24–37].

In the present review, we will focus on the best-known gene variants involved in the immunoinflammatory pathogenesis of AMD, particularly considering the regulation of both complement activity and cholesterol homeostasis.

2. Complement Activity

The complement system is one of the main components of the innate immune response and fulfils numerous functions, such as the recognition of foreign cells, communication with and activation of adaptive immunity, and the removal of cellular debris. Complement consists of over 40 proteins and cells, comprising a well-balanced network of circulating and cell-surface-bound proteins, which serve as substrates, enzymes, or modulators of a hierarchical series of extracellular proteolytic cascades. There are three well-known mechanisms of complement activation: classical, lectin, and alternative pathways. Each pathway is activated by different stimuli and the initial steps that trigger the complement activation differ considerably. The final stage of the enzymatic cascade of events is the lysis of bacteria or viruses and the opsonization, which consists in a sort of marking cells or molecules to be removed by the host [38–40].

The classical pathway is stimulated by the recognition of antigen-antibody complexes on foreign-cell surfaces by the hexameric complement component C1q. Similar pattern-recognition receptors, that is mannose-binding lectin (MBL) and ficolins, bind to carbohydrate ligands on microbial intruders and initiate the lectin pathway. Conversely, the alternative pathway is stimulated by the spontaneous hydrolysis of native C3 or the presence of foreign surface structures. Recent findings suggest that additional processes, such as the C2-bypass and extrinsic protease pathways or properdin-mediated direct convertase assembled on microbial surfaces, can also initiate complement activation [41–43].

All of the complement cascades end up in the central cleavage of C3 and in the generation of its active fragments C3a and C3b. Opsonization of foreign surfaces by covalently attached C3b fulfils three major functions:

(i) cell clearance by phagocytosis;

(ii) amplification of complement activation by the formation of a surface-bound C3 convertase;

(iii) assemblage of the C5 convertase.

Cleavage of C5 results in the formation of a multiprotein pore complex (MAC, membrane-attack complex), which leads to cell lysis. Both the covalent attachment of C3b and the stabilization of C3 convertase by the complement regulator properdin are markedly activated by hydroxyl-rich pathogen surfaces. A number of complement receptors mediate the recognition of opsonized cells by leukocytes, which induces phagocytosis and stimulation of the adaptive immune system (B and T cells). Finally, the anaphylatoxins

C3a and C5a are released during complement activation and trigger a range of chemotactic and proinflammatory responses, such as recruitment of inflammatory cells and increase of microvasculature permeability. In this way, the complement cascade also supports and promotes the function of downstream mechanisms of the immune response [44, 45].

Detrimental effects take place in case of exaggerated complement activation on self tissue. In addition to a location-based and time-based restriction to immediate sites of activation, a finely tuned set of soluble and membrane-bound regulators ensure that any overstated action of complement on host cells is either prevented or actively inhibited. There is a large number of regulators of complement activation, including complement receptor 1 (CR1), complement factor H (CFH), factor H-like protein-1 (FHL-1), C4-binding protein (C4BP), decay-accelerating factor (DAF), and membrane cofactor protein (MCP) [46].

Complement is the most important pathogenic pathway of the immune system involved in AMD [8, 47–53], clearly indicating that complement activation is implicated in its pathogenesis [54–58]. Although AMD is not a classic inflammatory disease, immunocompetent cells, such as macrophages and lymphocytes, are present in chorioretinal tissues with AMD [59, 60]. Specific alteration and/or dysregulation of innate immune system are observed in AMD eyes mainly at the level of complement pathway elements, such as complement components C3a and C5a, C5 and C5b-9 terminal complement complex, complement regulators or inhibitors (i.e., CFH, vitronectin, and clusterin), CR1, MCP, and DAF, but also at the level of C-reactive protein [61–68]. Activation products C3a, C5a, and C5b-9 are also systemically elevated in patients suffering from AMD [69–72]. Due to genetic evidence from GWAS as well as from common and rare variant analyses, the overactive alternative pathway has been investigated showing that its excessive engagement is a key component in AMD pathogenesis [24–37, 54–58, 73–77]. During AMD, several immunopathological phenomena occur within the structures of the macular area, especially due to the pathophysiologic effects of complement system, which have a main role in the parainflammation of the aging retina [8, 47–53]. In particular, reliably because the posterior retinal layers (i.e., photoreceptor outer segment, RPE, and Bruch's membrane) are more prone to environmental and/or blood-circulating oxidative stresses [78–82], they epigenetically represent the preordained site of onset of the elementary AMD lesions (drusen) [3–8]. In fact, unregulated choroidal blood flow may increase the fluctuations of oxygen and/or lipids concentration, leading to elevated generation of reactive oxygen species (ROS) [80–82]. Likewise, photooxidation in photoreceptors is associated with complement activation, which can increase MAC formation, an important trigger of those apoptotic processes inducing retinal degeneration [83–86]. In this pathogenetic context, the critical position of complement must be emphasized. In fact, exactly complement's dysregulation can lead to that autologous damage which, at macular level, provokes the development of drusen: the earliest hallmarks of AMD acting as foci of chronic inflammation [8, 49–53].

3. Cholesterol Homeostasis

Recent investigation array has highlighted that neural retina and RPE express most of the genes involved in cholesterol homeostasis [87]. Indeed, it has been reported that retina can synthesize cholesterol endogenously [88, 89] and express proteins mediating cholesterol transport [90–92] and removal [93–95]. At the present time, detection of several cholesterol-related genes suggests that cholesterol homeostasis in the retina might be considered relatively independent of the rest of the human body. Taking part, respectively, in internal and external blood-retina barriers, endothelial cells of neural retina (NR) and RPE cells synthesize and acquire cholesterol from low- and high-density lipoproteins (LDL and HDL) derived from blood circulation. However, the ratio between blood-borne cholesterol and endogenously synthesized is not well-known yet [87, 96, 97].

A large interindividual variability of cholesterol and lipoprotein metabolisms is unquestionable, but it is intriguing the fact that RPE has higher variations in expression of cholesterol-related genes than NR. It could be accounted for a sort of "gate-keeping" function of RPE controlling cholesterol and nutrient uptake from blood-stream to NR and reverse transport of metabolites from NR back to systemic circulation. At RPE level this gene expression is promptly modulated in response to fluctuations of blood lipids. Moreover, this adjustment varies in each individual depending on blood lipid profile, age, gender, lifestyle, and genetic background. There are a lot of fine mechanisms of regulation pertaining to cholesterol-related genes in both NR and RPE but, despite the many experimental findings, most of them are not currently well known [87]. At NR level, it seems that photoreceptor outer segment (OS) deals with intraretinal cholesterol transport by means of active and passive mechanisms [98]. The active transport of cholesterol from photoreceptor inner segment (IS) to the OS partially occurs via intracellular cholesterol transporter Niemann-Pick C1-like 1 [99–101]. Another modality of cholesterol mobilization involves scavenger receptors, especially that named scavenger receptor Class B Member 1, which mediates bidirectional cholesterol flux between cells and lipoproteins; in this manner, the photoreceptor OS can uptake lipids from the HDL-like particles and offload lipids to the same particles as well [102–107]. Regarding the passive mechanism, it is known that photoreceptor IS lies in a high-cholesterol environment than OS; hence, IS can provide cholesterol for OS just through passive diffusion. Because of loss of efficiency in either some of these systems or phagocytosis, the cholesterol accumulates in the basal OS disks dampening down the phototransduction cascade [106, 108, 109].

In the retina, the RPE plays a key role in cholesterol homeostasis controlling both cholesterol input and output [110, 111]. Experimental findings have indicated the presence of different pathways, even if the cholesterol offload via apolipoprotein B-mediated transport is regarded as one of the main mechanisms involved in AMD pathogenesis. In fact, with aging, the apolipoprotein B-containing particles pool in the Bruch's membrane forming esterified and unesterified cholesterol-enriched lipid deposits named drusen. Very little

is known about AMD and dysregulation of cholesterol-related genes, but it might be assumed that several affected individuals can be carrier of specific metabolic impairments in proteins determining cholesterol uptake (e.g., 3-hydroxy-3-methylglutaryl-CoA reductase and low-density lipoprotein receptor) and/or in those mediating cholesterol removal (e.g., ATP binding cassette transporter 1—*ABCA*1—cytochromes P*450*) [91, 92]. Although further investigation is needed to better elucidate these clinicogenetic relationships, recent GWAS identified four HDL-related genes as remarkable risk factors for AMD: *LIPC* (hepatic lipase), *CETP* (cholesteryl ester transfer protein), *ABCA1*, and *LPL* (lipoprotein lipase) [25, 31, 32].

In several age-related vascular disorders, increased levels of oxysterols play a crucial role provoking atherosclerosis with subsequent local and chronic inflammation. Homeostasis of cholesterol in blood vessel wall is of essential importance to regulate circulating cholesterol levels. A key event in the development of atherosclerosis is the uncontrolled uptake of oxidized LDL by macrophages recruited in the subendothelial space. The aberrant increasing of these lipid-loaded macrophages, termed foam cells, becomes a crucial condition causative of highly local inflammation [112–117].

Focusing on the lipoprotein retention in vascular wall, a parallel between atherosclerotic disease and AMD is identifiable. In atherosclerosis, apolipoproteins B cross the arterial endothelium, bind to proteoglycans, undergo oxidative and nonoxidative processes, and trigger downstream events, such as foam cells build-up and cytokine release [112, 118, 119]. In AMD, lipoprotein-like particles (enriched with esterified cholesterol) accumulate in the Bruch's membrane, especially in the space between the RPE basal lamina and the inner collagenous layer, forming lesions able to trigger inflammation, complement activation, and cytotoxicity (i.e., lipid-rich lesions, basal linear deposits and, finally, drusen) [3–8, 91, 92, 120–122]. RPE physiologically plays a critical role in the uptake, processing, and offload of retinal lipids. It uptakes the most part of oxidized lipoproteins via scavenger receptor Class B Member 3 and LDL receptors from the blood circulation, but it is also able to synthesize lipoproteins endogenously. On the other hand, aged or stressed RPE is unable to properly process the oxidized lipids, when the macrophages, which normally clean up these deposits, become less efficient and are slowly intoxicated by excessive levels of 7-ketocholesterol (7KCh) and other oxidized lipids [87–89, 96, 97, 123]. 7kCh is an oxidized form of cholesterol, that is, an oxysterol formed by auto-oxidation of cholesterol and cholesterol esters [124–127]; it is found in oxidized LDL deposits in the form of oxysteryl esters, covalently bound to oxidized unsaturated fatty acids [128–130]. Cholesteryl esters are particularly susceptible to oxidation and the cholesterol molecules in these esters can be oxidized to 7kCh [131–133]. In the primate retina, two main mechanisms for oxidation of cholesterol to 7kCh have been described: the Fenton reaction and the photooxidation [134–136]. The Fenton reaction requires a transition metal catalyst, such as iron and copper. Although the levels of these metals have not been measured in oxidized lipoprotein deposits, atherosclerotic plaques are known to contain relatively high levels of them [137]. By means of photooxidation and in presence of an adequate photosensitizer, cholesterol can be converted in a series of hydroperoxide intermediates that can further oxidize to 7kCh. Lipofuscin fluorophore A2E is one of the well-known photosensitizers, being involved in cholesterol ROS-mediated oxidation and also in the inhibition of the normal cholesterol efflux from RPE cells [138–140]. During the histopathologic evaluation of eyes affected by AMD, Lakkaraju and co-workers have documented that A2E induces aberrant cholesterol metabolism in RPE [140], which could contribute to AMD onset or progression also by means of inflammatory mechanisms.

4. Gene Variants Associated with AMD

Since 2005, several common variants in genes complement pathway have been consistently associated with the development of AMD. The common coding variant p.Tyr402His in the gene encoding complement factor H (*CFH*) was the first identified [64, 141–143]. A number of other polymorphisms in *CFH* [144], as well as in other genes involved in the alternative complement cascade, have also been demonstrated to affect AMD risk, including genes for complement component 2 (*C2*), complement component 3 (*C3*), and complement factor I (*CFI*) [29, 144–147]. More recently, common variants in genes encoding for cholesterol-related pathway, such as *LIPC* and tissue inhibitor of metalloproteinase 3 (*TIMP3*), have been reported to be associated with AMD in large GWASs [31, 32]. In the next subheadings, the genes implicated in phenotypic expression of AMD will be detailed, especially considering those main contributory variants at the basis of that immunoinflammatory dysregulation which, in AMD patients, can be labeled as inflammaging [148].

4.1. Complement Factor H. Originally known as β-1H globulin, CFH is a serum glycoprotein that regulates the function of the alternative complement pathway in fluid phase and on cellular surfaces. The binding of CFH to C3b reduces complement C3 activation, inhibits the formation of C3a, and lowers the production of IL-6 [149]. Besides, CFH accelerates the decay of the alternative pathway convertase C3bBb, and also acts as a cofactor for CFI, another C3b inhibitor [150, 151]. The *CFH* gene is located on chromosome 1q32, spans 94 kb, and comprises 23 exons. The *CFH* gene is located within a cluster of genes encoding the regulatory complement components of the activation of C3. This gene cluster includes the factor H-related genes *FHR1*, *FHR2*, *FHR3*, *FHR4*, and *FHR5* and the decay-accelerating factor, C4-binding protein (*C4BPA* and *C4BPB*), among others.

The c.1277 T-to-C transition in exon 9 of CFH gene (rs1061170) results in a substitution of histidine for tyrosine at codon 402 of the CFH protein (p.Tyr402His). This missense variant is located in the Short Consensus Repeat 7 (SCR7) that acts as a binding site of CFH to C-reactive protein (CRP) and heparin [152]. The binding of CFH to CRP or heparin increases CFH affinity for C3b and downregulates complement activity [153]. The p.Tyr402His can be

considered to be a functional protein variant, as the p.His402 allele impairs the binding of CFH to CRP, thus resulting in an enhanced complement activation and consequent tissue damage. At sites of tissue injury, the p.His402 variant does not dampen the alternative pathway of complement activation as efficiently as p.Tyr402 allele [154–158]. In Caucasian populations of European ancestry the p.His402 allele is very common, having a gene frequency in the range of 0.3-0.4. The p.His402 allele is likely replacing the major one because in early life it provides a survival advantage against streptococcal infections; for example, microbes bind CFH to their surface to inhibit complement activation [46, 159]. The CFH binding protein of group A beta hemolytic streptococcus has a lower affinity for p.His402 than for p.Tyr402. As a result, the host's complement system has greater activity against the pathogen if the host expresses p.His402, thereby reducing the microbes' ability to counteract the alternative pathway. CFH adheres to damaged eukaryotic cells and tissue debris via the same anionic (heparin) binding sites that microorganisms employ to attach it to their surface [160–162]. If on one hand the p.His402 allele is potentially giving some benefit, on the other hand it is one of the most significant known genetic contributor to AMD disease risk. In individuals bearing a p.His402/His402 homozygous genotype, the risk of developing all categories of AMD was estimated to be 3-fold increased. Higher odds ratio (OR) values, in the range of 3.5–7.4, were found if only advanced dry and wet forms of AMD were considered [64, 141–143]. The association between the p.His402/His402 genotype and AMD could be explained by a reduced capacity of the p.His402 variant of CFH to bind debris in a damaged retina. Differential binding of p.His402 versus p.Tyr402 to multiple constituents of a damaged retina has been demonstrated for DNA, RNA, lipids, CRP, necrotic and apoptotic cells, heparin and other glycosaminoglycans, lipofuscin, bis-retinoids, photooxidation byproducts, and amyloid beta. The common finding is that the p.His402 protein binds with a lower affinity than p.Tyr402. Therefore, in the retina of a p.His402/His402 homozygous individual there is a higher level of alternative pathway activation, leading to retinal debris accumulation and ultimately AMD development.

Recent evidence has been reported supporting the existence of multiple AMD susceptible alleles in the chromosome region of the *CFH* gene [163]. A case-control study of 84 single nucleotide polymorphisms located in a 123 kb genomic region in 1q32 including the *CFH* gene provided evidence that multiple *CFH* haplotypes associate with AMD risk independently from p.Tyr402His [143]. Functional variants within these haplotypes are likely to influence the expression of *CFH* gene and possibly also of other nearby genes of the C3-activation cluster. In particular, an A-to-G variant located in intron 14 of *CFH* gene (rs1410996) has been reported to associate with AMD [144]. In the recent GWAS of Fritsche and co-workers [24], the most strongly AMD-associated single nucleotide polymorphism in the *CFH* region—rs10737680—was not in disequilibrium with p.Tyr402His, which instead was tagged by a weaker signal. This evidence further supports the hypothesis that multiple functional gene variants in the *CFH* locus act as risk factors for AMD.

4.2. C2/CFB Cluster. The *C2* gene encodes for the complement component 2, spans 18 kb, and includes 18 exons. The *C2* gene maps in 6p21.33 and is adjacent to the *CFB* gene, which encodes for complement factor B, from which is separated by just 271 nucleotides. Common variants within the *C2/CFB* cluster have been recently confirmed as being significantly associated with risk of developing AMD [164].

A missense G-to-C variant in exon 7 of C2 (rs9332739) has a frequency of 0.067 among Europeans and causes the replacement of the glutamic acid residue at codon 318 with an aspartic acid one (p.Glu318Asp). The C-to-A substitution within intron 10 of C2 (rs547154) has a frequency of 0.062 among Europeans. Minor alleles of both rs9332739 and rs547154 have a protective effect and reduce by half the risk for developing AMD. A recent meta-analysis estimated that OR of C-allele of rs9332739 was 0.55 (95% confidence interval (CI): 0.46, 0.65), while minor allele at rs547154 carried an OR of 0.47 (95% CI: 0.39, 0.57) [27].

The CFB:c.26 T-to-A transversion (rs4151667) in exon 1 of *CFB* results in the substitution at codon 9 of leucine with histidine (p.Leu9His) that has a frequency of 0.067 among Europeans. A second missense variant in *CFB* gene, the CFB:c.95 G-to-A transition (rs641153), is located in exon 2 and determines a substitution of arginine at position 32 with a glutamine residue (p.Arg32Gln). The minor A-allele of CFB:c.26 T-to-A and the A-allele of CFB:c.95 G-to-A carried estimated risks of 0.54 (95% CI:0.45, 0.64) and 0.41 (95% CI:0.34, 0.51), respectively [27].

Haplotype analyses using two independent cohorts of AMD patients identified a statistically significant common risk haplotype and two protective haplotypes [145]. Both the haplotype including minor alleles of C2:c.954 G-to-C and CFB:c.26 T-to-A (H10 haplotype) and the haplotype including A-allele of rs547154 variant in intron 10 of C2 and the A-allele of CFB:c.95 G-to-A (H7 haplotype) confer a significantly reduced risk for AMD [145]. The protective effect of H7 haplotype has been confirmed in independent studies, and evidence has been reported suggesting that minor alleles of both variants contribute independently to the protective effect. To date it is not clear if the rs547154 variant in intron 10 of C2 has a functional activity or rather is in disequilibrium with a causal variant, but it is likely that its minor allele could be associated with a lower expression of C2. The CFB protein containing glutamine at position 32 has been reported to reduce hemolytic activity compared with the arginine containing form and to cause less efficient complement activation [165, 166]. This lower complement response determined by H7 haplotype could possibly explain the protective effect on AMD development. Combined analyses of the *C2/CFB* haplotypes and *CFH* variants showed that variation in the two loci can predict the clinical outcome in 74% of the affected individuals and 56% of the controls [167].

4.3. Complement Component 3. The *C3* gene encodes the complement component 3, a factor that plays important biological roles in the classical, alternative, and lectin activation pathways. The *C3* gene spans 41 kb on chromosome 9p13.3 and comprises 41 exons. The active C3 factor includes

an α-chain, encoded by the last 26 exons, and a β-chain, encoded by the first 16 exons, having exon 16 encoding both α and β-chain. The synthesis of C3 factor is induced during acute inflammation. C3 is produced mainly by liver but also by activated monocytes and macrophages. Mature C3 factor is obtained from the cleavage of a single chain 200 kDa precursor into the α (C3α) and β (C3β) subunits that are linked by disulfide bonds. C3 factor has a critical role in the complement system, and C3 deficiency makes people more susceptible to bacterial infection. The c.304 C-to-G substitution in exon 3 of *C3* gene (rs2230199) is a common missense variant that causes replacement of arginine residue at codon 102 with a glycine one (p.Arg102Gly). These two alleles correspond to the slow and fast electrophoretic variants of C3 factor. The p.Gly102 allele has a frequency of 0.175 among Europeans and is carried by more than 30% of individuals. Association between p.Arg102Gly and AMD has been confirmed in many studies on Caucasian populations [55, 146, 147, 164, 168, 169], but not in Asian populations, probably due to the lower frequency of the p.Gly102 allele [170]. Among Europeans, the OR for AMD has been reported to be 1.7 in p.Arg102/Gly102 heterozygotes and 2.6 in p.Gly102/Gly102 homozygotes, and the estimated population attributable risk for p.Gly102 was 22% [147].

4.4. Complement Factor I. The complement factor I (CFI) gene maps on chromosome 4q25, spans 63 kb, and comprises 13 exons. The first eight exons encode the heavy chain of CFI, while the light chain of CFI is encoded by the last five exons. The two chains are linked by disulfide bonds. CFI is a serine protease that plays a role in the complement pathway as it cleaves and inactivates C4b and C3b. A C-to-T transition (rs10033900) located 4.3 kb downstream the 3′ UTR of *CFI* gene has been shown to be independently associated with AMD [29, 31]. This variant could have a role in influencing CFI expression level or be in linkage disequilibrium with a functional regulatory variant.

4.5. ARMS2/HTRA1 Locus. A locus in 10q26.13 (LOC387715) has been identified as the second most important locus in the etiology of AMD [170, 171]. This locus includes the age-related maculopathy susceptibility (ARMS2) gene and the gene encoding for the high-temperature requirement factor A of serine peptidase 1 (HTRA1). A G-to-T transversion in exon 1 of ARMS2 (rs10490924) is a common missense variant that replaces an alanine residue with a serine (p.Ala69Ser). The p.Ser69/Ser69 homozygotes were reported to have a significant 7.6-fold increased risk of developing AMD [171], and this association has been next confirmed in independent case-control studies. Only 4.2 kb separate *ARMS2* gene from the near *HTRA1* gene, and the ARMS2:p.Ala69Ser variant is located just 6.3 kb from a G-to-A variant in the promoter region of HTRA1 gene (rs11200638). These two variants are in strong linkage disequilibrium ($r^2 = 0.90$), and it is difficult to determine which one could be the causal variant in this locus [24]. Therefore, it is still under debate to definitively establish which gene, *ARMS2*, *HTRA1* or possibly also others, is responsible for the genetic association with AMD [8].

The *HTRA1* gene encodes a member of the trypsin family of serine proteases. The HTRA1 protein is a 50 kDa secreted enzyme that cleaves substrates involved in the complement pathway, such as clusterin, vitronectin and fibromodulin, and could theoretically play a role in the pathogenesis of AMD. The G-to-A substitution in the promoter region of HTRA1 has been initially considered a functional variant as it is located in a conserved CpG island and resides within a putative binding site for the transcription factor adaptor-related protein complex-2α and could possibly regulate the expression level of HTRA1 [172, 173]. However, later studies showed that this variant does not affect the transcription level of HTRA1 in several cell lines [174] nor alters HTRA1 mRNA or protein expression in human retina-RPE-choroid samples [175]. Therefore, it is unlikely that rs11200638 is the functional variant that accounts for the strong association between the *ARMS2*/*HTRA1* locus and the risk of developing AMD.

ARMS2 is a small gene—just 2.7 kb wide—that includes only two exons and a single intron. The encoded 107-amino acid peptide is expressed in the outer membrane of mitochondria and in the citosol. The p.Ala69Ser variant could affect the conformation of protein and eventually modify mitochondria function [174]. A second variant, an insertion/deletion (indel) polymorphism in the 3-prime untranslated region (3′UTR) of *ARMS2* (ARMS2:c.372_815del443ins54), has been strongly associated with risk of developing AMD ($P = 4.1 \times 10^{-9}$) [164]. The association between del443ins54 indel and AMD has been replicated in different populations [176]. This indel variant removes the polyadenylation signal in the 3′ UTR of ARMS2 and replaces it with a 54 bp element known to mediate rapid mRNA turnover. The expression of ARMS2 transcript is lost in homozygous carriers of the del443ins54 indel. This variant is located between ARMS2:c.269 G-to-T and HTRA1:c.-625 A-to-G, and the haplotype including minor alleles (T-indel-A) was reported to be associated with a significant 3-fold increased risk for AMD [176]. Considering the deleterious effect of del443ins54 indel to the expression of *ARMS2* transcript, we could suggest that this indel polymorphism could be the actual variant causing the increased risk of AMD associated with the *ARMS2/HTRA1* locus.

The actual function of ARMS2 protein is unknown, but it is thought to play a role in diseases in the elderly [8]. *ARMS2* transcripts have been detected in retina and in a variety of other tissues and cell lines [174], and it has been proposed that ARMS2 could play a key role in AMD through mitochondrial-related pathways [164]. So far, very little is known about the function of ARMS2, and more investigations are needed to determine if variants in this gene have causal role in the pathogenesis of AMD.

4.6. Tissue Inhibitor of Metalloproteinase 3. The tissue inhibitor of metalloproteinase 3 (*TIMP3*) gene belongs to a family of genes encoding for inhibitors of matrix metalloproteinases, a group of zinc-binding endopeptidases involved in the degradation of the extracellular matrix. TIMP3 is also a potent angiogenesis inhibitor, as it blocks the binding of VEGF to VEGFR2 and inhibits downstream signaling leading to VEGF-mediated angiogenesis [177]. The *TIMP3*

gene spans 55 kb on chromosome 22q12.3 and includes 5 exons. *TIMP3* mutations are causing a Mendelian early onset form of macular degeneration often complicated by CNV, known as Sorsby's fundus dystrophy (MIM #136900). *TIMP3* has been considered a putative candidate for AMD susceptibility, but early studies failed to find association between TIMP3 and AMD [178, 179]. Recent evidence has been reported indicating that an A-to-C substitution located far upstream (113 kb) of TIMP3 gene (rs9621532) within an intron of the synapsin III gene (SYN3) is associated with a reduced risk of developing AMD [31]. This variant influences the expression of TIMP3 transcripts in cultured primary human fetal RPE cells, and the protective C-allele of rs9621532 was associated with mRNA expression [180]. However, the genetic association between rs9621532 and AMD has not been confirmed in Asian population, and the role of TIMP3 in AMD etiology still remains controversial [181].

4.7. Hepatic Lipase. *LIPC*, a novel AMD gene, is involved in HDL cholesterol metabolism. The gene spans 60 kb on chromosome 15q21.3, includes 9 exons, and encodes a hepatic triglyceride lipase which is expressed in liver. LIPC enzyme is also a triglyceride hydrolase and a ligand/bridging factor for receptor-mediated lipoprotein uptake. Rare deficiencies of LIPC are associated with pathologic levels of circulating lipoprotein. Expression of LIPC in the retina has been reported [167]. Two variants in *LIPC* putative promoter, an A-to-G substitution (rs493258) located 14 kb from LIPC transcription start site and a C-to-T substitution (rs10468017) 22 kb upstream of *LIPC*, were reported to be associated with advanced AMD in two independent European cohorts, indicating that common variants in LIPC gene could play a role as genetic risk factor for AMD [31, 32, 182]. These variants are thought to regulate the expression of *LIPC* and therefore influence the metabolism of HDL cholesterol. The T-allele of rs10468017 has been reported to have a protective effect for advanced wet and dry AMD by influencing LIPC expression in serum and increasing HDL levels [32].

5. Final Remarks

This etiogenotypic excursus has been first and foremost aimed to speculatively interconnect two different types of gene polymorphism, which are able to alter either complement or cholesterol pathway and, consequently, to predispose to AMD via inflammation and parainflammation. Several clinicogenetic studies show increased OR to develop AMD in individuals carrying more risk genotypes [167, 183]. In particular, the carriers of combination of peculiar *CFH*, *ARMS2/HRTA1*, and *C2/CFB* genotypes have been reported to have high OR values, although significance level of these findings were relatively low, mainly due to the low number of patients included in the investigations. Calculating a risk score including genetic information across the nineteen top loci resulting from a very recent GWAS [24], Fritsche and coworkers reported that a multiple combination of genotypes could distinguish AMD patients from healthy controls (area under the receiver operator curve = 0.74) and suggested that similar scores could be used to identify and prioritize at-risk individuals, in order to provide them preventive treatment before the disease onset. The development of an efficient tool able to predict the development of AMD is strongly awaited, as it could have a remarkable impact on the health systems. However, several biases can counteract the expectation to achieve reliable data on this complex topic. Many small clinicogenetic studies and, despite adequate statistical protection from multiple comparisons, some GWASs are at risk of findings by chance or of overestimating marker effects [184, 185]. Therefore, correct translational information from genomic marker research to clinical practice of AMD will be more rapidly available if biomedical community works together in carrying out large-scale consortium of trials designed to concomitantly verify the weight of both clinical [13] and genotypic [24] risk factors in AMD patients, as recently performed by Seddon and co-workers in a quite numerous sample population [186]. Before long, the validation of risk prediction models, inclusive of proteomic biomarkers, will be useful for the managing of research, clinical trials, and personalized medicine not only in AMD, but also in other frequent causes of legal blindness such as diabetic retinopathy, glaucoma, and pathologic myopia [1]. In particular, AMD risk scores based only on simple sums of genotypes are unlikely to turn out effective, probably because the complex nature of AMD etiology includes synergistic interactions both among gene variants and among these and environmental conditions. A more comprehensive exploratory approach on the relationship between the chief AMD-risk genotypes, the underlying immunoinflammatory endophenotypes, and the networks of interaction with acquired or epigenetic factors is likely to provide, in the near future, the knowledge for the development of useful predictive algorithms, able to guide in the direction of an effective primary and secondary prevention of AMD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Systemic Treatments for Noninfectious Vitreous Inflammation

Angela Jiang,[1] Jillian Wang,[1] Malav Joshi,[2] and John Byron Christoforidis[2]

[1] *Department of Ophthalmology, The Ohio State University Wexner Medical Center, 915 Olentangy River Road, Suite 5000, Columbus, OH 43212, USA*
[2] *Department of Ophthalmology, The University of Arizona Medical Center, 655 North Alvernon Way, Suite 108, Tucson, AZ 85711, USA*

Correspondence should be addressed to John Byron Christoforidis; jbchristo@hotmail.com

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Vitreous inflammation, or vitritis, may result from many causes, including both infectious and noninfectious, including rheumatologic and autoimmune processes. Vitritis is commonly vision threatening and has serious sequelae. Treatment is frequently challenging, but, today, there are multiple methods of systemic treatment for vitritis. These categories include corticosteroids, antimetabolites, alkylating agents, T-cell inhibitors/calcineurin inhibitors, and biologic agents. These treatment categories were reviewed last year, but, even over the course of just a year, many therapies have made progress, as we have learned more about their indications and efficacy. We discuss here discoveries made over the past year on both existing and new drugs, as well as reviewing mechanisms of action, clinical dosages, specific conditions that are treated, adverse effects, and usual course of treatment for each class of therapy.

1. Introduction

Vitreous inflammation, or vitritis, may result from many causes, including both infectious and noninfectious. Epidemiologic studies indicate that uveitis accounts for 2–10% of prevalent blindness in the European and North American population and is therefore an underrated and significant public health problem [1]. Infectious etiologies include bacterial Lyme, syphilis, or *Bartonella*; viruses HSV, VZV, and CMV, and a variety of fungal and parasitic causes. Noninfectious etiologies include rheumatologic and autoimmune processes, examples being sarcoidosis, systemic lupus erythematosus, multiple sclerosis, and Behcet's disease. However, idiopathic vitritis without associated systemic disease is most common. Vitritis is sometimes visionthreatening, due to sequelae such as cystoid macular edema (CME), vitreous opacities, and retinal detachment, ischemia/neovascularization, or pigment epithelium changes. Glaucoma and cataracts may also form. With such serious sequelae, there are multiple methods of systemic treatment for vitritis. On the other hand, mild vitritis without vasculitis or CME can sometimes be followed closely without any treatment. The goal of all types of treatment is to rapidly alter and stop the course of intraocular inflammation but at the same time minimize any side effects from these systemic drugs. We reviewed these treatment categories last year, but, even over the course of just a year, many therapies have made progress, as we have learned more about their indications and efficacy [2].

2. Initial Treatment: Corticosteroids

The first line of treatment for noninfectious uveitis is corticosteroids. This group of drugs is used to suppress inflammation, either systemically or intraocular. The accepted algorithm for treatment begins with topical glucocorticoids, with frequency depending upon severity and not necessarily etiology. However, topical corticosteroids have been shown to have poor penetration into the posterior segment and are thus not used often for posterior segment disease; they are more commonly used to reduce anterior chamber inflammation and have only a minor effect on vitreous inflammation [3]. Oral or intravitreal corticosteroids are therefore used to treat cases of posterior segment disease. Oral prednisone (1 mg/kg/day with gradual tapering) is often the first therapeutic agent used [4].

Table 1: Disease indications for immunosuppressive agents.

Strong Indications	Relative Indications
Behcet's disease with retinal involvement	Noninfectious uveitis
Vogt-Koyanagi-Harada syndrome	Retinal vasculitis with central vascular leakage
Sympathetic ophthalmia	Severe chronic iridocyclitis
Juvenile idiopathic arthritis-associated uveitis	Relapsing polychondritis with scleritis
Ocular manifestations of Wegener's granulomatosis	Ocular cicatricial pemphigoid
Rheumatoid necrotizing scleritis or peripheral ulcerative keratitis	Serpiginous choroiditis

Intravitreal delivery systems include injection or implantation of periocular or intravitreal steroid compounds (triamcinolone acetonide) [5]. There are several different types of systems, either nonbiodegradable or biodegradable; a more extensive review of drug delivery implants is reviewed in our other paper. Although previous studies raised concern for recurrence of inflammation as intravitreal steroid concentration decreases, some recent trials elude that this may no longer be the case [6]. Patients undergoing treatment with local delivery methods will usually have minimal adverse events. It has however been reported that localized side effects may occur, such as cataract formation, increased intraocular pressure, and transient vitreous hemorrhage.

On the other hand, those undergoing systemic corticosteroid therapy often encounter nonocular adverse events, such as arthralgia and hypertension. Other common complications range from those affecting the musculoskeletal system (osteoporosis, aseptic bone necrosis, and myopathy), gastrointestinal system (ulcers and pancreatitis), endocrine (hyperglycemia and cushinoid features), infectious, (delayed wound healing, secondary infection, and reactivation of latent herpes simplex or tuberculosis), or even psychosis. If patients develop adverse effects, or are refractory to treatment with corticosteroid therapy, switching to an intravitreal delivery system or considering systemic immunosuppressive therapy is indicated [7].

3. Immunosuppressive Treatment

Systemic immunosuppressive therapy can either supplement or completely replace corticosteroid therapy, for the reasons touched upon above. There are several conditions that have been found to be refractory to corticosteroid treatment but instead respond to immunosuppressives. Examples of these conditions ran the gamut of several autoimmune diseases such as Behcet's, Wegener's, or juvenile idiopathic arthritis-associated uveitis [8]. Other conditions that indicate immunosuppressive therapy are found in Table 1.

There are several categories of immunosuppressive agents: antimetabolites, alkylating agents, T-cell inhibitors/ calcineurin inhibitors, and biologic agents. Information about these categories is available in Table 2, while newer biologics and investigations are discussed below. Table 3 addresses ocular diseases and which groups of immunosuppressive agents are used to treat them.

In general, treatment with immunosuppressives starts after or with corticosteroid therapy, with local treatment attempted before systemic treatment, if the disease process is amenable. Systemic treatment attempts to start with the least toxic medications in the case of mild-moderate disease; methotrexate and cyclosporine are most commonly used after corticosteroids, followed by more antimetabolites. Severe, vision-threatening disease may require the use of biologic or cytotoxic agents, although they are avoided whenever possible due to their severe adverse effects.

3.1. Leflunomide. Leflunomide is a noncytotoxic drug that works on both the cellular and humoral immune response. It is most commonly used for systemic rheumatologic diseases, examples being severe rheumatoid or psoriatic arthritis. Ocular use in treating chronic inflammation associated with sarcoidosis is currently under investigation [9]. Recently, Leflunomide was proven as both safe and efficacious for long-term therapy treating chronic anterior uveitis associated with juvenile idiopathic arthritis [10]. Most patients maintained an ocular response to the drug and underwent only a few mild adverse effects. Common adverse effects of Leflunomide include hepatotoxicity with known fatalities, myelosuppression with resulting opportunistic infection and anemia, interstitial lung disease, alopecia, and skin reactions (Stevens Johnson and toxic epidermal necrolysis). Leflunomide is also a teratogen (pregnancy class X), and patients need to be on contraception during treatment. Overall, it is a promising form of treatment, as methotrexate is currently the first and was previously the only choice for patients with juvenile idiopathic arthritis.

3.2. Biologic Agents. Biologic agents are one of the newest classes of therapeutic proteins. They were originally developed for preventing organ transplant rejection but were found to be useful for treating systemic inflammatory diseases as well. They are now used off label in treating uveitis, and have been used with some success for refractory cases. Biologic agents' major mechanisms of action all revolve around targeting specific inflammatory molecules, with the goal of inhibiting mediators or cytokines. Examples of these inflammatory mediators include tumor necrosis factor alpha and interleukin-2. Due to their strong immunologic suppression, serious adverse effects revolve around infectious processes or malignancies such as lymphoma. Latent and opportunistic infections are especially important to monitor for and include those such as tuberculosis, histoplasmosis, coccidiomycosis and herpes viruses.

Biologic agents are categorized into two groups: monoclonal antibodies and fusion proteins. Monoclonal antibodies are further classified and suffixes named based on their regions (either human, murine, or a combination of regions). Fusion proteins are created by joined genes, and are a combination of a receptor and another protein fragment.

3.2.1. Adalimumab. Adalimumab is a recombinant, full-length humanized immunoglobulin directed against tumor

Table 2: Immunosuppressive agents, organized into categories, and with information on mechanism of action, administration, side effects, and clinical management.

	Mechanism of action	Indications	Administration	Side effects	Management
			Antimetabolites		
(1) Methotrexate	Folic acid analog; dihydrofolate reductase inhibitor, thus inhibiting synthesis of purines and therefore DNA, RNA, thymidylate, and proteins [7]. Reduces T-cell role in inflammation by inhibiting its activation and suppressing intercellular adhesion molecule expression [37]. With all administrations of methotrexate, it is critical to supplement folinic acid, to restore thymidylate and purine biosynthesis.	(i) Vitritis (ii) Vasculitides (iii) Anterior uveitis (iv) Orbital pseudo-tumor (v) Sarcoidosis	(i) Oral (ii) Subcutaneous (iii) IM (iv) IV Dose: 7.5–25 mg/week and May require 3–8 weeks for effects to take full effect. Course: two years after reduction of inflammation, to avoid recurrence [38].	(i) Common: fatigue, nausea, vomiting, and anorexia [39] (ii) Rare: hepatotoxicity, marrow suppression, and vasculitis (cutaneous) (iii) Teratogen Overall, long-term side effect profile is preferable compared to high-dose steroids.	Baseline: CBC, serum chemistry, BUN, Cr, LFT it, UA, pregnancy test. Follow-Up: CBC and LFT's every 4 weeks, with dose adjustment if LFT's double on two measurements. Stopped if LFT's stay elevated even after dose reduction [40].
(2) Azathioprine	Imidazolyl derivative; active metabolite is a purine synthesis inhibitor. Since lymphocytes have no method of nucleotide salvage, they are particularly affected [41].	(i) Serpiginous choroiditis (ii) Multifocal choroiditis (iii) Panuveitis (iv) Ocular cicatricial pemphigoid (v) Juvenile idiopathic arthritis [42–44]	Oral Dose: initially 2-3 mg/kg/day. Course: two years after reduction of inflammation, to avoid recurrence [45].	(i) GI upset (ii) Hepatotoxicity, bone marrow suppression, alopecia, and pancreatitis [46].	Baseline: CBC, LFT's, thiopurine methyltransferase enzyme activity (If low enzyme activity withhold treatment [46].) Follow-Up: CBC and LFT's every 4–6 weeks, with dose adjustment or temporary stop if abnormalities arise [47].
(3) Mycophenolate mofetil	Reversibly inhibits guanosine nucleotide synthesis, which particularly affects B- and T-cells [48]. it disrupts cellular adhesion to vascular endothelial cells, thus affecting lymphocytic chemotaxis [49].	(i) Chronic ocular inflammation [50] (ii) Scleritis, uveitis; used with cyclosporine and methotrexate [50].	(i) Oral (ii) IV Dose: initially 500 mg twice daily, thereafter increaseing to 1 g twice daily if well tolerated [45]. Course: two years following ocular quiescence [45].	(i) GI upset (nausea, vomiting, and diarrhea) (ii) Bone marrow suppression, hepatotoxicity [8]	Baseline: CBC, LFTs Follow-Up: CBC weekly for first month, twice monthly for next two months, and then monthly. LFT's monthly for duration of treatment [51].
(4) Leflunomide	Pyrimidine synthesis inhibitor, by inhibiting dihydroorotate dehydrogenase. In this manner, it suppresses B- and T-cell proliferation by interfering with cell cycle progression [52]. Nonlymphoid cells use a salvage pyrimidine pathway to synthesize ribonucleotides [52]. Leflunomide also has proven anti-inflammatory action, due to suppression of lymphocyte proliferation, tyrosine kinase, cyclooxygenase, and histamine release [53, 54].	Systemic rheumatology (severe rheumatoid and psoriatic arthritis). Ocular use in treating chronic inflammation associated with sarcoidosis is currently under investigation (see main text).	Oral Dose: loading dose100 mg and then 10–20 mg daily. A loading dose may result in initially increased adverse effects, but more rapid efficacy [55, 56]. To increase tolerability, patients may be given prednisolone rather than a loading dose [55]. Course: currently not certain.	(i) Serious hepatotoxicity (jaundice, hepatitis, and fatalities) (ii) Bone marrow suppression, interstitial lung disease, paresthesias, and headaches (iii) Teratogen [57] Due to its hepatotoxic effects, concurrent use with methotrexate is not recommended.	Baseline: CBC and LFTs. Follow-Up: both biweekly for the first six months, then bimonthly for the duration of treatment.

Table 2: Continued.

	Mechanism of action	Indications	Administration	Side effects	Management
			Alkylating agents		
(1) Cyclophosphamide	Cytotoxic properties are due to addition of an alkyl group to the guanine base of DNA and forming irreversible inter- and intrastrand DNA cross-links at guanine positions. This results in toxicity to rapidly-dividing cells (lymphocytes) and suppression of antibody production and delayed type hypersensitivity [58].	(i) Behcet's disease (ii) Polyarteritis nodosa (iii) Wegener's granulomatosis (iv) Mooren's ulcer [59–64]	IV Dose: starts at 1 g/m^2 and adjusted on response and side effects [51]. At the beginning of treatment, given biweekly. Discontinued if hematuria occurs, with urology consult indicated if hematuria persists beyond three weeks [51]. Course: once ocular quiescence is achieved, space treatment intervals to every 3-4 weeks continued for 1 year.	(i) Bone marrow suppression (ii) Hemorrhagic cystitis (iii) Secondary cancers (bladder, AML) (iv) Testicular atrophy (v) Ovarian suppression (vi) Known teratogen	Baseline: CBC, LFTs, UA Follow-Up: CBC and urinalysis are initially repeated weekly then spaced out to monthly intervals when blood counts are stabilized.
(2) Chlorambucil	Cytotoxic properties from addition of an alkyl group and forming DNA crosslinks [65].	(i) Sympathetic ophthalmia (ii) Behcet's disease (iii) Serpiginous choroiditis [66, 67]	Oral Dose: two treatment algorithms. One starts at 0.1 mg/kg/day; maximum dosage 12 mg daily. The other uses short-term higher doses for 3–6 months [52]. Course: one year after ocular quiescence [47].	(i) Heme/Onc: myelosuppression, bone marrow aplasia, and secondary cancers (ii) Endocrine: male sterility, amenorrhea (iii) GI: hepatotoxicity (iv) CNS: seizures (v) Infectious: reactivation of latent herpes simplex virus [52, 68, 69].	Baseline: CBC w. differential, LFT's. Follow-Up: CBC initially repeated weekly, then spaced out to monthly intervals after stable dose. LFTs monthly.
			T-cell inhibitors/calcineurin inhibitors		
(1) Cyclosporine	Suppresses T lymphocyte activity and thus the immune response. Binds lymphocytic protein cyclophilin, which inhibits calcineurin. Since calcineurin normally activates interleukin-2 transcription, there is decreased T lymphocyte function [70].	(i) Behcet's disease (ii) Sympathetic ophthalmia (iii) Sarcoidosis (iv) Birdshot retinochoroidopathy (v) VKH [71, 72]	Oral Dose: initially 2.5 mg/kg/day, increased in increments of 50 mg; maximum 5 mg/kg/day [47]. Course: two years after ocular quiescence [47].	(i) Hypertension, gingival hyperplasia, lymphoma nephrotoxicity (ii) Myalgia, tremor, or paresthesias	Baseline: LFT's, CBC w. differential, BUN, Cr, UA, blood pressure Follow-Up: blood pressure and electrolytes initially repeated biweekly spaced out to monthly after dose is stable. Other labs monthly [51].
(2) Tacrolimus	Macrolide antibiotic, whose mechanism is similar to that of cyclosporine; both inhibit calcineurin and suppress T-cell signaling and IL-2 transcription [73].	Used with systemic corticosteroids [73]. Often used when cyclosporine treatment fails [74, 75].	(i) Oral (ii) IV Dose: 0.10–0.15 mg/kg/day. The more serious adverse effects are seen at higher doses [76–78].	Hypertension, nephron-toxicity, electrolyte abnormalities, anorexia, neurologic (insomnia, confusion, depression, catatonia, tremors, and seizures), non-Hodgkin's lymphoma	Similar to cyclosporine.

Table 2: Continued.

	Mechanism of action	Indications	Administration	Side effects	Management
(3) Rapamycin	Inhibits cellular response to IL-2 and inhibits activation of B and T lymphocytes. Rapamycin acts on "mammalian target of rapamycin" (mTOR), rather than on a calcineurin inhibitor, as cyclosporine and tacrolimus do.	Used with other immunosuppressive agents [79, 80].	Oral Dose: loading 6 mg; daily 2–6 mg/day [79].	Elevated LFT's, anemia, thrombocytopenia, hypercholesterolemia, nausea, abdominal pain, eczema, and increased risk of malignancy Markedly less nephrotoxic than other calcineurin inhibitors.	Similar to cyclosporine and tacrolimus
			Biologic agents		
(1) Etanercept	Targets TNF-α and TNF-β receptor, preventing molecules from binding, thus inactivating TNF. Thus it suppresses neutrophil migration and cytokine synthesis.	Indeterminate; see paper	Subcutaneous Dose: 25 mg twice a week, for two years.	Infection, increased risk for latent TB and hepatitis B reactivation, CNS demyelination, pancytopenia, congestive heart failure, and lymphoma [81, 82].	Baseline: CBC, LFT's, TB skin test, hepatitis B serologic testing Follow-Up: monthly CBC and LFTs [52, 83].
(2) Infliximab	Binds to and inhibits TNF-α (bound or circulating) [84].	(i) Sarcoidosis (ii) Wegener's granulomatosis (iii) Juvenile inflammatory arthritis (iv) Behcet's disease [85–89]	Intravenous Dose: loading infusions weeks 0, 2, and 6; maintenance infusions every eight weeks [89]. For monotherapy, dose of 5 mg/kg; for concurrent noncorticosteroid treatment, dose of 3 mg/kg. Treatment for two years after ocular quiescence is achieved [40].	Infection (urinary tract, upper respiratory), GI (nausea, emesis), vasculitis, anemia, and thrombocytopenia [89–91].	Baseline: CBC, LFT's, TB skin test Follow-Up: monthly CBC and LFTs.
(3) Adalimumab	Binds to and inhibits TNF-α [92].	(i) Birdshot retinochoroidopathy (ii) VKH (iii) Behcet's disease (iv) Rheumatoid arthritis scleritis [12–16].	Subcutaneous Dose: 40 mg every two weeks [93]. Course: 2 years after ocular quiescence is achieved [40].	Injection site reactions, infections (urinary tract, upper respiratory), headache and confusion, CNS demyelination, hepatotoxicity, congestive heart failure, and lymphoma [94, 95].	Similar to infliximab.
(4) Daclizumab	Binds to CD25, a subunit of the IL-2 receptor on T lymphocytes [96].	(i) Birdshot retinochoroidopathy (ii) Posterior uveitis (iii) Juvenile inflamm arthritic uveitis [97–99].	Intravenous Dose: 1 mg/kg every two weeks; maximum daily dose of 200 mg [100]. Dose independent of concurrent immunomodulatory treatment. Course: two years after ocular quiescence is achieved [97].	Rash, lymphadenopathy, chest discomfort, and fever [101].	Baseline: CBC, LFTs Follow-Up: repeat baseline labs prior to each infusion.

Table 2: Continued.

	Mechanism of action	Indications	Administration	Side effects	Management
(5) Rituximab	Binds to CD20, found on B lymphocytes. It thus suppresses B-cell differentiation, and decreased production of IgG and IgM [102].	(i) Wegener's granulomatosis [19] (ii) Retinal vasculitis [20] (iii) Ocular cicatricial pemphigoid [22]		(i) Death from infection (*Pneumocystis jiroveci*, progressive multifocal leukoencephalopathy) (ii) Toxic epidermal necrolysis (iii) Pulmonary toxicity [103, 104] (iv) Severe infusion reaction, cytokine release syndrome, and acute renal failure [22].	
(6) Tocilizumab	Blocks T/B-lymphocyte and monocyte IL-6 receptors, hindering its expression and proinflammatory effects. it increases Th1 cell specific regulatory binding protein of retinal photoreceptors, suggesting possible treatment of refractory uveitis associated with inflammatory or autoimmune processes [105].	(i) Rheumatoid and systemic juvenile idiopathic arthritis [23] (ii) Refractory uveitis [25]		(i) Common: infections, hypertension, headache, and transient increases in ALT [106] (ii) Rare: neutropenia, thrombocytopenia, GI (perforations or gastritis), infections (TB, fungal) [107]	
(7) Gevokizumab	Binds IL-1b and downregulates its activity.	Behcet's		None known currently	
			Other		
(1) Interferons	Endogenous cytokines released in response to external pathogens.	Nonophthalmologic [28, 29]: (i) Melanoma (ii) Hepatitis C (iii) Multiple sclerosis Ophthalmologic [30–33]: (i) Behcet's disease (IFN-α 2a) (ii) Multiple sclerosis uveitis (IFN-β 1a)	Dose: IFN-α 2a given at 3–6 million international units, with frequency ranging from daily to three times weekly [108]. Course: maintain treatment after ocular inflammatory quiescence achieved for two years [7].	(i) Common: fever, chills, myalgias, alopecia, and depression [109]. (ii) Interferon retinopathy Unlike other immunosuppressants and biologic agents, IFNs rarely cause infectious complications and are also not carcinogenic.	Baseline: CBC, LFTs, and thyroid function tests Follow-Up: CBC and LFTs every four weeks; thyroid function tests every three months.
(2) Anakinra	IL-1 receptor antagonist; competitively inhibits binding of IL-1 to its receptor. IL-1 has been found to have significance in systemic autoinflammatory diseases, where excessive IL-1 signaling will occur [36].				

Table 3: Categories of vitritis drugs and what diseases they are indicated for.

Drug	Indications
	Antimetabolites
Methotrexate	Noninfectiouschronic uveitis, ocular inflammation, ocular sarcoidosis
Azathioprine	Chronic uveitis, Behcet's, choroidal neovascularization, ocular cicatricial pemphigoid, retinal vasculitis, serpiginous choroiditis
Mycophenolate mofetil	Chronic uveitis, noninfectious ocular inflammation, refractory uveitis, scleritis
Leflunomide	Sarcoidosis
	Alkylating agents
Cyclophosphamide	Refractory uveitis, nonnfectious ocular inflammation, ANCA-associated vasculitides
Chlorambucil	Serpiginous choroiditis, refractory uveitis, Behcet's
	T-cell inhibitors/calcineurin inhibitors
Cyclosporine	Serpiginous choroidopathy, Behcet's, scleritis, rheumatoid arthritis, nonnfectious uveitis
Tacrolimus	The above indications but usually in conjunction with systemic corticosteroids or adjunct immunosuppressants
Rapamycin	
	Biologic agents
Etanercept	Juvenile idiopathic arthritis, noninfectious uveitis, ocular inflammatory disease
Infliximab	Refractory uveitis, childhood uveitis, Behcet's
Adalimumab	Refractory uveitis, ankylosing spondylitis, juvenile idiopathic arthritis
Daclizumab	Juvenile idiopathic arthritis, recalcitrant ocular inflammation, birdshot chorioretinopathy
Rituximab	Primary Sjogren's syndrome, thyroid eye disease, Wegener's granulomatosis
Tocilizumab	Severe refractory posterior uveitis
Gevokizumab	Behcet's
	Other
Interferons	Behcet's, noninfectious uveitis
Anakinra	Behcet's, refractory juvenile idiopathic disease

necrosis factor (TNF). It is able to bind with both high affinity and specificity to soluble TNFα or β, thus neutralizing the biological function of TNF, as well as modulating biological responses that TNF is responsible for inducing or regulating [11]. It is currently used with increasing frequency for treating several autoimmune diseases such as Behcet's, juvenile idiopathic arthritis-associated uveitis, Vogt-Koyanagi-Harada (VKH) disease, and birdshot retinochoroidopathy [12–16]. A recent multicenter trial found it to be a useful treatment for patients with refractory uveitis, with a 10-week success rate of 68% [17].

A more recent retrospective analysis of 60 patients, the largest case series to date, showed a positive effect of adalimumab in 82% of these patients with different uveitis types, independent of additional systemic disease [11]. This study found that those who had been treated with infliximab and etanercept with insufficient response were effectively treated with adalimumab in 92% of cases. Another interesting finding was that patients pretreated with other TNF agents still had good results; thus, it is reasonable to switch to another TNF agent if the first was ineffective. In this study, no major infections nor serious complications known to TNF inhibitors (demyelinating disease, reactivation of TB) occurred. This is a significant finding, as adalimumab may thus be a better option than infliximab, although follow-up was short and the study's power would need to be increased in a further study.

Another prospective study evaluated the efficacy and outcomes of using adalimumab to treat uveitis associated with juvenile idiopathic arthritis [18]. Ocular symptom improvement was seen in 76% of cases, with anterior uveitis flare rate reduced after starting treatment. This study also confirmed a lack of serious sideeffects and infections and fewer hypersensitivity reactions than infliximab. Overall, this study concluded that adalimumab was a reasonable adjuvant therapy for treating uveitis.

3.2.2. Rituximab. Rituximab is an antibody that binds CD20, with many effects. Most commonly used in hematologic and autoimmune disorders, it has been found to be effective as a sole treatment for Wegener's uveitis and retinal vasculitis [19, 20]. The value of rituximab in Behcet's disease is yet to be determined, due to limited evidence [21]. In addition, it has also been used with intravenous IgG to treat ocular cicatricial pemphigoid [22].

3.2.3. Tocilizumab. Tocilizumab is a humanized antibody that binds both to IL-6 receptors, originally used for treating rheumatoid arthritis and systemic juvenile idiopathic arthritis [23]. IL-6 has a role in proliferation and differentiation

of T- and B-cells, with persistent production demonstrated in chronic inflammatory diseases. Although ophthalmologic usage is currently limited, patients with active posterior uveitis have been found to have elevated IL-6 levels in serum and intraocular, although levels were not specifically correlated with a clinical diagnosis [24].

In one retrospective study, tocilizumab was found to be efficacious in treating uveitis patients with cystoid macular edema that was refractory to intraocular steroids or other immunosuppressive therapies [25]. These patients were found to have complete resolution after six months of therapy and were also found to have no recurrence of inflammation at follow-up, suggesting that it is able to maintain disease remission. In another recent case study, a patient with severe refractory posterior uveitis improved, with decreasing levels of IL-6 after treatment [26].

3.2.4. Gevokizumab. IL-1β is an inflammatory cytokine produced in large amounts in Behcet's patients. Gevokizumab is a recombinant anti-IL-1β antibody, which modulates cytokine activity. It is a new therapy whose indications and efficacy are still being studied; a recent pilot study for patients with refractory Behcet's disease showed promising results, with only two infusions needed to render patients attack-free for several months [27]. Patients tolerated the infusions well, with no reported drug-related side effects. Treatment led to a rapid reduction in manifestations of intraocular inflammation, without the rebound attacks associated with discontinuation of corticosteroid use. This was thought to be in part due to accumulation of gevokizumab in ocular tissues, thus being able to sustain its therapeutic effect with an infrequent dosing interval.

3.3. Other

3.3.1. Interferons. Interferons (IFN) are endogenous cytokines, released in response to external pathogens. IFN-α 2a, IFN-α 2b, IFN-β 1a, and IFN-β 1b are the classes most commonly used in therapy. Interferons are commonly used to treat conditions ranging from malignancy (cutaneous melanoma), infection (hepatitis C), and inflammatory (multiple sclerosis) [28, 29]. As far as ophthalmologic uses, IFN-α 2a has successfully treated Behcet's disease, and IFN-β 1a reduced uveitis recurrences in multiple sclerosis patients [30–33]. In Behcet's disease, interferon demonstrated significant benefit by decreases in aphthous ulceration and the number of lesions [34]. Several studies consistently reported that many patients had durable remissions of ocular inflammatory disease after discontinuation.

3.3.2. Anakinra. Anakinra is an interleukin-1 receptor antagonist, which competitively inhibits IL-1 binding to its receptor. IL-1 has been found to have significance in systemic autoinflammatory diseases, where excessive IL-1 signaling will occur. It plays a key role in auto inflammatory diseases such as Muckle-Wells and neonatal onset multisystem inflammatory disease (NOMID), which are rare causes of uveitis in childhood [35]. It may in the future be used to treat refractory juvenile idiopathic and Behcet's disease, for which it is currently in phase III clinical trials [36].

4. Conclusion

Uveitis is a vision-threating group of diseases that encompasses a variety of etiologies, which are either infectious or noninfectious. Both groups are commonly treated with steroids. Uveitis resulting from infection, however, focuses on eradicating the source with antibiotics or antivirals. Those of noninfectious origin may need additional immunosuppressive agents. These antimetabolites, cytotoxic agents, biologics, and immunomodulators can be used either alone or together, to control inflammation of the vitreous. As with any medication, especially immunosuppressants, side effects must be balanced with therapeutic benefit—a determination still in process for many drugs and indications. The complexities in investigating these therapies result from the innate heterogeneity of uveitis. Even with its difficulties, research on expanding indications for existing therapies and the discovery of new systemic agents continues to progress.

Conflict of Interests

The authors declare that there is no conflict of interests related to any topic in this paper.

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Review Article

Inflammatory Mechanisms of Idiopathic Epiretinal Membrane Formation

Malav Joshi, Shivi Agrawal, and John Byron Christoforidis

Department of Ophthalmology, University of Arizona Medical Center, Tucson, AZ 85711, USA

Correspondence should be addressed to John Byron Christoforidis; jbchristo@hotmail.com

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The pathogenesis of idiopathic epiretinal membranes (iERMs), a common pathology found in retina clinics, still eludes researchers to date. Ultrastructural studies of iERMs in the past have failed to identify the cells of origin due to the striking morphologic changes of cells involved via transdifferentiation. Thus, immunohistochemical techniques that stain for the cytostructural components of cells have confirmed the importance of glial cells and hyalocytes in iERM formation. The cellular constituents of iERMs are thought to consist of glial cells, fibroblasts, hyalocytes, etc. that, in concert with cytokines and growth factors present in the vitreous, lead to iERM formation. Recently, research has focused on the role of the posterior hyaloid in iERM formation and contraction, particularly the process of anomalous PVD as it relates to iERM formation. Recent advances in proteomics techniques have also elucidated the growth factors and cytokines involved in iERM formation, most notably nerve growth factor, glial cell line-derived growth factor, and transforming growth factor β1.

1. Introduction

Epiretinal membranes (ERMs) are classified as idiopathic when they are not associated with any other ocular disease processes such as retinal detachment, intraocular inflammation, trauma, and retinal vascular diseases. Idiopathic epiretinal membranes (iERMs), whose exact pathogenesis still remains unknown, are characterized by the growth of fibrocellular tissue on the inner limiting membrane (ILM). They can range from subtle cellophane-like films without visual consequences to markedly contractile membranes that can cause metamorphopsia and decreased visual acuity [1]. Several theories of the responsible pathologenic mechanisms have been proposed, including the role of glial cells, fibroblasts, hyalocytes, and so forth assisted by cytokines and growth factors present in the vitreous fluid; however, the debate regarding the types of cells that produce iERMs and the means by which they reach the retinal surface has continued for decades. With recent improvements in imaging techniques coupled with immunocytochemistry and proteomic techniques, the understanding of the development of iERMs has evolved. This review summarizes the prior and latest developments in understanding the inflammatory mechanisms of idiopathic ERMs.

2. Cellular Constituents in iERM Formation

During the past decades, significant progress has been made elucidating the pathogenic mechanisms in iERM formation; however, many fundamental questions still remain unanswered. One of the significant impediments to a greater understanding of how and why iERMs occur is the accurate identification of the cells that participate. Morphologic analysis of surgically excised ILM specimens has demonstrated a variety of cells in iERM, including glial cells (Müller cells, fibrous astrocytes, and microglia), hyalocytes, retinal pigment epithelial (RPE) cells, fibroblasts, and myofibroblasts [2]. However, because the cells in the vitreous commonly undergo striking morphologic changes via transdifferentiation, morphologic criteria alone have proven inadequate for identifying the origin of cells [3]. In fact, Vinores et al. confirmed that when glial cells, fibroblasts, and RPE

cells are cultured on vitreous, they undergo time-dependent changes in morphology and are essentially indistinguishable from each other by ultrastructural criteria [4]. Therefore, recent research has focused on using immunohistochemical markers of structural proteins such as intermediate filament proteins to assist in cell-type determination.

Commonly used antibodies against structural proteins and their respective target cells are listed in Table 1. Glial cells usually predominate in iERMs with little traction while myofibroblasts are the major cell type in membranes with significant traction [4]. In a study by Zhao et al., Müller cells and hyalocytes were found to be the predominant cell type in macular pucker specimens. All surgically removed iERM specimens were found to have positive immunostaining for glial fibrillary acidic protein (GFAP), CD45, CD68, CD163, vimentin, and cellular retinaldehyde binding protein (CRALBP), indicating the presence of glial cells and hyalocytes [5]. Kir4.1 was also found in iERMs, which is reported to be found on Müller cell end-feet membranes [6]. Immunostaining for pan-cytokeratin was negative, predicting little if any role of RPE cells in iERMs. The importance of Müller cells is highlighted by the fact that all immunomarkers for Müller cells were positive in this study including GFAP, CRALBP, vimentin, and Kir4.1. All hyalocyte markers were also immunopositive. Interestingly, this study also found colocalizations of GFAP and hyalocyte markers CD45 and CD163 in 20% of specimens. These double-labeled cells may represent hyalocytes since hyalocytes with positive GFAP expression have already been described in other species [7, 8]. Hyalocytes are considered to be of macrophage lineage, so they could have phagocytosed GFAP positive debris or apoptotic cells, which could explain their immunopositivity for GFAP [9]. Colocalization of CD163 and α-SMA was also seen in single cases, which most likely indicated hyalocytes that might have transdifferentiated into myofibroblast-like cells. These results support the hypothesis that hyalocytes and Müller cells constitute the major cell type in iERM.

A study by Schumann et al. also confirmed the presence and importance of Müller cells as a component of iERM [2]. They tested surgically excised flat-mounted ERM specimens for GFAP, hyalocyte markers (CD45 and CD64), vimentin, CRALBP, and α-SMA. They also found the colocalization of GFAP and the hyalocyte markers, which are presumed to be hyalocytes that could have phagocytosed GFAP positive debris or apoptotic cells. Additionally, cells colocalized with GFAP/vimentin and GFAP/CRALBP were also found and thought to represent Müller cells, and, finally, cells positive for GFAP but not for hyalocyte markers conceivably signified Müller cells as well. Thus, these findings also highlight the importance of Müller cells in ERM proliferation.

The importance of glial cells in iERM formation cannot be denied. However, there is disagreement when it comes to deciding which type of glial cell, Müller cells versus astroglia, is the major cell type involved. According to Foos, it is unlikely that ERMs derive from Müller cells since they are anchored in the outer retina and attached to photoreceptor cells [10]. On the other hand, Kase et al. claim that Müller cells and their processes are the main constituent cells in iERMs [11]. They used immunohistochemical staining for glutamine synthetase (GS) (expressed specifically in Müller cells and processes and not astrocytes) on surgically excised iERMs. All the iERMs demonstrated a continuous, isodense pattern of immunoreactivity for GS, indicating that Müller cells are the main cell type responsible for iERM formation, not astrocytes. However, due to the continuous appearance of GS immunoreactivity in the collagenous tissues of the ERMs, these most likely represented extensions of Müller cell processes through the ILM, not the actual Müller cells as a whole. There was also a minor part of the ERM that showed no immunoreactivity for GS, which likely represented hyalocytes, myofibrocytes, and so forth. Rentsch also believes that Müller cell processes, not the entire cells, extend into the vitreous cavity through the ILM and serve as scaffolds for the migration and proliferation of other cells [12].

Table 1: Antibodies used for immunocytochemical staining.

Antibodies	Target cells/structure
Glial fibrillary acidic protein (GFAP)	Glial cells
Vimentin	Glial cells
Cellular retinaldehyde binding protein (CRALBP)	Glial cells/Retinal pigment epithelial cells
Kir4.1	Muller cell end-feet membranes
CD 45	Hyalocytes
CD 64	Hyalocytes
CD 168	Hyalocytes
Pan-cytokeratin	Retinal pigment epithelial cells
Neurofilament	Retinal ganglion cells
α-Smooth muscle actin (α-SMA)	Fibroblasts, Myofibroblasts
CD 68	Macrophages and Microglia

Hyalocytes, named for their location in the posterior hyaloid, are considered to be one of the macrophage lineages, and accumulating evidence has emphasized the importance of their role in iERM formation. In a study by Kohno et al. [9], immunohistochemistry performed on surgically excised iERMs demonstrated the presence of GFAP and α-smooth muscle actin (α-SMA) immunopositive cells in all ERMs. GFAP is an intermediate filament protein that is found in glial cells, while α-SMA is thought to be an intermediate filament protein presumed to be essential for extracellular matrix contraction by fibroblasts [13]. Interestingly in the study, the α-SMA positive cells were located mainly at the contracted focus of the ERM, while the GFAP positive cells were present at the peripheral, noncontracted areas of the ERM in all samples. In order to figure out whether these α-SMA positive cells were transdifferentiated hyalocytes or glial cells, a collagen gel contraction assay was performed using cultured bovine hyalocytes or normal human astrocytes to evaluate the contractile property of the cells in the presence of transforming growth factor β2 (TGF β2). TGF β2 is thought to stimulate transdifferentiation of cells into myofibroblasts. The bovine hyalocytes showed strong contractile activity of collagen gels and overexpression of α-SMA in the presence

of TGF β2 while the human astrocytes did not. One can deduce from this that hyalocytes, which transdifferentiate into myofibroblasts in the presence of TGF β2, might play an important role in ERM contraction. The authors also claimed that the GFAP positive cells found in the periphery were astrocytes not Müller cells. When they stained for a Müller cell marker (antiglutamine synthetase), they only occasionally detected immunopositive cell processes and no obvious Müller cell aggregation was seen. Thus, one can surmise that while Müller cell processes might play a role in ERM formation, the majority of GFAP positivity seen in iERMs represents accessory glia [9].

The potential for transdifferentiation to myofibroblasts is not exclusive to hyalocytes. In a study done by Guidry [14], who evaluated Müller cells as potential sources of contractive cells in proliferative diabetic retinopathy, Müller cells were shown to lose their GFAP and GS expression with concurrent increase in α-SMA immunoreactivity, thereby demonstrating transdifferentiation into myofibroblast-like cells capable of contractile properties.

An interesting finding in a study by Lesnik Oberstein et al. suggested the possible role of retinal ganglion cells in iERM formation. Their study demonstrated neurofilament processes of ganglion cells in all of the 32 iERMs examined by immunohistochemistry. The ERMs were labeled with antibodies for neurofilament protein, presumed to originate from retinal ganglion cells. Previous studies on feline retina have shown that neurites from ganglion cells and horizontal cells can be found after experimental retinal detachment next to Müller cells [15]. However, in these cases, the growth of these neurites was thought to result from a reaction to retinal injury. This study showed growth of neurites into iERMs with no history of trauma in the patients. Interestingly, these neurites were only found in regions where Müller cells were present, suggesting that some type of signal from Müller cells stimulates the ganglion cells to sprout neurites. However, the reason for this type of neurite growth is not known. In fact, in a series by Parolini et al., in which ERMs were removed from patients with idiopathic lamellar holes, antineurofilament staining was not demonstrated [13].

3. Pathophysiology of Idiopathic ERMs

Structurally speaking, there are two types of iERMs that have different clinical presentations: simple and tractional ERMs. Simple ERMs are membranes with delicate cellophane-like films on the internal limiting membrane (ILM) with mild to no visual symptoms. These membranes are usually composed mostly of glial cells. On the other hand, tractional iERMs are thicker with contractile properties that cause surface wrinkling of the retina and are usually accompanied by decreased vision and metamorphopsia. They are composed of glial cells plus contractile cells [16]. The two main components of an ERM are extracellular matrix structures such as fibronectin and collagen and cells of extraretinal and retinal origin such as glial cells, fibroblasts, and hyalocytes [17].

The complete pathogenesis of iERM is unknown, but many theories have been proposed. The most widely accepted theory is that iERM is a consequence of surface breaks formed in the ILM by posterior vitreous detachment (PVD) that allows glial and other cells from the underlying retina to migrate through the defect and proliferate on the ILM [1]. Some of the original studies of iERMs were performed by Foos, in which he carried out an ultrastructural study of 8 cases of simple ERMs using an electron microscope. In his studies, he found simple ERMs to only contain glial cells. He also hypothesized that an initial event damages the superficial retina and leads to glial cell proliferation and migration through the defect. Because of the defect on the retinal surface, glial cells react through extension and hypertrophy of their processes in an effort to repair the defect. Meanwhile, some of the other cells divide and contribute to the substance of the ERM. He goes on to say that the breaks in the ILM, following the formation of ERM, can heal and make it difficult to find them later on [10].

In more recent years, attention has been placed on attempting to understand the role of vitreous in iERM formation, since most cases of iERM seem to occur in patients with a PVD. In fact, Foos [18] reported the presence of condensed collagen fibrils indistinguishable from vitreous collagen in premacular fibrosis. Bellhorn and colleagues [19] also identified vitreous in variable amounts within the ERM of a lesion they studied with electron microscopy. The role of vitreous was further elucidated when Kishi and Shimizu [20] reported oval or round defects in detached posterior hyaloid membranes of patients with idiopathic preretinal fibrosis. They postulated that a premacular oval defect in the detached posterior hyaloid membrane plays a key role in the development of idiopathic preretinal macular fibrosis. In their study, they found that 31 (65%) of 48 eyes with a PVD and idiopathic preretinal macular fibrosis had an oval or round defect and 12 (25%) of 48 eyes had a break in the premacular area. This implies that a majority of the eyes with a PVD and a defect in the premacular cortical vitreous develop idiopathic preretinal macular fibrosis. The theory is that in some cases the posterior cortical vitreous may remain attached to the retina during PVD development, which leads to the defect in the premacular detached cortical vitreous. But more importantly, it is the remnants of the cortical vitreous on the premacular ILM that then serve as a structural component and provide a medium upon which glial cells and hyalocytes can proliferate to form an iERM. Histologic studies have supported this theory and have shown that a portion of posterior cortical vitreous does remain attached to the premacular ILM after a PVD [21]. Hikichi et al. [22] conducted an in vivo study to further elucidate the relationship between premacular cortical vitreous defects and their relationship to idiopathic premacular fibrosis. They also found that the incidence of the defect in the detached premacular cortical vitreous was significantly higher in eyes with idiopathic premacular fibrosis than in eyes without. However, 27 (75%) of 36 eyes with premacular fibrosis did not exhibit the defect in the premacular cortical vitreous.

It was Sebag who later unified this concept and coined it anomalous PVD. According to Sebag, for an uncomplicated PVD to occur, two processes must occur concurrently: weakening of vitreoretinal adhesion and vitreous liquefaction [23]. An anomalous PVD occurs when the extent of vitreous

liquefaction exceeds the degree of weakening of vitreoretinal adhesion and leads to posterior vitreoschisis. This is when splitting of the posterior cortical vitreous occurs and forward displacement of the vitreous body leaves the outer layers of posterior vitreous cortex (which contains hyalocytes) still attached to the retina, potentially resulting in the formation of macular pucker. Exactly how these hyalocytes cause ERM is not known, but, according to Kampik, these hyalocytes stimulate Müller cells to send processes through an intact ILM to form the scaffolding which allows other cells to then be taken up into the membrane [24]. Sebag has proposed pharmacologic vitreolysis as a way to weaken the vitreoretinal adhesion to safely detach the posterior vitreous cortex and prevent an anomalous PVD [23].

In a order to validate the theory of anomalous PVD as the initiating event for the formation of iERM, Sebag et al. studied 44 eyes with macular pucker using combined optical coherence tomography and scanning laser ophthalmoscopy to look for vitreoschisis. Vitreoschisis was detected in 19 out of 44 eyes (43.2%) with macular pucker. The authors considered vitreoschisis to be present only when they saw two membranous layers of the posterior vitreous cortex join into one, forming a "Y" shaped configuration. However, the authors stated that there were many cases where a clear cut "Y" shape was not seen but a distinct thin membrane of posterior vitreous cortex was visible anterior to the surface of the retina [25]. Future studies with high-resolution OCTs are needed to investigate whether the incidence of vitreoschisis is even greater than that observed in this study.

Going along with this theory, Kampik also believes that the role of vitreoschisis is likely responsible for iERM formation. In the many specimens he has examined, he has rarely encountered a break in the ILM, and it is therefore unlikely to be a mechanism for iERM formation. According to his findings, there are two types of iERM membranes: type I is when there is vitreous collagen sandwiched between the ILM and the ERM, and type II is when the cells proliferate directly on the ILM surface with sparse or no collagen layer in between [24]. Since the posterior vitreous cortex is composed of many thin lamellae, very few delicate lamellae would actually be present on the ILM if the vitreoschisis occurs in the very posterior portion of the cortex. This would explain why, in type II iERMs, there is sparse to no vitreous seen in some areas.

One can speculate that pharmacologic vitreolysis could potentially have a therapeutic role in type I iERMs, whereby the enzyme plasmin could act and detach the ERM. However, this would not be possible in type II membranes. Surgically speaking, this could explain why some membranes are easier to peel than others. Additionally, one would have to peel two membranes to prevent recurrence and to rid sources causing traction in type I membranes, whereas, in type II membranes, one would only have to peel one membrane—the ILM. In fact, in a study by Gandorfer et al., simple ERM removal leaves 20% of total cell count behind on the ILM in 2 of 3 patients with iERM [26]. These most likely represent type I membranes and peeling just the ERM in these patients would leave the residual cells to proliferate and cause ERM recurrence. Thus, ILM staining with subsequent removal is important for recurrence prevention. According to Kampik, the cells need the scaffolding of either the ILM or the native vitreous in order to proliferate, and by peeling the ILM both are taken away [24]. Kenawy et al. reported the difficulty of removing the ILM in the presence of ERM due to the deeper cleavage plane in ILM peeling [27]. Interestingly, they found that patients with ERMs tended to have glial and/or neuronal cells on the retinal surface as well as the vitreous surface of the ILM. These cells found on the retinal surface of the ILM account for the deeper cleavage plane in ILM peeling. This study also suggests that iERM formation, which previously was considered to be predominantly epiretinal, may have a significant intraretinal component.

4. Cytokines and Growth Factors

There is very limited data available on the proteomics of iERMs alone. However, according to a study by Mandal et al. [1], high abundance proteins found in undiluted vitreous samples from patients with iERMs include α-antitrypsin, apolipoprotein A-1, transthyretin, and serum albumin. They also compared these results to vitreous samples from patients with idiopathic macular holes and found no significant difference between the two. This leads one to speculate that both iERM and macular holes involve similar inflammatory processes.

Since it is known that glial cells are one of the most important cellular components of iERM, understanding the role of molecules involved in glial signal transduction is important. Basic fibroblast growth factor (bFGF) is such a molecule. It supports the survival and maturation of both neurons and glial cells and may play an important role in the regeneration after neural injury [28]. In a study by Harada et al. [29], polymerase chain reaction (PCR) analysis revealed bFGF mRNA expression in 10 of 15 (67%) iERMs and 13 of 19 ERMs from proliferative diabetic retinopathy (PDR) patients. Chen et al. [30] found bFGF immunoreactivity in five of seven (71%) iERMs and four of eight (50%) PDR membranes.

Nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) also may be involved in ERM formation. In the same study by Harada et al. [29], they examined the expression of receptors for neurotrophins (trkA, trkB, trkC, and $p75^{NTR}$) and GDNF (GFRα1, GFRα2, and Ret) in ERMs obtained from PDR and iERM patients. Expressions of neurotrophin receptor mRNAs were similar in both groups. A study by Iannetti et al. [31] also studied the role of NGF in iERMs and found the levels to be significantly higher in iERM samples versus control groups (patients without ERMs who underwent vitrectomy for primary retinal detachment within 72 hours of onset). In terms of GDNF, the expression of GFRα1 receptor mRNA was surprisingly higher in iERMs (12 of 15 cases) compared to PDR ERMs (eight of 19 cases). On the other hand, GFRα2 expression levels were significantly higher in PDR ERMs (17 of 19 cases) versus iERM (two of 15 cases). Despite the above findings, few other studies have shown GDNF levels to be far below the sensitivity threshold in iERM samples [31, 32]. The discrepancy in the study results might relate in part to the methods used to process the samples (i.e., ELISA versus

PCR). Further studies are necessary in order to elucidate the role GDNF and neurotrophins play in iERM formation.

Recently, a more advanced technique for proteomics using liquid chromatography mass spectrometry and multiplex protein assays was utilized by Pollreisz et al. [17] to study the aqueous and vitreous fluids from patients with iERMs. The majority of proteins identified were involved in the classical and alternative pathway of complement activation, proteolysis, and cell adhesion. Most of the proteins were found in similar quantity between the aqueous humor and vitreous sample; however, there were 8 proteins that were expressed at a lower level in the aqueous fluid compared to vitreous fluid. Of these proteins, fibrinogen A was the most highly expressed protein in the vitreous compared to the aqueous fluid. Fibrinogen has been implicated in the development of vitreous membrane formation in a rat model [33]. The other 7 proteins have not been reported to play a role in iERM formation. Multiplex protein array analysis showed similar concentrations of cytokines and growth factors in the aqueous versus vitreous fluids, except for platelet-derived growth factor A (PDGF-A). This factor was expressed at a higher level in the vitreous fluid. Cassidy et al. have reported higher levels of PDGF in vitreous fluids of eyes with proliferative vitreoretinopathy after retinal detachment compared to healthy controls [34].

Nerve growth factor (NGF) and transforming growth factor β1 (TGFβ1) both play a crucial role in fibroblast activities. According to a study by Minchiotti et al., both TGF β1 and NGF mRNA were found in all 8 iERMs evaluated [35]. In fact, every iERM displayed α-smooth muscle actin (α-SMA) positive myofibroblasts that expressed NGF and its receptors $trkA^{\mathrm{NGFR}}$ and $\mathrm{p75}^{\mathrm{NTR}}$. Biologic effects of NGF include fibroblast migration, differentiation into myofibroblasts, and extracellular matrix contraction. Thus, it is reasonable to suppose that TGF β1 and NGF could target glial cells and stimulate them to transdifferentiate into myofibroblasts and could also stimulate myofibroblasts to turn on their contractile actions.

The study by Iannetti et al. also reported the role of transforming growth factor β1 (TGFβ1), β2, and nerve growth factor (NGF) in the pathogenesis of iERM [31]. They reported much higher TGFβ2 levels in patients with iERMs compared to controls, whereas the levels of TGFβ1 was similar to controls. This is in contradiction with what was reported by Minchiotti et al., which reported TGFβ1 expression in all iERM specimens. According to Iannetti, TGFβ2 is the most important growth factor in the pathogenesis of iERM and possibly stimulates the differentiation of specific types of glial cells or hyalocytes into myofibroblasts, inducing ERM contraction. The previously reported study by Kohno et al. also demonstrated the importance of TGFβ2 in iERM contraction [9]. One can speculate on the efficacy of therapeutic agents against TGFβ2 in preventing iERM formation and contraction.

Vascular endothelial growth factor (VEGF), one of the most extensively studied vitreoretinal growth factors, has also been reported in iERMs. In a study by Mandelcorn et al., 11 (85%) of 13 iERMs stained positively for VEGF, but there was no statistically significant relationship between the presence of VEGF and leakage on fluorescein angiogram [36]. Positive VEGF immunoreactivity of iERMs was also found in a study by Chen et al. [30]. However, since retinal glia have been known to produce VEGF, this is not surprising [37]. What is puzzling is why there are no blood vessels in iERM despite the presence of VEGF. One possibility is that there are other cells in the iERM besides endothelial cells that are targeted by VEGF. It is also plausible that the presence of endothelial growth inhibitory factors, such as TGF-β, may prevent VEGF from exerting its angiogenic activity [38].

5. Conclusion

Despite the advances in imaging technology, immunohistochemistry, and proteomics, the exact mechanism of iERM formation is still unclear. We have come a long way in understanding the cell types involved, but much of our understanding related to the interdependence of cytokines and growth factors involved in iERM production is incomplete. Further studies are needed to evaluate the cells, cytokines, and growth factors involved in iERM formation.

Conflict of Interests

The authors declare that there is no competing/conflict of interests related to any topic in this paper.

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Review Article

Vitreous Inflammation Associated with Intravitreal Anti-VEGF Pharmacotherapy

Shivi Agrawal,[1] Malav Joshi,[1] and John B. Christoforidis[2]

[1] *Department of Ophthalmology, University of Arizona Medical Center, Tucson, AZ 85711, USA*
[2] *Retina Division, Department of Ophthalmology, University of Arizona Medical Center, 655 N. Alvernon Way, Suite 108, Tucson, AZ 85711, USA*

Correspondence should be addressed to John B. Christoforidis; jbchristo@hotmail.com

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Vascular endothelial growth factor (VEGF) is a potent promoter of angiogenesis involved in a wide variety of physiologic processes. Intravitreal injections targeting VEGF have transformed the treatment of neovascular retinal diseases. Currently, there are four anti-VEGF agents in use: bevacizumab, ranibizumab, pegaptanib, and aflibercept. The success and frequency of anti-VEGF therapy have made the ocular safety profile of these agents of vital importance. This paper focuses on sterile endophthalmitis. In this paper, we compare the incidences of posttreatment sterile endophthalmitis among the four agents, review the mechanism of actions, and discuss the most prevalent hypotheses leading to sterile endophthalmitis.

1. Introduction

Vascular endothelial growth factor-A (VEGF) is the master regulator of angiogenesis [1]. Pharmacotherapy utilizing intravitreal injections of antivascular endothelial growth factor (anti-VEGF) agents has revolutionized the treatment of neovascular retinal disorders by inhibiting angiogenesis. Bevacizumab was the first intravitreal agent utilized for the treatment of macular edema secondary to a branch retinal vein occlusion and age-related macular degeneration [2, 3]. Today, multiple anti-VEGF agents have been developed including bevacizumab, pegaptanib, ranibizumab, and aflibercept. These agents have shown promising results in the treatment of various retinal diseases including age-related macular degeneration, diabetic retinopathy [4], neovascular glaucoma [5], retinopathy of prematurity [6], and intraocular tumors [7]. Today, the use of intravitreal anti-VEGF agents is the most common intravitreal procedure performed by ophthalmologists. The recognition of adverse effects from the use of these medications and appropriate treatment has become increasingly important. In this paper, we will compare the inflammatory effects of the different anti-VEGF agents, differentiate their clinical features, and review the possible mechanisms involved in the development of posttreatment sterile inflammation.

2. Definition of Sterile Endophthalmitis

Sterile endophthalmitis (also known as "pseudoendophthalmitis") is described as any acute intraocular inflammation without infection that resolves without antibiotic treatment, unlike true endophthalmitis. A review of the literature published on PubMed between 1945 and June 2013 was conducted using combination keywords such as sterile endophthalmitis, anti-VEGF, pegaptanib, bevacizumab, ranibizumab, aflibercept, and ocular inflammation. Only the articles written in English were included. Also, in order to avoid confusion, only the studies reporting noninfectious endophthalmitis were included.

3. Sterile Endophthalmitis versus Infectious Endophthalmitis

Infectious endophthalmitis is the most feared complication after intravitreal injections. It is important to differentiate infectious endophthalmitis from sterile endophthalmitis, as the management and prognosis of these two entities vary vastly. While infective endophthalmitis cases are heavily treated by intravitreal antibiotics, the treatment of sterile endophthalmitis has shown prompt improvement with

Table 1: Clinical characteristics of noninfectious versus infectious endophthalmitis.

	Noninfectious endophthalmitis	Infectious endophthalmitis
Pain	± [14, 17, 20]	++ [14]
Onset	<1 day [10, 14, 16, 20, 21] to 1 week [11, 15, 17, 22]	2.5 days (range: 1–6 days) [14, 15, 23]
Signs	Blurred vision [11], anterior segment inflammation greater than posterior inflammation [10, 17, 18, 21, 22]	Decreased vision, severe anterior segment reaction (fibrin and hypopyon), and vitritis [14]
Time to resolution	2–12 weeks [11, 15, 17, 18, 20, 24]	Extremely variable
Prognosis	Preinjection visual acuity [10, 11, 14, 16–18, 21, 22]	Severely depressed [22]

Table 2: Sterile inflammatory rates between anti-VEGF agents.

Study	Anti-VEGF agent	Number of patients	Number of injections	Percentage (%) of inflammation
Chong et al. (2010) [11]	Bevacizumab	—	16116	0.40%
Georgopoulos et al. (2009) [25]	Bevacizumab	—	2500	0.03%
Shima et al. (2008) [16]	Bevacizumab	707	1300	0.28%
Wickremasinghe et al. (2008) [10]	Bevacizumab	—	1278	1.49%
Johnson et al. (2010) [26]	Bevacizumab	173	693	1.30%
Sato et al. (2010) [22]	Bevacizumab	35	35	14.3%
Yamashiro et al. (2010) [19]	Bevacizumab	15	20	73%
Wang et al. (2013) [24]	Bevacizumab	116	116	69%
Wu et al. (2008) [27]	Bevacizumab	1173	4303	0.09%
Chong et al. (2010) [11]	Ranibizumab	—	3839	0.03%
Regillo et al. (2008) [28]	Ranibizumab	184	—	0%
Holz et al. (2011) [29]	Ranibizumab	514	—	0%
Busbee et al. (2013) [30]	Ranibizumab	1098	—	0.4%
Rosenfeld et al. (2006) [12]	Ranibizumab	716	—	2.6%
Brown et al. (2006) [13]	Ranibizumab	280	—	0.35%
Heier et al. (2006) [31]	Ranibizumab	105	—	11.4%
Antoszyk et al. (2008) [32]	Ranibizumab	105	—	9.5%
Rosenfeld et al. (2006) [18]	Ranibizumab	29	—	86%
Chun et al. (2006) [33]	Ranibizumab	10	30	50%
Chakravarthy et al. (2012) [34]	Bevacizumab and ranibizumab	610	—	0.16%
Ladas et al. (2009) [35]	Bevacizumab and ranibizumab	450	2000	1.90%
Sharma et al. (2012) [36]	Bevacizumab and ranibizumab	524	1584	1.90%
Hahn et al. (2013) [37]	Aflibercept	—	30000	0.05%
Ho et al. (2013) [38]	Aflibercept	85	—	0%
D'Amico et al. (2006) [39]	Pegaptanib	1190	—	0%
Singerman et al. (2008) [40]	Pegaptanib	161	1254	9%

topical steroid therapy [8]. The clinical features can help when attempting to differentiate the two (Table 1).

In the literature, the incidence of sterile endophthalmitis after intravitreal anti-VEGF therapy ranges between 0.033% and 2.9% [9–14]. Meta-analyses reports have shown variability in the incidence of sterile endophthalmitis between the different anti-VEGF agents (Table 2). It typically presents 24 hours to 7 days after injection [10, 15], with or without pain. Pain may be an indication of the severity of the inflammation in the anterior chamber and vitreous cavity. The most common presenting symptoms are blurred vision and floaters [11]. The time between symptom presentations after injection ranges from 1 day to 1 week [10, 11, 14–18]. Visual acuity at presentation is substantially reduced compared with preinjection acuity and typically returns to preinjection acuity after resolution of the inflammation [10, 11]. The average time to resolution of inflammation ranges from 2 to 12 weeks [11, 15, 17] and recovery of visual acuity occurs between 7 and 9 weeks [11]. Moreover, the time from injection to presentation with inflammation does not seem to affect the extent of visual recovery; it only affects the length of time to recovery [10]. In addition, history of prior intravitreal anti-VEGF injections

does not increase the risk or severity of ocular inflammation in subsequent injections [11, 19].

The clinical course of sterile endophthalmitis varies based on the management of the clinical practitioner. Management includes the use of topical medications, intravitreal antibiotics, and pars plana vitrectomy with or without intravitreal antibiotics. The time to resolution based on this can vary from two to 42 days (Table 1). The median duration of inflammation was six days in patients undergoing vitrectomy, seven days in patients receiving triple intravitreal injections, and four days in patients receiving topical corticosteroids [24]. While these results may imply that treatment with topical corticosteroids is the most effective, this is not accurate. Chong et al. [11] reported only 0.27%, 14 of 16166 cases, of sterile endophthalmitis resolving with topical antibiotics alone. It is difficult to generalize the treatment with the time to resolution because typically the most severe cases were chosen for pars plana vitrectomy with intravitreal antibiotics. Shah et al. [15] reported in a retrospective case series that the clinical difference between these two entities was not significant and therefore a low threshold for vitreous tap with intravitreal antibiotic injection might be warranted.

4. Pharmacokinetics of Anti-VEGF Agents

Before discussing possible mechanisms of inflammation after intravitreal injection, it is important to examine the pharmacokinetic properties of these agents, especially in regard to the duration of activity within the vitreous. The intravitreal half-lives of bevacizumab using ELISA methods range from 4.32 to 9.82 days [41–43]. Similarly, the intravitreal half-life of ranibizumab was approximately 7.15 days [44]. In a rhesus monkey study, the vitreous half-life of pegaptanib was found to be approximately 3.9 days [45]. There are currently no reports on the pharmacokinetic properties of aflibercept using ELISA. Utilizing PET/CT to detect I-124 labeled anti-VEGF agents, Christoforidis et al. reported the intravitreal half-lives of ranibizumab, bevacizumab, and aflibercept [46, 47] to be 2.82, 4.22, and 4.58 days, respectively. Their findings corroborate the previously described presence of a two-compartment pharmacokinetic decay model with an initial rapid phase followed by a slower phase described by Zou et al. [48].

5. Antivascular Endothelial Growth Factor Drugs

Targeting anti-VEGF in the treatment of ocular neovascular diseases first requires an understanding of the human VEGF-A gene. The human VEGF-A gene is composed of eight exons with six principle amino acid isoforms (121, 145, 165, 183, 189, and 206) [1]. VEGF121 is freely diffusible, while VEGF189 and VEGF206 are primarily bound and sequestered in the extracellular matrix. This is due to the heparin-binding domain found in the larger isoforms of VEGF such as VEGF189 and VEGF206. VEGF165 has properties of both the diffusible and bound form of VEGF [1]. There are four anti-VEGF agents currently utilized in the treatment of ocular diseases, which differ in their isoform-binding specificities.

Pegaptanib (Macugen; Eyetech/OIS Pharmaceuticals, Melville, New York, USA) was the first anti-VEGF therapy approved for the treatment of wet AMD. It is an aptamer that selectively binds to and neutralizes VEGF-A165 while sparing smaller isoforms that lack the heparin-binding domain such as VEGF121 and VEGF110 [49]. The large-scale, randomized controlled VISION trials reported twelve cases of 1190 cases of endophthalmitis (1%) although nine of the twelve were likely associated with violations of the injection preparation protocol, such as failure to use an eyelid speculum. In year 2, there were four cases in 1024 patients of endophthalmitis (0.4%) [39]. There were no reported cases of sterile endophthalmitis [39, 40].

Ranibizumab (Lucentis; Genentech, South San Francisco, California) was designed as a potent inhibitor of all VEGF isoforms with an affinity-matured antigen-binding fragment (Fab) derived from bevacizumab. It was developed as a Fab fragment because it was thought that its smaller-size would increase its diffusion capacity as an anti-VEGF-A agent compared to its parent bevacizumab. Ranibizumab, compared to its parent bevacizumab, has a higher affinity for VEGF with a greater potency. As an antibody-binding fragment, it lacks the domain that activates compliment-mediated cytotoxicity and Fc receptors on immune cells [50]. The primary ocular adverse event associated with ranibizumab is ocular inflammation. Many large-scale studies have reported the frequency of ocular inflammation or presumed endophthalmitis to be between 0 and 12.7%. The results of these trials have been outlined below.

The rate of intraocular inflammation in the MARINA trial using ranibizumab was found to be 2.6% [12]. In the PIER study, no cases of serious uveitis or endophthalmitis were noted with the use of ranibizumab [28]. In the IVAN trial, only one case of 610 (0.1%) developed uveitis [34]. The HARBOR study group reported a 0.7% rate of endophthalmitis and a 0.4% rate of inflammation with intravitreal ranibizumab [30]. The SUSTAIN study utilized ranibizumab and reported no episodes of sterile endophthalmitis in the 249 patients studied [29]. The first year results of the FOCUS study revealed that the more frequently associated serious ocular adverse events were intraocular inflammation (11.4%) and endophthalmitis (1.9%; 4.8% including presumed cases) [31]. In the two-year FOCUS study, endophthalmitis and serious ocular inflammation occurred in 2.9% and 12.4%, respectively, in the ranibizumab + PDT patient groups [32]. It should be noted that this study used a lyophilized formulation of ranibizumab that was discontinued afterwards. Among the 280 patients treated in the ANCHOR trial, presumed endophthalmitis occurred in 2 patients (0.7%) and serious uveitis occurred in 1 patient (0.35%) at year 1 [13]. Ladas et al. reported a 1.9% frequency of ocular inflammation after intravitreal ranibizumab and bevacizumab injection with no reported statistical difference between the two medications [35]. Overall, the rates of presumed endophthalmitis or severe inflammation were similar between the two drugs.

Bevacizumab (Avastin; Roche, Basel, Switzerland) was initially a drug approved by the Food and Drug Administration for the treatment of glioblastoma, metastatic colon cancer, advanced nonsquamous non-small cell lung

cancer, and metastatic kidney disease. It is a full-length murine-derived humanized, monoclonal, nonselective antibody against VEGF-A. It is a significantly larger molecule with potentially less effective retinal penetration and binding affinity to VEGF. In comparison to ranibizumab (Lucentis), which is an antibody fragment, bevacizumab has an Fc fragment which may make it more immunogenic or proinflammatory. Larger molecules with Fc constant fragments and antibody-binding Fab fragments are more immunogenic than those with the antibody-binding fragment alone.

A retrospective single center study conducted by Johnson et al. reported the incidence of intraocular inflammation after bevacizumab injection to be 1.3% after 693 injections [26]. Similarly, Georgopoulos et al. reported a 0.3% incidence of intraocular inflammation after 2500 injections of bevacizumab [25] and Shima et al. reported a 0.2% incidence of ocular inflammation after 1300 injections [16]. In a smaller study that used the same lot of bevacizumab, 5 of the 35 (14.3%) of the patients developed severe intraocular inflammation [22]. In this study however, 80% of the patients had received bevacizumab injections previously without an intraocular inflammatory episode. A similar incidence of lot specific intraocular inflammation has been reported in China where 80 patients of 116 (69%) developed postinjection intraocular inflammation [24]. This study implicated endotoxin as the cause of the inflammation.

Aflibercept (Eyelea, Regeneron, Inc., Tarrytown, NY) utilizes the fusion of multiple endogenous receptor components creating what is called a VEGF Trap. It binds with higher affinity to multiple isoforms of VEGF-A as well as VEGFR1 ligands, VEGF-B, and placental growth factor (PIGF). Consistent with this higher affinity, VEGF Trap demonstrates a higher ability in blocking VEGF-mediated mobilization and migration of human endothelial cells [51].

It was approved by the FDA in November 2011, and within the first three months after its approval, a cluster of small cases were reported with injection-related ocular inflammation [37]. This report indicated that aflibercept was associated with sterile inflammation in 0.05% of cases and associated with pain far more than the other anti-VEGF agents (60%). Prior to approval of aflibercept, the clinical characteristic of pain could often be used to distinguish between sterile inflammation and endophthalmitis. This report has led clinicians to be more cautious and more apt to treat with intravitreal antibiotics sooner. Subgroup analysis in this study did not detect any variables significantly affecting visual outcome or number of days to resolution. Moreover, Ho et al. looked at the short-term outcomes of aflibercept in 245 patients for five months and reported no cases of endophthalmitis [38]. More recently, The American Society of Retina Specialists Therapeutic Surveillance Committee (ASRS TSC) reported at the annual ASRS meeting in August 2013 that there were at least 41 cases of sterile endophthalmitis among more than 800,000 aflibercept injections given in the United States between December 2011 and June 2013. While some of these reported cases responded to topical corticosteroid treatment and observation alone, others were associated with more severe inflammation that resembled infectious endophthalmitis. The ASRS TSC concluded that there was no clear pattern detected to predict these events.

6. Hypotheses

There are several hypotheses pertaining to the etiology of sterile inflammation secondary to intravitreal anti-VEGF injections. The manufacturer's guidelines for anti-VEGF agent preparation state that the medication should be refrigerated at 2 to 8 degrees C (36 to 46 degrees F), protected from the light, stored in the original carton until used, and used within 8 hours of being opened [10]. Any variance from this protocol could result in degradation of the agent with increased immunogenicity [52, 53].

The eye may mount an immune response to the antibody molecule after prior exposure to the drug. One report found an 83% incidence of sterile inflammation after intravitreal injection of ranibizumab. One of the 29 patients (0.03%) had to be permanently withdrawn from the study secondary to the severe inflammation. In this study, the inflammation was low-grade and self-limited and did not increase with repeated injections or increasing doses [18]. While these results do not support this immune mediated hypothesis, the immunogenicity varies between different anti-VEGF agents.

Bacterial endotoxin contamination has been reported in the pharmaceutical production phase of antibody preparation [24, 54]. In a study by Wang et al., a total of 116 patients were injected from 3 vials of counterfeit bevacizumab with 80 patients subsequently developing intraocular inflammation. The presence of endotoxin in vitreous specimens was confirmed by laboratory testing. They concluded that endotoxin testing should be considered as part of the laboratory investigation in patients who develop noninfectious inflammation. These studies demonstrated that endotoxin contamination of individual aliquots is possible during preparation. While this occurrence could explain clusters of sterile endophthalmitis cases in patients treated with injections from the same batch, it is unlikely to explain the cause of sporadic cases.

While systemic use of anti-VEGF agents has not been described to entice an inflammatory response, in the closed system of the eye, it may mount a significant response to the anti-VEGF. Multiple case series have described a high percentage (35–78%) of sterile ocular inflammation after intravitreal injections from a single lot of anti-VEGF agents.

Yamashiro et al. reported 14 consecutive cases of endophthalmitis after intravitreal injection of bevacizumab from the same lot. Of the 19 eyes, 14 showed ocular inflammation after injection. Vitreous samples from these patients revealed the etiology to be noninfectious [19]. Similarly, in a report by Sato et al., 14.3% (five of 35 cases) were noted to develop a severe intraocular inflammation after intravitreal injection of bevacizumab. Vitrectomy was performed in all 5 cases with no growth of any causative organisms or microbes [22]. All five cases were resolved with treatment with steroids. These reports support the possibility of trace endotoxin contamination resulting in sterile endophthalmitis. Wang et al. published a retrospective paper where 116 patients were injected with counterfeit bevacizumab. Of these patients, 69% developed a sterile endophthalmitis with endotoxin levels (endotoxin units) detected as high as 36 Eu/mL (standard of bevacizumab <2 Eu/mL) [24]. These patients shared the typical clinical features described above with 78% of the affected patients returning to pre-injection visual acuity.

In summary, sterile inflammation is an adverse event of intravitreal anti-VEGF injection that should be included in the patient consent in all anti-VEGF agents. Acute intraocular inflammation is most frequently following bevacizumab [36], possibly due to the less stringent purification process of the medication. In most cases, the inflammation resolves and vision returns to baseline. A history of prior inflammation does not increase the risk with subsequent injections.

Conflict of Interests

The authors declare that there is no competing/conflict of interests related to any topic in this paper.

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Review Article

Inflammation and Macular Oedema after Pars Plana Vitrectomy

Vito Romano,[1] Martina Angi,[2] Fabrizio Scotti,[3] Renata del Grosso,[4] Davide Romano,[4] Francesco Semeraro,[5] Paolo Vinciguerra,[3] Ciro Costagliola,[6] and Mario R. Romano[3]

[1] *Second University of Naples, Via Pansini 5, 80100 Napoli, Italy*
[2] *Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK*
[3] *Department of Ophthalmology, Istituto Clinico Humanitas, Rozzano, Milan, Italy*
[4] *Ophthalmology Unit, San Sebastiano Hospital, Caserta, Italy*
[5] *Eye Clinic, Department of Neurological Sciences and Vision, University of Brescia, Brescia, Italy*
[6] *Eye Clinic, Department of Health Sciences, University of Molise, Campobasso, Italy*

Correspondence should be addressed to Vito Romano; vito.romano@gmail.com

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Cystoid macular oedema (CMO) is a major cause of reduced vision following intraocular surgery. Although the aetiology of CMO is not completely clarified, intraocular inflammation is known to play a major role in its development. The macula may develop cytotoxic oedema when the primary lesion and fluid accumulation occur in the parenchymatous cells (intracellular oedema) or vasogenic oedema when the primary defect occurs in the blood-retinal barrier and leads to extracellular fluid accumulation (extracellular oedema). We report on the mechanisms of CMO formation after pars plana vitrectomy and associated surgical procedures and discuss possible therapeutic approaches.

1. Introduction

Macular oedema results from serous exudation of incompetent intraretinal capillaries localized between the retina's outer (plexiform) and inner (nuclear) layers, as well as from swelling in retinal Müller cells. Cystoid macular oedema (CMO) is a localized expansion of the extracellular, and sometimes intracellular, space in the macular area of the retina and has a characteristic radially orientated cystic pattern with perifoveal cyst-like spaces [1]. The empty space may result in lamellar holes or full-thickness oedema, which consequently damages the outer retinal layers resulting in permanent central vision impairment [1–3]. CMO can arise in cases of central or branch retinal vein occlusions, diabetic retinopathy, and retinal traction disorders due to blood-retinal barrier (BRB) alterations [4].

BRB alterations are the result of cytotoxic insult that is secondary to intraocular inflammation. The same mechanism appears to be responsible for iatrogenic damage after cataract extraction and other kinds of intraocular surgeries, such as vitreoretinal surgery [2]. The BRB is located on two levels: the chorioepithelial interface and the retinal vessels, forming the outer and inner BRB, respectively. The retinal pigment epithelium of the outer BRB is comprised of cells linked by tight junctions, adherent junctions, and desmosomes. The endothelial membrane of the retinal vessels of the inner BRB is comprised of cells linked by tight junctions. Together, the retinal pigment epithelium and the endothelial membrane form the BRB's main structures. Under physiological conditions, the BRB separates blood from the surrounding retinal tissue and maintains environmental stability for ocular neurons and photoreceptors by controlling the movement of proteins and cells from the blood into these tissues [5]. Additionally, every neuron and glial cell has a membrane transport system that balances ion and water movement in and out of the cell [5].

Under pathological conditions, the retina may develop cytotoxic oedema, where the primary lesion and fluid accumulation occur in the parenchymatous cells (intracellular oedema), or vasogenic oedema, where the primary defect occurs in the BRB and leads to extracellular fluid accumulation (extracellular oedema) [6]. The vasogenic damage

that occurs in vasogenic oedema is governed by inflammatory cells, such as macrophages, neutrophils, and several other inflammatory mediators. These mediators include angiotensin II, vascular endothelial growth factor (VEGF), prostaglandins, cytokines, chemokines, matrix metalloproteinases, interleukins, P-selectin, E-selectin, VCAM-1, and ICAM-1 [7, 8]. Typically, although some conditions primarily cause extracellular oedema or intracellular oedema, a hybrid of both types of oedemas occurs simultaneously.

In this paper, we report on the mechanisms of CMO formation after pars plana vitrectomy and associated surgeries and discuss possible therapeutic approaches.

2. Cystoid Macular Oedema after Pars Plana Vitrectomy

The overall incidence of CMO after pars plana vitrectomy (PPV) is not easily determined, as it is often related to previous conditions, such as central or branch retinal vein occlusions, diabetic retinopathy, and retinal traction disorders. The most accurate data come from patients undergoing PPV for vitreous floaters, where any postoperative CMO is clearly linked to this surgical procedure. The work carried out by de Nie et al. on this topic showed that CMO after PPV occurred in 5.5% of cases. All patients were successfully treated with medical treatment, except two cases that needed a second surgery [9]. Other studies with the same inclusion criteria did not record any case of CMO after PPV [10–12]. These data show that the technical developments over the past years have made vitrectomy a mini-invasive type of surgery, improving the risk/benefit equation.

3. Cystoid Macular Oedema after Pars Plana Vitrectomy with Internal Limiting Membrane Peeling

Optical coherence tomography (OCT) and histological findings provide detailed retinal microstructure imaging. They help in delineating any inflammatory damage occurring after PPV, the role played by the internal limiting membrane (ILM), and any benefits of ILM removal during surgery. The interstitial pathway from the vitreous cavity to the subretinal space is formed by an external and an internal limiting membranes. The junctions between the photoreceptors and the Müller cells of the external limiting membrane (ELM) are not sealed and, consequently, can only partially limit the movement of large molecules. However, the ILM has no significant influence on water movement. The balance between static and dynamic vitreous tractional forces determines whether CMO forms a macular hole or becomes a chronic tractional CMO [13].

ILM peeling may have beneficial effects on CMO because it removes tangential traction, increases retinal oxygenation, reduces VEGF production, and allows intraretinal fluid from the macula to reach the vitreous cavity [14]. Studies have shown that the Müller cells immediately swell (intracellular oedema) after PPV with ILM peeling and that this swelling persists. However, Kado et al. showed that the period of macular oedema (extracellular oedema) could be shortened by reducing the centripetal traction transmitted to the Müller cells by vitreous fibres inserted into the macula [15]. Additionally, ILM removal may also help preventing postoperative complications [16, 17]. Spaide recently observed an inner retinal dimple along the path of the nerve fiber layer in 52% of the eyes treated with ILM peeling [18]. The Müller cell footplates run over the inner surface of the nerve fiber layer, having the ILM as a basement membrane. The patients developed a radiating pattern of darker spots within a thin superficial grayish lamina. This pattern has been called dissociated optic nerve fiber layer (DONFL) appearance and it seems to be related to the impact of Müller cell footplates avulsion [18]. DONFL has been also described by Tadayoni et al. after epiretinal membrane (ERM) removal [19]. The authors described slightly darker arcuate striae in the direction of the optic nerve fibers. This feature had no functional effect on postoperative functional prognosis [18, 19].

PPV with ILM peeling in retinal vein occlusions removes traction and reduces VEGF and IL-6 production, two factors responsible for inducing vascular permeability [15, 20, 21]. Mandelcorn et al. [22, 23] have hypothesized that PPV-ILM peeling decompresses retinal blood vessels, thereby facilitating the release of extracellular fluid and blood into the vitreous cavity, where it can be more easily removed. Other authors have also highlighted the lack of ERM formation and CMO recurrence following this surgery [24]. Raszewska-Steglinska et al. reported that 68% of patients in their series had improved visual acuity after PPV-ILM peeling and that the best results were obtained in patients treated within 1 month of CMO onset [16].

ILM peeling has also been associated with PPV for the treatment of retinal detachment (RD) with proliferative vitreoretinopathy (PVR), in the hope of reduceing postoperative CMO. A retrospective study of 90 eyes demonstrated a reduction in CMO in some patients; however, PPV-ILM peeling was still not enough to eliminate this complication in 47% of cases [25]. Better results were shown by Schocket et al., who reported CMO in only 12% of eyes treated for RD [26], and by Kiss et al. (17%) [27]. The RD duration, the numbers of surgeries, and the mechanical activities related to ILM peeling were important in both these situations [25]. Chang et al. further confirmed that apoptosis and macular oedema begin a few hours after RD and that apoptosis and oedema severity only increase by time to significantly influence visual acuity [28, 29].

In contrast to the above, the postoperative retinal thickness and visual acuity of diabetic patients after PPV-ILM peeling were not significantly better than those of the ILM-preserved group in two Japanese studies [30, 31]. In these patients, however, attention must also be paid to preexisting ocular conditions (i.e., diabetic retinopathy, uveitis, and/or a preexisting ERM) and systemic risk factors (e.g., renal failure and hypertension) because these can influence the prognosis of diabetic CMO [32]. These conditions can lead to vascular instability, mostly due to endothelial cell damage by advanced glycosylation end-products, which predispose the BRB to breaking down.

An immunohistochemical study of ILMs peeled during vitrectomy for various aetiologies found strong adhesions between ILM cells and, consequently, that ILM peeling increases the risk of removing inner retinal structures [33]. ERM formation involves epiretinal glial proliferation and induces significant intraretinal changes [34]. This has been associated with increased expression of the intermediate filament protein GFAP in both Müller cells and astrocytes [35]. GFAP forms bridges between the cytoskeleton, epiretinal receptors, and the extracellular matrix [36]. Thus, the GFAP within Müller cells may alter adhesion between these cells and the ILM. Consequently, removing the ILM may damage Müller cells and transmit a focal force towards the inner retina that results in the avulsion of some retinal cells and the loss of competent retinal structure. This may increase the propensity for developing CMO if additional intraocular inflammation occurs [33, 37].

4. Cystoid Macular Oedema after Pars Plana Vitrectomy and Cataract Surgery

Patients who have already undergone PPV with epiretinal peeling have a higher incidence of CMO after a second intraocular surgery [38]. A prospective, nonrandomized, controlled clinical study found that 26% of eyes developed CMO after successful cataract surgery when eyes had been previously treated with PPV and ERM and ILM peeling [38]. In contrast, no cases of CMO were observed in the control group. The problem is mainly related not to the combination of surgeries but to the lack of vitreous and of a competent retinal structure. Therefore, cataract surgery should be avoided after vitrectomy and, instead, be planned before or at the same time of PPV [38, 39].

Even though combined vitrectomy presents its advantages in regard to CMO formation, it has several disadvantages as well. The main disadvantages are increased postoperative inflammation and the complications related to such inflammation. This holds particularly true in diabetic patients, where a higher incidence of postoperative complications (such as synechia formation and fibrinous uveitis) has been reported following combined phaco/vitrectomy; especially if the retinopathy is very active, a large amount of intraoperative laser is needed or tamponade is used [40–47]. In such patients subconjunctival and topical steroids can be used at the end of the surgery to lessen the incidence of these complications.

Jiramongkolchai et al. retrospectively evaluated the incidence of macular oedema and cataract formation after PPV in diabetic patients who required cataract surgery. Macular oedema incidence was 6% six months after PPV and 30% six months after cataract surgery in the same patients. This suggests that factors independent of the vitreous, such as inflammation, are mainly involved in the pathogenesis of macular oedema after cataract surgery in diabetics [48]. Additionally, according to Bhatnagar et al., patients who have already undergone surgery for macular holes have an increased risk for macular hole recurrence after cataract surgery [49]. This is most likely due to ILM peeling causing a loss of retinal structure and greater responsiveness to inflammatory stimuli. Consequently, CMO recurs and the macular hole reopens. In other studies, no association was found between cataract extraction and macular hole reopening [50–52]. However, this situation is unclear, because several differences exist between the design and inclusion criteria of these studies that may explain the discrepant results.

5. Cystoid Macular Oedema after Silicone Oil Removal

The use of silicon oil (SiO) as a long-term intraocular tamponade may lead to macular changes such as CMO. A comparative analysis of macular microstructures before and after SiO removal reported that microstructural changes were associated with the duration of SiO tamponade and that most of the microstructural changes were reversed upon SiO removal. Under SiO tamponade, the OCT identified CMO in 19.6% of cases. In most cases, however, visual acuity was significantly improved after SiO removal in correlation with the decrease of CMO [53]. In one retrospective interventional case series, complicated RD with PVR macular changes was observed in 87% of patients following SiO removal, and 18% of those had CMO that required additional treatment [27]. Cox et al. also showed that the CMO is not related to epiretinal traction since ERM formation was not statistically related to the type of tamponade (SiO versus gas) [54].

SiO impurities, such as the oil's low molecular weight components (LMWC) and residual catalysts, are thought to cause the ocular inflammation. Using gas chromatography, Nakamura et al. analysed SiO up to two years after injection and found evidence of decreased LMWC concentrations. LMWC likely diffused from the oil into the ocular tissues, resulting in chronic ocular toxicity [55]. Furthermore, histopathological analysis of an ERM that developed after intraoperative use of perfluorocarbon liquids identified an inflammatory reaction with foreign body response to intraocular tamponade [56].

6. Cystoid Macular Oedema after Pars Plana Vitrectomy for Retained Lens Fragments

Clinical CMO occurs in fewer than 2% of eyes after an uneventful cataract surgery and rarely becomes chronic [57, 58]. Conversely, clinical CMO is reported in up to 28% of eyes after PPV for retained lens fragments and becomes chronic in about 20% of these eyes [59]. If residual fragments were not removed from the eye, the incidence of CMO would likely be even higher [60]. Moreover, after vitreous removal, the eye behaves like a single compartment. Therefore, in vitrectomized eyes, inflammatory mediators can more easily diffuse from the iris and anterior chamber to the macula, causing CMO [61]. Furthermore, the lens epithelial cells (LECs) are responsible for synthesis of prostaglandins and cytokines such as PGE2, IL-1, and TGF-beta [62].

Posterior dislocation of nuclear lens fragments is associated with a worse visual outcome than that of nonnuclear fragments. This is likely due to direct mechanical damage

to the retina, a stronger inflammatory response, or a more traumatic vitrectomy procedure [63]. A retrospective study of 91 patients who had PPV for retained lens fragments observed that CMO developed in only 8% of patients with a sulcus-fixated posterior chamber intraocular lens. In contrast, CMO developed in 46% of patients with aphakia or an anterior chamber intraocular lens (IOL) [64]. In these cases, long-term anti-inflammatory therapy should be considered because of the high rate of CMO recurrence.

The timing of surgical retained lens fragment removal remains a multifactorial decision involving surgeon and patient preferences, situational logistics, and clinical judgment. A systematic review and meta-analysis of retrospective interventional cases found evidence that postoperative outcomes, such as visual acuity, RD, increased intraocular pressure, and intraocular infection/inflammation, are better with early PPV [65]. However a retrospective study on 569 eyes found similar visual acuity outcomes and complication rates in patients undergoing same-day or a later PPV [66].

7. Cystoid Macular Oedema in the Presence of Epiretinal Traction

Some reports emphasize the role of mechanical factors in clinical CMO. These factors include tractional forces on the macula (i.e., ERM or vitreomacular traction) that pull on the retinal surface resulting in vascular damage and in the release of mediators which lead to the breakdown of the BRB. Vitreomacular traction syndrome (VMT) can, therefore, cause both tractional and exudative CMO [67, 68]. Prognosis and treatment options depend on the size and configuration of the residual vitreomacular adhesion and on the consequential anatomical macular changes [69]. This type of CMO can be easily confused with postoperative, uveitic, or retinal vascular CMO [70]. Important clinical clues of tractional etiology may include metamorphopsia, subtle asymmetry of the cystoid foveal thickening, and the absence of leakage via fluorescein angiography. Surgical intervention for this CMO appears to benefit the majority of patients with significant associated visual loss.

8. Medical Treatment for Cystoid Macular Oedema

The rationale for pharmacological CMO treatment after vitreoretinal surgery is based on understanding the aetiology and inhibition of these pathophysiological mechanisms. The main factor triggering CMO is the release of inflammatory mediators; vitreous traction does not always play a role in the CMO pathogenesis. Other possible mechanisms include photoretinal stress and pathologic evidence of Müller cell damage. However, more research is needed to better understand the cause of CMO and its pathophysiology [33].

CMO treatment aims to reduce the release of inflammatory mediators which results from the breakdown of the BRB. These mediators generate vasogenic damage such as vasodilation, increased capillary permeability, leukocyte migration, and finally CMO [71].

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase 1 and 2 and, therefore, prostaglandin production. Thus, NSAIDs modulate fluid movement coupled with chloride movement. Cyclooxygenase inhibitors (e.g., indomethacin and other NSAIDs) reduce the incidence of angiographic CMO [72]. The ability of topical NSAIDs to penetrate ocular tissues, including retinal tissue, is an important factor for treating and preventing CMO. NSAID use has been beneficial for chronic postoperative macular oedema. Flach found that a topical NSAID (0.5% ketorolac tromethamine) was effective and that treatment duration of three months provided a more persistent benefit than one or two months [73, 74]. Baklayan et al. showed that Xibrom, a highly lipophilic ophthalmic solution of 0.09% bromfenac, rapidly penetrates ocular tissues [75]. This resulted in both rapid and sustained detectable drug levels in all relevant ocular tissues, including the retina, for over 24 h following a single topical administration. The efficacy of topical NSAIDs in treating CMO has been reviewed in great detail elsewhere. The general consensus is that, despite the paucity of well-designed studies, NSAID treatment is beneficial by reducing macular oedema and possibly improving vision, at least in the short term [72].

Corticosteroids are also well known for their effects on inflammation and cellular proliferation. Corticosteroids block phospholipase A, which acts upstream the arachidonic acid cascade. Consequently, they also block prostaglandin and leukotriene production, downregulate VEGF, and decrease occludin phosphorylation, thereby increasing the tightness of the BRB [76, 77]. Systemic steroid treatment does not seem to significantly improve the anatomic and functional outcomes of CMO [78]. However, periocular application or intravitreal injections appear to be effective for CMO management [79–82]. A prospective randomized, controlled trial of 315 patients with persistent macular oedema due to uveitis or Irvine-Gass syndrome showed that 700 mg of intravitreous dexamethasone over 90 days was well tolerated and resulted in statistically significant improvements in visual acuity and vascular leakage compared to a 350 mg dose [83].

Experimental studies have shown that the vitreous half-life of different drugs after intravitreal injection decreases after PPV [84]. The corticosteroid triamcinolone acetonide has been used during vitrectomy to prevent postoperative inflammatory complications [85]. Intravitreal triamcinolone acetonide was more rapidly cleared in vitrectomized patients, though. Schinder et al. suggested that triamcinolone acetonide in the empty vitreous cavity can circulate more easily and faster than that in the normally viscous vitreous [86]. The vitreous is made of highly viscous, gel-like materials, and intravitreal corticosteroids are condensed into a small space. Consequently, highly viscous vitreous likely has a very slow gel circulation. Therefore, the widespread distribution and increased circulation of triamcinolone acetonide in an empty vitreous cavity may be responsible for its rapid clearance.

In contrast, Chang-Lin et al. reported that the vitreoretinal pharmacokinetic profiles of a dexamethasone intravitreal implant were similar between nonvitrectomized and vitrectomized eyes [87]. In both groups, the decrease in central retinal thickness was usually accompanied by improved

visual acuity, and no systemic side effects were observed. However, ocular side effects developed in 70.6% of patients, including increased intraocular pressure (47.1%), transient hypotony (11.8%), displacement of the implant into the anterior chamber in aphakic eyes (5.9%), and RD (5.9%) [87].

Topical betaxolol is a β1-selective adrenoceptor antagonist with ocular hypotensive and retinal neuroprotective effects. It is also a vasodilator that acts by blocking Ca^{2+} channels. Consequently, betaxolol may play a role in relaxing retinal microarteries, which would improve ocular circulation, resolve macular oedema, and restore retinal function. A randomized clinical trial noted that topical betaxolol appeared to have a favourable effect for eyes with macular oedema [88].

Carbonic anhydrase inhibitors are widely used for modulating the polarized distribution of carbonic anhydrase in retinal pigment epithelium. This occurs via extracellular pH gradients and stimulates fluid resorption from the retina to the choroid. Anti-VEGF agents can also restore occludin proteins in the BRB and reduce protein kinase C activation.

Heier et al. suggested that a combination of topical ketorolac and steroids appeared to offer benefits over monotherapy for acute CMO [89]. Additionally, three additional small studies, which could not be directly compared, have also indicated that using topical anti-inflammatory drugs in combination with topical steroids has therapeutic benefits [3].

Evidence for treating acute CMO remains insufficient for recommending any practices as an adequate solution.

9. Prevention of Cystoid Macular Oedema

CMO can lead to permanent structural damage of the outer nuclear layers therefore causing irreversible visual loss. Minimally traumatic and fast vitreoretinal surgery is the primary means of preventing CMO.

Attention must be paid to preexisting systemic conditions such as diabetes and cerebrovascular and cardiovascular diseases as well as to preexisting ocular conditions [90]. In these cases, using NSAIDs as a prevention strategy may be effective for preventing CMO [73, 74, 91, 92]. Several topical NSAIDs are commercially available for ophthalmic use. Heier et al. measured vitreous drug levels in patients who received either 0.4% ketorolac, 0.09% bromfenac, or 0.1% nepafenac for three days before vitrectomy surgery. All three NSAIDs were able to penetrate the vitreous cavity. Additionally, they found that ketorolac might have a clinical impact on managing prostaglandin-mediated diseases, including CMO [93]. Preoperative NSAID use can also stabilize pupillary dilation during intraocular surgery and reduce postoperative inflammation, pain, and the occurrence of CMO [39, 72].

In conclusion, preventing intraocular inflammation appears to be more successful than curing CMO. Prevention should be initiated 6 weeks in advance for uncomplicated surgery or 3 months for complicated surgery and in cases where risk factors are a concern.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

TNF-Alpha Levels in Tears: A Novel Biomarker to Assess the Degree of Diabetic Retinopathy

C. Costagliola,[1] V. Romano,[2] M. De Tollis,[3] F. Aceto,[1] R. dell'Omo,[1] M. R. Romano,[1] C. Pedicino,[3] and F. Semeraro[4]

[1] *Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy*
[2] *Second University of Naples, Via Pansini 5, 80100 Naples, Italy*
[3] *"Casa di Cura Villa Maria", Campobasso, Italy*
[4] *Department of Medical and Surgical Specialities, Radiological Specialities and Public Health, Eye Clinic, University of Brescia, Brescia, Italy*

Correspondence should be addressed to V. Romano; vito.romano@gmail.com

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We assess the level of tumour necrosis factor alpha (TNF-alpha) in tear fluids and other serum parameters associated with diabetes in different degrees of diabetic retinopathy. We have performed a prospective, nonrandomized, observational study. Study population consisted of 16 healthy subjects (controls) and 32 type 2 diabetic patients: 16 affected by proliferative diabetic retinopathy (PDR) and 16 with nonproliferative retinopathy (NDPR, background/preproliferative). Body mass index, urinary albumin, blood glucose, HbA1c, and tear levels of TNF-alpha were measured in all subjects. The value of glycaemia, microalbuminurea, and Body mass index in diabetic retinopathy groups were higher than those in control group ($P < 0.05$). Glycemia in NPDR: 6.6 mmol/L (range: 5.8–6.3); in PDR: 6.7 mmol/L (range: 6.1–7.2); in control: 5.7 mmol/L (range: 4.9–6.1); microalbuminurea in NPDR: 10.6 mg/L (range: 5.6–20); in PDR: 25.2 mg/L (range: 17–40); in control: 5.3 mg/L (range: 2.6–10); Body mass index in NPDR: 26 Kg/m^2 (range: 20.3–40); in PDR: 28 Kg/m^2 (range 20.3–52); in control: 21 Kg/m^2 (range 19–26). The TNF-alpha concentrations in tears increase with the severity of pathology and were lower in control group than in diabetic subjects. In the end, the level of TNF-alpha is highly correlated with severity of diabetic retinopathy and with nephropathy. Tear fluid collection may be a useful noninvasive method for the detection of proliferative diabetic retinopathy.

1. Introduction

Diabetes is a pathologic condition strongly associated with both microvascular (involving small vessels, such as capillaries) and macrovascular (involving large vessels, such as arteries and veins) complications. Microvascular complications include retinopathy, nephropathy, and neuropathy; whereas macrovascular complications include cardiovascular diseases, strokes, and insufficiency in blood flow to legs. These complications are triggered by the same culprit: elevated blood glucose levels. In fact, chronic hyperglycaemia plays a major role in the beginning of diabetic vascular complications through many metabolic and structural derangements, that is, the production of advanced glycation end products, abnormal activation of signaling cascades, elevated production of reactive oxygen species, and abnormal stimulation of hemodynamic regulation systems. Thus, metabolic control in diabetes can delay the onset and progression of these complications [1, 2].

Diabetic retinopathy (DR) is a leading cause of blindness and visual disability. DR is a serious consequence of long-standing and poorly controlled diabetes and the most important cause of vision loss in the working-age population in developed countries [3]. One hundred and fifty million of people in the world are affected, and in 10–15 years the World Health Organization predicts that the number of affected people will be doubled [4]. DR is classified according to the presence or absence of abnormal new vessels as nonproliferative (background/preproliferative) retinopathy and proliferative retinopathy [5]. Different studies reported that

the prevalence of any retinopathy in diabetic patients is more than 30% [6–8]. Chronic hyperglycaemia drew metabolic and haemodynamic derangements, such as impaired blood flow regulation, increased vascular permeability, capillary basement membrane thickening, microaneurysm formation, microvascular cell death, and eventually widespread nonperfusion and ischemia of the inner retina [9], leading to dysfunctional responses in a range of cell types including neurons, glial cells, and supporting microvasculature [10]. While the precise pathogenesis of DR remains incompletely understood, inflammation and related processes are now thought to contribute to neuronal, glial, and microvascular lesions [10–12]. However, the role played by inflammation in DR has not yet been completely clarified [13, 14].

TNF-alpha is an inflammatory cytokine, strongly correlated with insulin resistance and chronic inflammation [15]. Recently, the molecular mechanisms of TNF-alpha function have been intensively investigated. Many studies demonstrated increased circulating levels of TNF-alpha both in animals [16, 17] and humans [18–24], as well as in the retina of diabetic rat [25]. Moreover, TNF-alpha also has a role in the development of insulin resistance; in fact, it affects insulin sensitivity by changing the phosphorylation of insulin receptor substrate-1 and interferes with the insulin signaling cascade, thereby leading to insulin resistance [26, 27]. TNF-alpha is a potent mediator of the leukostasis induced by VEGF, interleukin-1 alpha, and platelet-activating factor in the retinal vasculature [28], and it also mediates the cell death/apoptosis of retinal neurons and vascular endothelial cells in DR [29]. These studies suggest that the retinal leukostasis and apoptosis mediated by TNF-alpha contribute to blood retinal barrier (BRB) breakdown in DR. Previous studies have shown that the level of soluble TNF receptors increases in the serum and vitreous of patients with proliferative diabetic retinopathy [30, 31]. These findings provide a potential link between inflammation and insulin resistance, further confirmed by the evidence that TNF-alpha inhibition prevents the pathologic events related to the development of early DR, including BRB breakdown [16].

The present study was designed to verify the feasibility of using the tear TNF-alpha assay as a novel biomarker to assess the degree of eye involvement in type 2 diabetic patients with and without proliferative diabetic retinopathy.

2. Patients and Methods

2.1. Subjects and Methods. The study was conducted in accordance with the tenets of the Declaration of Helsinki and the Institutional Review Board approved the study protocol. All participating subjects gave their informed consent after a detailed description of the procedure used and of the aim work. Thirty-two diabetic patients and 16 healthy subjects were recruited for this study. Diagnosis of type 2 diabetes was carried out according to the guidelines of American Diabetes Association [32]. Diabetic retinopathy classification and grading was performed according to the ETDRS criteria [33]. The study was conducted at the Operative Unit of Ophthalmology, "Casa di Cura Villa Maria", University of Molise, Campobasso, Italy. Diabetic patients were divided into 2 groups: 16 patients presented nonproliferative retinopathy (NPDR, background/preproliferative retinopathy, 9 : 7, male : female; 54 years: range 49–67), whereas the remaining 16 patients were diagnosed as affected by proliferative diabetic retinopathy (PDR, 10 : 6, male : female; 59 years: range 52–73). Before study inclusion, each patient underwent a baseline eye check including best corrected visual acuity (BCVA) measured at 2 m with a standard ETDRS chart, biomicroscopy and fundus examination, applanation tonometry, fluorescein angiography, and evaluation of foveal thickness by optical coherence tomography (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany). The control group consisted of 16 healthy, age-matched, randomly selected University of Molise employers.

Body height and weight and waist circumference were measured to calculate the Body Mass Index (BMI). The following formula was used: weight in kilograms/height in meters squared (kg/m^2). The BMI results are classified as: (i) underweight (BMI < 18.5); (ii) healthy weight (BMI between 18.5 and 24.9); (iii) overweight (BMI between 25 and 30); (iv) obese (BMI > 30); (v) morbidly obese (BMI > 40). Blood glucose, HbA1c (NycoCard HbA1c test and NycoCard Reader, Axis-Shield PoC AS), blood urea nitrogen (BUN), and protein urine (Micral-Test, Roche Diagnostics Germany) were performed to all subjects. The occurrence of nephropathy was assessed measuring the glomerular filtration rate (GFR), which was calculated according to the Cockcroft formula [34]. A value of 90 mL/min/1.73 m^2 or higher was considered normal.

Nonstimulated tears were collected from each eye from the inferior tear meniscus between the 6 o'clock and lateral canthus positions using a standard clinical Schirmer's strip. Following collection, the strip was immediately transferred into a sterile labelled tube and frozen at −40°C until analysed. The wet portion of each Schirmer's strip was then cut into smaller pieces and soaked in 50 μL/5 mm of phosphate-buffered saline (PBS) for 3 h to elute tear proteins. TNF-alpha was measured using a 96-well plate coated with antibody specific for human TNF-alpha, according to manufacturer's instructions (Human TNF-alpha ELISA Development Kit PEP-900-K25, Li StarFish S.r.l.Cernusco S/N, Milan, Italy).

Data were recorded in an Excel 2007 spreadsheet and analyzed by SPSS statistical software. For analysis of statistical differences, we used the Wilcoxon signed-rank test to evaluate the intragroup significance between variables. Conversely, the Mann-Whitney U-test was used to compare the control and DR groups. A P value of <0.05 was considered statistically significant.

3. Results

BMI, blood glucose, HbA1c, diabetes duration, BUN, protein urine, and GRF data are showed in Table 1. In diabetic patients, blood glucose levels were slightly higher than those recorded in the control group: NPDR: 6.6 mmol/L (range: 5.8–6.3); PDR: 6.7 mmol/L (range: 6.1–7.2); controls: 5.7 mmol/L (range: 4.9–6.1); P = not significant. The blood glucose difference between diabetic patients with and without proliferative retinopathy was also not significant. The HbA1c

Table 1: Demographic and laboratory results of cohort.

	Controls Healthy subjects	Cases Diabetic patient		P value
		NPDR	PDR	
No. of subjects	16	16	16	N.S.$^{\#}$ N.S.*
Age, yrs (range)	53 (47–58)	54 (49–67)	59 (52–73)	N.S.$^{\#}$ N.S.*
Gender (M/F)	(8/8)	(9/7)	(10/6)	N.S.$^{\#}$ N.S.*
BMI (Kg/m^2)	21 (19–26)	26 (20.3–40)$^{\#}$	28 (20.3–52)$^{\#}$	<0.05$^{\#}$ N.S.*
Glucose (mmol/L)	5.7 (4.9–6.1)	6.6 (5.8–6.3)	6.7 (6.1–7.2)	N.S.$^{\#}$ N.S.*
HbA_{1c} (%)	5.6 (5.3–6.1)	7.2 (6.5–11.5)$^{\#}$	7.8 (7.2–12.5)$^{\#}$	<0.05$^{\#}$ N.S.*
DM duration (yrs)	Not available	14.8 ± 8.7$^{\#}$	16.5 ± 11.3$^{\#*}$	<0.001$^{\#}$ <0.05*
BUN (mg/dL)	15 (6–20)	30 (24–48)$^{\#}$	44 (26–51)$^{\#*}$	<0.001$^{\#}$ <0.001*
Protein urine (mg/L)	5.3 (2.6–10)	10.6 (5.6–20)$^{\#}$	25.2 (17–40)$^{\#*}$	<0.001$^{\#}$ <0.001*
GFR (mL/min/1.73 m^2)	95 (73–91)	82 (46–89)$^{\#}$	64 (56–78)$^{\#*}$	<0.001$^{\#}$ <0.001*

BMI: Body Mass Index; DM: Diabetes Mellitus; BUN: Blood Urea Nitrogen; GRF: Glomerular Filtration Rate.
$^{\#}$Mann-Whitney U-test: diabetic patients versus controls; *Wilcoxon signed-rank test: patients with PDR versus patients with NPDR; NS: not significant.

percentage was significantly higher in the groups of diabetics than in controls: NPDR: 7.2% (range: 6.5–11.5); PDR: 7.8% (range: 7.2–12.5); controls: 5.6% (range: 5.3–6.1); $P < 0.05$. However, there were no significant intragroup differences (NPDR versus PDR patients, P = not significant). Diabetic subjects exhibited levels of protein urine significantly higher ($P < 0.001$) than controls: NPDR: 10.6 mg/L (range: 5.6–20); PDR: 25.2 mg/L (range: 17–40); controls: 5.3 mg/L (range: 2.6–10). The intra-group difference (PDR versus NDPR) was also highly significant ($P < 0.001$). The values of BMI between diabetic and nondiabetic subjects were significantly different: NPDR: 26 Kg/m^2 (range: 20.3–40); PDR: 28 Kg/m^2 (range 20.3–52); control: 21 Kg/m^2 (range 19–26); $P < 0.05$. Contrarily, the difference between diabetic patients with and without proliferative retinopathy was not significant. Duration of diabetes was higher in patients with PDR ($P < 0.05$). Lastly, diabetic patients showed a significant reduction ($P < 0.001$) of GFR: from 95 mL/min/1.73 m^2 (73–91) of controls to 82 mL/min/1.73 m^2 (46–89) and 64 mL/min/1.73 m^2 (56–78) of NPDR and PDR patients, respectively. The intra-group difference (PDR versus NDPR) was also highly significant ($P < 0.001$). Tears TNF-alpha levels were lower in control than those in PDR group ($P < 0.05$) and the TNF-alpha concentration significantly increased along with the severity of pathology: control: 1.9 Kg/mL (range 1.1–6.9); NPDR: 2.8 pg/mL (range: 1.2–5.5); PDR: 13.5 pg/mL (range 9.2–21.7). (Figure 1).

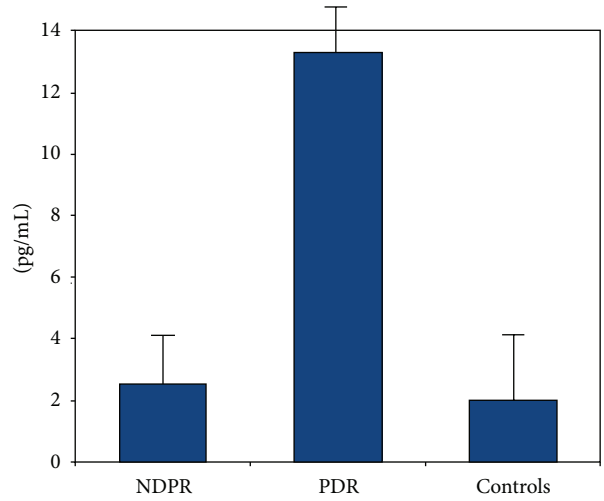

Figure 1: TNF-alpha levels in tears of the three different study groups. NPDR: non proliferative diabetic retinopathy; PDR: proliferative group diabetic retinopathy. The values are given as media and standard deviation.

4. Discussion

To the best of our knowledge, this is the first report in which TNF-alpha in tears from patients with type 2 diabetes has been measured. The levels of TNF-alpha were associated with

the degree of DR, being lower in NPDR and higher in PDR. These findings of ours tally with those reported by previous investigators which showed that proangiogenic cytokines are more highly represented than antiangiogenic cytokines in the tears of type 2 diabetic patients with retinopathy [35, 36].

Several studies have shown the association between TNF-alpha and diabetic vascular damage [16, 37]. Kuo et al. demonstrated that the levels of soluble TNF 1 and 2 receptors are highly correlated with the severity of DR, suggesting a role of TNF-alpha in the development of DR [38]. Also other authors have found that the inflammatory marker TNF-alpha was associated with the severity of DR in type 1 diabetic participant with kidney disease [14]. Limb et al. demonstrated that the type 1 diabetic patients with PDR exhibited significantly higher serum levels of soluble TNF-receptors 1 and 2 than those recorded in patients without retinopathy or in healthy individuals. Our results strengthen the important role of TNF-alpha in DR. Furthermore, another important finding is the strong association between nephropathy and retinopathy; in fact, in our patients a significant correlation among HbA1c, body mass index (kg/m^2), glomerular filtration rate (GFR), albuminuria, blood urea nitrogen, and levels of TNF-alpha in the tears has been found, further confirming that TNF-alpha tear levels may be considered a good predictor of microvascular complications. TNF-alpha is a central regulator of inflammation, and TNF-alpha antagonists may be effective in treating inflammatory disorders in which TNF-alpha plays an important pathogenetic role. Strong correlations between enhanced inflammatory biomarkers, including TNF-alpha, and the occurrence of diabetic retinopathy have been reported through extensive studies by many researchers [39, 40]. In the retina, it was shown that diabetes activates induction of proinflammatory mediators such as monocyte chemoattractant protein-1, interleukin-6, intercellular adhesion molecule-1, inducible nitric oxide synthase, matrix metalloproteinase-9, and TNF-alpha [40]. High glucose and advanced glycation end products stimulate monocytes/macrophages and their release of sTNF-alpha [41]. Serum concentrations of sTNF-alpha is increased in type 1/type 2 diabetic animals and diabetic patients with microangiopathy. Patients with proliferative DR show significantly higher serum TNF-alpha compared with that of nonproliferative DR patients [39]. TNF-alpha is elevated approximately 3-fold, and the receptors for TNF-alpha were increased by 40% in poor glycemic control retina compared with normal rat retina [42]. Altogether these findings indicate a role for TNF-alpha in DR pathogenesis and therapy and as a potential diagnostic target.

Early detection and treatment of diabetes can decrease the risk of developing its complications. Randomized trials have established the benefits of interventions to prevent or delay diabetes and reduce diabetes-related complications [43]. Screening for undiagnosed diabetes is generally considered to be safe. Most screening procedures begin with a risk assessment that relies on routinely collected demographic and clinical examination information followed by blood testing in high-risk individuals. There are also well-established and accepted diagnostic criteria for making a diagnosis of type 2 diabetes [44]. The American Diabetes Association has recommended that nondiabetic individuals ≥45 years of age be screened for diabetes at least every 3 years. Despite frequent screening and appropriate targeting of high-risk patients, followup of patients with abnormal results is uncommon and the yield of screening is low [45]. It is mandatory to diagnose DR as soon as possible, since early detection and timely treatment can prevent vision loss. Shortening the diagnostic delay, it is possible to act against the diabetes complications. It has been shown that an intensive treatment designed to keep glucose levels close to normal reduces the risk of developing long term complications, including retinopathy, and slow the progression of preexisting retinopathy in diabetic patients [46]. In this way we can reduce the number of diabetes patients with visual impairment [47] and the cost of diabetes related blindness that requires approximately $500 million/annually in USA [48]. For this reason there is a great interest to find biomarkers suitable for the prediction of vision loss as soon as possible [13, 14, 30, 31, 49].

The proposed method does not replace eye exams by an ophthalmologist, but it could be seen as a "filter" to exclude diabetic patients without diabetic retinopathy. Furthermore, increasing rate of patients with diabetes soon will outpace the supply of eye care providers, and then some communities have poor or even no access to ophthalmologic care. It was seen that the tear levels of TNF-alpha increases in various inflammatory pathologies of the ocular surface (such as Sjogren's syndrome, rosacea, and dry eye) [50–52]. Once these diseases are ruled out, TNF-alpha can be used as a biomarker for DR screening. Thus, the medical costs could be reduced, and from a patient's perspective it could be favourable because the required pupil dilation may be uncomfortable. Last but not least, in this way all diabetic patients could be subjected to a very easy and noninvasive screening procedure to assess the presence of DR. In conclusion, data from this study indicate that TNF-alpha levels are associated with DR after adjusting for potential confounders. Level of TNF-alpha may be correlated with clinical disease severity and with predictors of kidney microvascular damage, BMI, and HbA1c. This finding needs to be replicated in prospective studies performed on a higher number of subjects.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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Research Article

Expression of VEGF-A, Otx Homeobox and p53 Family Genes in Proliferative Vitreoretinopathy

Claudio Azzolini,[1] Ilaria Stefania Pagani,[2] Cristina Pirrone,[2,3] Davide Borroni,[1,2] Simone Donati,[1] Muna Al Oum,[1] Diana Pigni,[2] Anna Maria Chiaravalli,[4] Riccardo Vinciguerra,[1] Francesca Simonelli,[5] and Giovanni Porta[2]

[1] *Department of Surgical and Morphological Sciences, Section of Ophthalmology, University of Insubria, Ospedale di Circolo, Via F. Guicciardini 9, 21100 Varese, Italy*
[2] *Department of Clinical and Experimental Medicine, University of Insubria, Via O. Rossi 9, 21100 Varese, Italy*
[3] *Ph.D. Program in Biotechnology, School of Biological and Medical Sciences, University of Insubria, Via O. Rossi 9, 21100 Varese, Italy*
[4] *Pathology Institute, Ospedale di Circolo, Via F. Guicciardini 9, 21100 Varese, Italy*
[5] *Eye Clinic, Second University of Napoli, Via S. Pansini 5, 80131 Napoli, Italy*

Correspondence should be addressed to Claudio Azzolini; claudio.azzolini@uninsubria.it

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Introduction. Proliferative vitreoretinopathy (PVR) is a severe inflammatory complication of retinal detachment. Pathological epiretinal membranes grow on the retina surface leading to contraction, and surgery fails in 5% to 10% of the cases. We evaluated the expression of VEGF-A, Otx1, Otx2, Otx3, and p53 family members from PVR specimens to correlate their role in inducing or preventing the pathology. *Methods*. Twelve retinal samples were taken from patients affected by PVR during therapeutic retinectomies in vitreoretinal surgery. Gene expression was evaluated using quantitative real-time reverse transcriptase PCR analysis and immunohistochemistry, using four healthy human retinae as control. *Result*. Controls showed basal expression of all genes. PVR samples showed little or no expression of Otx1 and variable expression of VEGF-A, Otx2, Otx3, p53, and p63 genes. Significant correlation was found among VEGF-A, Otx2, p53, and p63 and between Otx1 and Otx3. *Conclusions*. Otx homeobox, p53 family, and VEGF-A genes are expressed in PVR human retina. We individuated two possible pathways (VEGF-A, Otx2, p53, p63 and Otx1 and Otx3) involved in PVR progression that could influence in different manners the course of the pathology. Individuating the genetic pathways of PVR represents a novel approach to PVR therapies.

1. Introduction

Proliferative vitreoretinopathy (PVR) is a complication of a retinal detachment and occurs in approximately 8–10% of patients developing retinal detachment [1–3]. In retinal detachment, a full-thickness retinal break exposes cells, allowing macrophages, retinal pigment epithelial cells, glial cells, and fibroblasts to migrate into the vitreous, a rich source of growth factors and cytokines correlated to PVR activity [4–6]. All these cells proliferate in the vitreous, survive, form extracellular matrix proteins, and assemble into membranes. These membranes contract on the retina, causing PVR and subtending chronic inflammation [7]. In the anterior segment of the eye, elevated laser flare photometry value in aqueous humor corresponds to an altered profibrotic intraocular cytokines milieu [8].

PVR can be divided into multiple categories based on the configuration of the retina and the location of the scar tissue [6, 9], with surgery as unique therapeutic option [10]. Despite advances in surgical techniques, the percentage of unhealed PVR remains high, causing from 5% to 10% failures in retinal surgical repairs. For the above reasons, in the recent years it has become increasingly important to individuate the inflammatory and genetic mechanisms involved in the pathogenesis of PVR, so as to highlight a possible design to be exploited in clinical trials [11–16].

Vascular endothelial growth factor A (VEGF-A), platelet-derived growth factors (PDGFs), and non-PDGFs (growth factors outside of the PDGF family) are relevant to PVR pathogenesis because of their role in suppressing p53 levels through different pathways [17]. This promotes an environment of cell survival, proliferation, organization into a membrane, and subsequent membrane contraction, all processes relevant and intrinsic to PVR pathogenesis.

The dynamic regulation of VEGF by the p53 family members makes its regulation complex, especially given the fact that all of the three transcription factors (p53, p63, and p73) are able to induce and repress VEGF, which appears to be dependent on cellular context and stimulus [18]. The OTX family includes an important class of Homeodomain-containing transcription factors involved in the induction and in the morphogenesis of the neuroectoderm, leading to the formation of the vertebrate central nervous system that includes the retina. The Otx1 gene is expressed in the rostral part of the neural tube and is required for corticogenesis and sense organs development. Postnatally, it is a marker of the anterior part of the retina, which then will develop into the ciliary body. Otx2 plays a role in the functional development of the retina, in which it is expressed at both prenatal and adult stage; it is necessary for the development and differentiation of rod and cone photoreceptors and bipolar cells, and it is detected in the retinal pigment epithelium. In addition, Otx3 expression was observed in the eye development during embryogenesis [19].

The aim of the present study is to evaluate the expression levels of VEGF-A, Otx1, Otx2, Otx3, and p53 family genes in adult healthy human retinae in comparison with retinae affected by PVR and to try to understand their role in inducing or preventing this eye pathology.

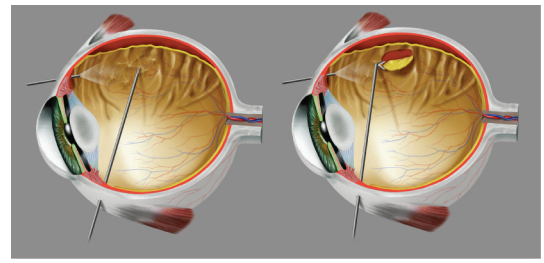

Figure 1: Important surgical steps. Left: peeling of epiretinal membranes using endoillumination and surgical instruments entering the eye through sclerotomies. Right: cut of peripheral retina (retinectomy) allowing further retinal reattachment.

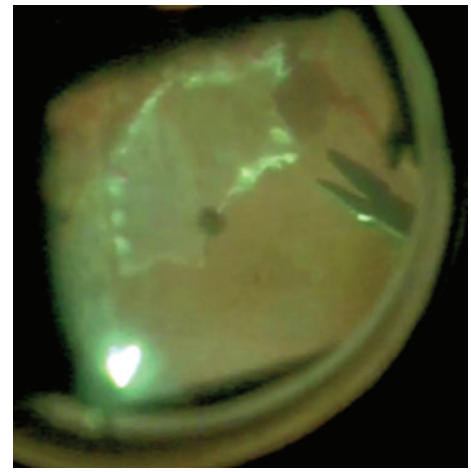

Figure 2: Retina grasp. After retinectomy, a small retinal portion is grasped with nontraumatic forceps and is removed from the eye.

2. Materials and Methods

2.1. Retina Samples. Twelve human retinae samples were taken from twelve patients affected by PVR during vitreoretinal surgery by the same operating surgeon (CA). PVR was graded according to the Retina Society Terminology Committee [9]. Patients' data are shown in Table 1. All patients were instructed and they signed the informed consent (Ospedale di Circolo, Varese, Italy). Four adult human healthy retinae from autopsy were used as control tissue. The research was approved by the local ethical committee and followed the tenets of the Declaration of Helsinki.

2.2. Surgery. Surgical procedures were similarly conducted in all patients. After accurate vitreous and vitreous base removal with vitrectomy (Stellaris, Bausch&Lomb, Rochester, New York, USA), endoilluminator and room temperature infusion fluid at surgical microscope (OPMI 1, Carl Zeiss, Jena, Germany), and accurate epiretinal membranes' peeling with surgical instruments, it was impossible to reattach the peripheral retina because of strong epi- and intraretinal PVR tissue in peripheral retina. Therefore, peripheral retinectomy preceded by endodiathermy of adjacent retina tissue was necessary in each patient to allow final total retinal reattachment at the end of surgery (Figure 1). Retina removed from retinectomy is usually destroyed for preventing other PVR occurrences and for avoiding anterior neovascularization of the eye stimulated from VEGF coming from the ischemic retinal portion. In these cases, the little retinal specimens were isolated, grasped with nontraumatic instruments (Figure 2), and removed via pars plana through the sclerotomy holes used for entering surgical instruments. The retina sample was placed in RNA later solution (Ambion, Austin, TX, USA) and stored at −20°C until RNA extraction. Surgery continued with the use of perfluorocarbon liquid [20] which allows subsequent drainage of subretinal fluid by a Charles flut-needle and appropriate laser endophotocoagulation [1, 21]. Perfluorocarbon liquid-silicone oil 1000 centistokes exchange was later performed to have the final postoperative stable retina reattachment. Silicone oil was surgically removed after two to three months to reach stable retina reattachment and final visual acuity.

2.3. RNA Isolation and Reverse-Transcription. RNA was isolated using EuroGold Total RNA Mini Kit (Euroclone, Milan, Italy) and quantification of total RNA was performed by NanoDrop ND 1000 spectrophotometer (Thermo Scientific, Wilmington, USA). Total RNA (1 μg) was reverse transcribed

Table 1: Patients' data in PVR surgery.

Patients	Age*	Sex	Refraction (SE)	Visual acuity before surgery*	PVR stage*	Total number of vitreoretinal surgeries*	Visual acuity final outcome**
1	65	F	−1.00	0.4	C2	2	0.4
2	58	M	−1.50	0.2	C2, CA1	2	0.3
3	67	M	−1.00	0.3	C3	2	0.2
4	62	M	+1.75	0.1	C6, CA2	5	0.1
5	62	M	+2.00	0.1	C8, CA3	6	0.05
6	45	M	−7.50	0.02	C7	2	HM
7	80	M	+1.00	HM	C6	4	HM
8	70	F	0	0.02	C5	2	HM
9	72	M	+1.00	0.2	C4	1	0.2
10	51	M	−1.00	HM	C6	4	0.05
11	77	M	0	HM	C7	2	HM
12	57	M	−6.00	HM	C11, CA4	2	LP

SE: spherical equivalent. HM: hand motion. LP: light perception. PVR: proliferative vitreoretinopathy. PVR stage: presence of preretinal or subretinal membranes posterior (C) or anterior (CA) to the equator of the eye, and number of clock hours involved (from 1 to 12). *at the time of retinectomy in PVR surgery. **six months after surgery.

using High Capacity cDNA Kit (Applied Biosystems, Foster City, CA, USA) according to protocol.

2.4. Quantitative Real-Time Reverse Transcriptase PCR. Quantitative real-time reverse transcriptase PCR (qRT-PCR) was performed by TaqMan technology using ABI Prism 7000 apparatus (Applied Biosystems, Foster City, CA, USA). Gene expression analyses were done with TaqMan Assays-on-Demand containing primers and fluorescent probe mix (Applied Biosystems, Foster City, CA, USA). PCR reaction mix contained 12.5 μL of TaqMan Universal PCR Master Mix, no AmpErase UNG (Applied Biosystems, Foster City, CA, USA), 1.25 μL Assays-on-Demand, 3 μL of cDNA, and 8.25 μL of nuclease-free water. Thermocycler program consists of an initial hot start cycle at 50°C for 2 minutes and 90°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and a final cycle at 60°C for 1 minute. For all genes, reactions were performed in triplicate. Negative control consists of PCR mix without cDNA. Human beta-actin gene was used as endogenous control to normalize gene expression levels for relative quantitative analysis through comparative cycle threshold (ΔCt) method. Finally, $\Delta\Delta$Ct method was used to compare gene expressions between human adult healthy retina and human retinal tissues from PVR patients.

2.5. Statistical Analysis. Statistical correlations between gene expressions were calculated with dispersion plots. We correlated two genes for graphic and values were considered significant with a linear regression coefficient (R) > 0.8 [22].

2.6. Immunohistochemistry. Immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded sample of healthy human retina used as control. Three μm sections were mounted on poly-L-lysine-coated slides, deparaffinized and hydrated through graded alcohols to water. Endogenous peroxidase activity was blocked with 3% aqueous hydrogen peroxide for 10 minutes. Antigen retrieval was performed with citrate buffer (10 mM, pH 6.0) inside a 720W domestic microwave oven. The section was incubated overnight at 4°C with rabbit anti-Otx2 polyclonal antibody (Chemicon International, Temecula, CA, cat. no. AB9566) at a dilution of 1 : 2000 and later with Ultravision Detection System Kit (Thermo Scientific, Fremont, CA) according to the manufacture's suggestion. The immunoreaction was developed using 0.03% 3.3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO, USA), and nuclei were counterstained with Harris hematoxylin. The primary antibody was produced using full-length recombinant human Otx2 as immunogen. Due to amino acid sequence homology between the Otx1 and the Otx2 proteins, the antibody recognized both of the proteins. Negative specificity control was performed by omission of primary antibody and substitution with a nonimmune serum with the same dilution.

3. Results

3.1. Gene Expression Analysis in Adult Healthy Human Retina and in PVR Patients. To our knowledge, this is the first time that the expression of Otx genes is found in human adult retina and in PVR tissue. In the control samples, we found basal expression of all genes. In PVR samples, we found little (samples 1, 2, 3, 4, 8, 9, and 10) or no expression (samples 5, 6, 7, 11, and 12) of Otx1, attesting the nondifferentiated state of retinal tissues [23], and variable expressions of VEGF-A, Otx2, Otx3, p53, and p63. We found high Otx3 levels in samples with little levels of Otx2 (samples 3 and 4) (Figure 3). In particular, VEGF-A, Otx2, p53, and p63 genes showed the same expression trend (Figure 4). We found higher levels of VEGF-A, Otx2, p53, and p63 genes inversely to Otx1 and Otx3 expression in PVR samples affected by more severe features of the disease (more severe PVR, more number of surgical

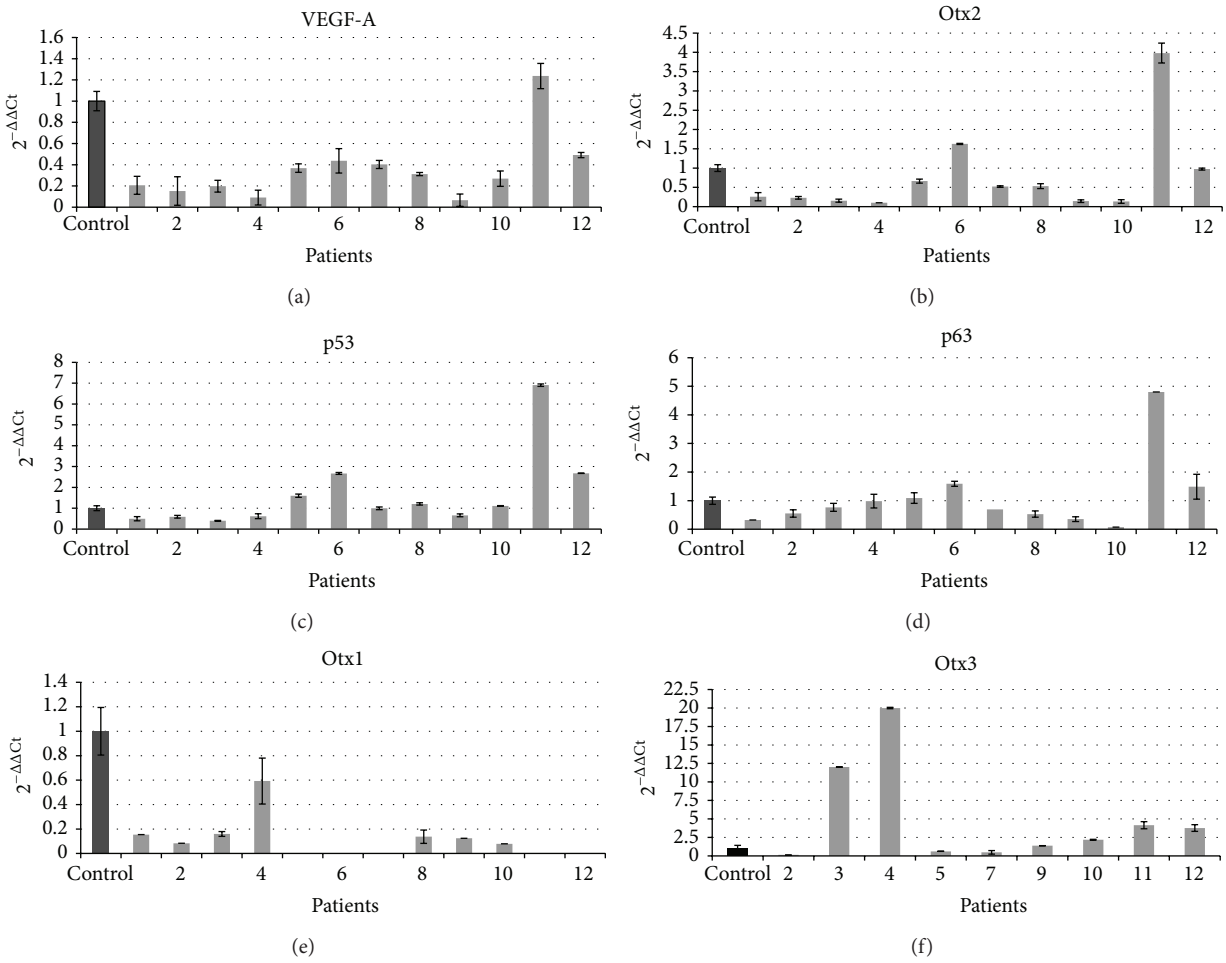

Figure 3: Gene expression levels in adult healthy human samples and in patients affected by PVR. Gene levels were detected by quantitative PCR. $2^{-\Delta\Delta Ct}$ values representing gene expression are shown on y-axis. Black column: adult human samples as controls; gray columns: PVR affected patients. VEGF-A, Otx2, p53, and p63 show the similar expression trend, inversely to Otx1 and Otx3.

operations) (samples 5, 6, 7, 11, and 12). We could not evaluate Otx3 levels in patients 1, 6, and 8 due to the low amount of samples.

3.2. Statistical Analysis. qRT-PCR showed a correlation ($R > 0.8$) among VEGF-A versus Otx2, p53, and p63; Otx2 versus VEGF-A, p53, and p63; p53 versus VEGF-A, Otx2, and p63, and finally p63 versus VEGF-A, Otx2, and p53. Moreover we found a close correlation between Otx1 and Otx3. No statistical relationships were found between Otx1, Otx3, and all other genes (Figure 4).

3.3. Immunohistochemistry. By immunohistochemical analysis we observed positivity for anti-Otx2 antibody in different retinal layers of adult healthy human sample. Photoreceptors (rods and cones), horizontal cells, bipolar and ganglion cells showed positivity for the Anti-Otx2 antibody, on the contrary of Muller cells. We confirmed the presence of Otx proteins in nucleus and cytoplasm of human retina (Figure 5).

4. Discussion

Zebrafish, like many members of the ray-finned fish (teleosts), have the innate capacity to regenerate tissues (e.g., fins, heart, and eye) [24]. In teleost fish, retinal neurogenesis continues in adult life beyond embryogenesis development. In addition, following the destruction of retinal neurons, the retina can regenerate and restore visual function [25]. Following injury, Muller glia cells dedifferentiate into a stem-like state and proliferate to replace lost retinal cells [24]. In the retina of the posthatch chick, the Muller glia demonstrates the ability to dedifferentiate into retinal progenitor, but significant regeneration of retinal neurons does not occur [26]. In contrast to the persistent neurogenesis in the retina of teleosts, neurogenesis in the retina of mammals is completed during pre- and perinatal development [27], and there is as yet no evidence for continual neurogenesis or regeneration in the adult retina.

PVR is a proliferative disease and the knowledge of expressions of some key regulatory genes could be useful to understand the disease and, hopefully, retina regeneration.

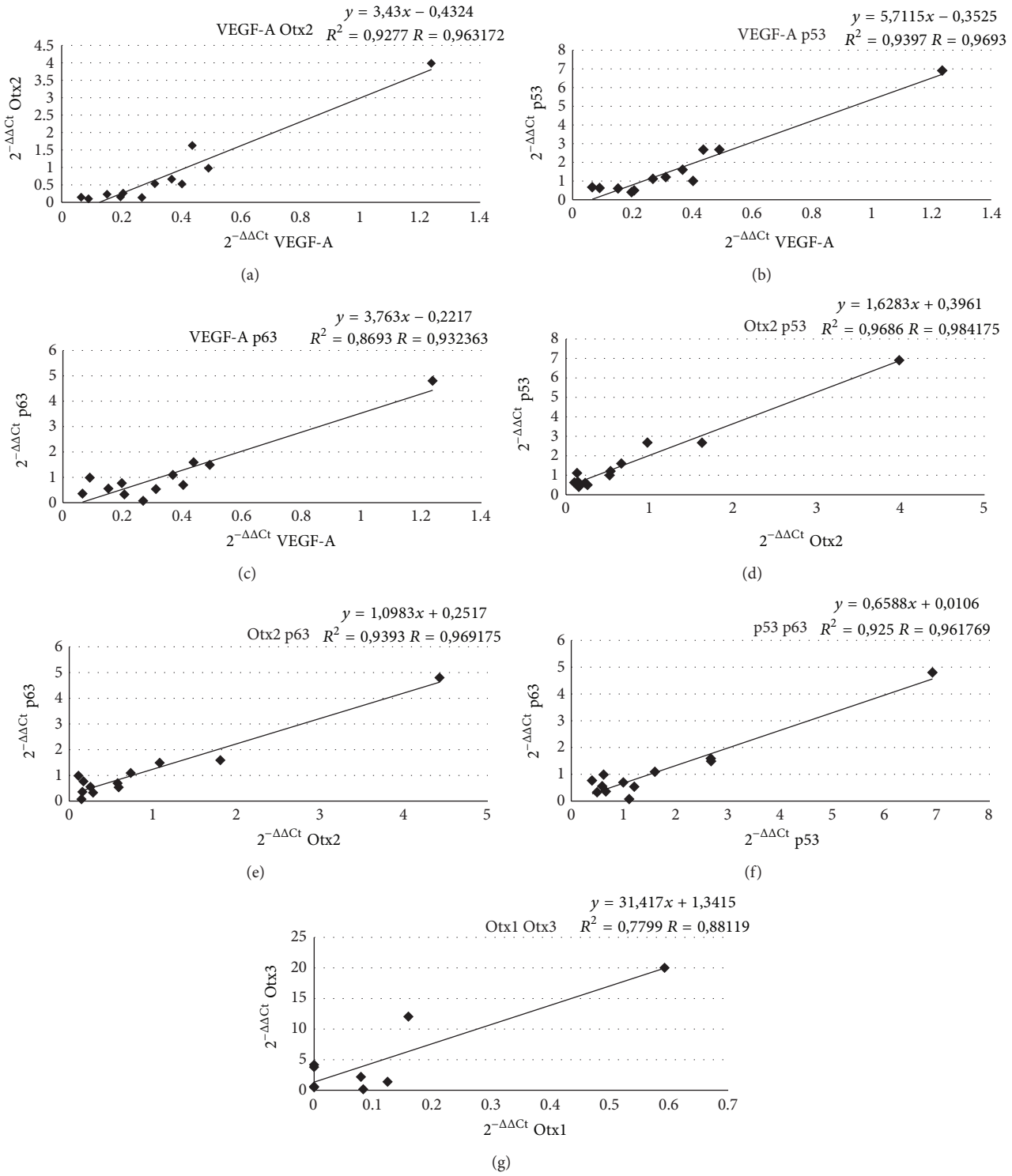

Figure 4: Statistical correlation among VEGF-A, Otx2, p53, and p63 genes and between Otx1 and Otx3 genes. Dispersion plots correlate two genes for graphic. x- and y-axes indicate $2^{-\Delta\Delta Ct}$ values that represent gene expression levels. Linear regression coefficient (R) > 0.80 indicates a statistically significant correlation.

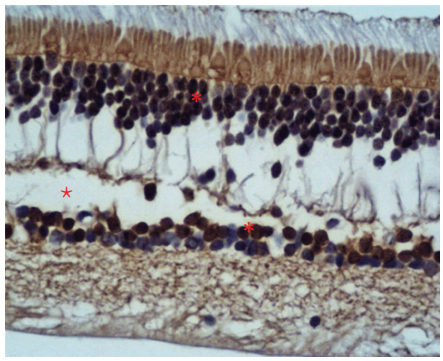

Figure 5: Histological section of healthy adult human retina. The different retinal layers appear marked in dark-marron with rabbit anti-Otx2 polyclonal antibody. Positivity is shown (from top to bottom) in photoreceptors, horizontal, bipolar, and neuronal cells (asterisks). An artifact (empty space) is present due to fixating procedure (star).

The purpose of the present study was to evaluate the expression of VEGF-A, Otx1, Otx2, Otx3, p53, and p63 genes in adult healthy human retina from autopsy and in surgically removed retinae of PVR affected patients.

We confirmed the presence of VEGF-A and p53 family members and, for the first time, we found the expression of Otx genes in controls, also confirmed by immunohistochemical analysis, and in PVR tissue (Figures 3 and 5).

Statistical analysis showed a correlation among VEGF-A, Otx2, p53, and p63 and between Otx1 and Otx3 (Figure 4). These data could individuate two possible pathways involved in the pathogenesis of PVR. Molecular genetic pathways represent a hypothesis or model of how the expression of different genes in a series of biochemical relationships influences each other and eventually leads to a specific phenotypical expression [28].

The two groups of genes showed reverse trends. In fact, patients with higher expressions of VEGF-A, Otx2, p53, and p63 (samples 5, 6, 7, 11, and 12) showed no expression of Otx1 and low levels of Otx3 in comparison to the other samples. On the contrary, patients showing low levels of VEGF-A, Otx2, p53, and p63 genes (samples 3 and 4) had higher levels of Otx1 and Otx3. Thus, Otx1 and Otx3 showed a significant correlation, inversely to what is observed in Otx2 expression. In fact, it is reported that Otx3 significantly suppresses Otx2-induced transcription activity, suggesting that Otx3 functions as a transcription repressor of Otx2 by acting competitively on the consensus TAATCC sequence [15]. Moreover, patients who showed a very severe PVR and underwent an elevated number of surgical procedures (samples 5, 6, 7, 11, and 12) had higher levels of VEGF-A, Otx2, p53, and p63 genes.

In our case series, the functionality of the retinae undergoing genetic study is similar at the time of surgery and at six months followup (results shown in Table 1): this evidence reflects the final outcomes of the literature, as expected, because we performed a standard surgery without new therapies. Aggressive surgery succeeded in stopping PVR development in most of the cases. The cases with more severe PVR and higher levels of VEGF-A, Otx2, p5, and p63 genes showed a worse final functional outcome.

It was demonstrated that p53 can influence VEGF-A expression both increasing and repressing its levels and that, likewise, the different isoforms of p63 have different effect on VEGF-A expression [17]. Further studies are needed to better understand the effective role and correlation among VEGF-A and p53 family members in retina and in retina affected by PVR.

During standard vitreoretinal surgeries using room temperature infusion fluid, the vitrectomy cavity and retina reach deep hypothermia. After closing the infusion line, intraocular tissue rewarms rapidly [29, 30]. These temperature fluctuations could influence many biological functions like bleeding, expression of cytokines in the vitreous, induction of specific patterns of proteins, and neuroprotection. Low temperature causes lower VEGF expression [31]. On the contrary, in our study we found higher VEGF gene expression, highlighting our results.

Immunohistochemical analysis showed the presence of Otx proteins in the internal part of photoreceptors, in horizontal cells, in bipolar cells, and in neuronal cells. Despite the fact that the role of Otx1, Otx2, and Otx3 has been proved during embryo development, little is known about their functions in adult human retina. We hypothesize that Otx2 could have a function of maintenance in the identity and survival of adult retinal differentiated cells, and that its high expression in PVR patients correlates with a reentry of differentiated cells into proliferation cycle and staminal status. Precursor cells, probably in an attempt to regenerate death retina cells, on the contrary could mature in fibroblasts that produce fibrocellular membranes. Interestingly, in very severe PVR cases this process is more stimulated and it is accompanied by higher expression of Otx2.

One of the main targets of genetic studies is to translate evidence and benefits into clinical practice. In our study, we attempted to reach this objective in two different ways. Firstly, our study shows the elevated expression of VEGF-A in PVR patients, and VEGF-A was found to promote the bioactivity of vitreous in patients and rabbits with PVR. In the latter, the anti-VEGF ranibizumab injected intravitreally was found to neutralize PVR [17]. Therefore, in line with the results and the authors' opinion, it can be postulated that intravitreal ranibizumab could be effective in protecting patients from developing PVR. The intravitreal use of ranibizumab should be considered in the presence of PVR predisposing factors (i.e., retinal detachment secondary to trauma, long-lasting intraocular surgical procedures, or visible signs of PVR during standard surgery and during postsurgical followup). Secondly, during retinal detachment surgery, we could perform an intraoperative extemporaneous low-cost examination of retinal gene expression and, if high levels of PVR-related genes are shown, it could be useful to perform a more aggressive surgery instead of a low invasive one. Nowadays, technical and organizational hurdles do not allow performing this procedure in a useful timeframe; the exploitation of the above mentioned technique would be a valid attempt to try in the near future.

5. Conclusions

We found expressions of VEGF-A, p53 family, and, for the first time, Otx homeobox genes in healthy adult human retinae and in retinae affected by PVR. We individuated two possible pathways, VEGF-A, Otx2, p53, and p63 and Otx1 and Otx3 involved in PVR genes pattern that could influence in different manners the course of the pathology. In particular, in many samples of patients with more severe features of PVR, we found higher levels of VEGF-A, Otx2, p53, and p63 genes inversely to Otx1 and Otx3 expression. The anti-VEGF ranibizumab molecule, neutralizing retinal VEGF, could protect retina from PVR.

The immunohistochemical analysis of human healthy retina showed the presence of Otx proteins in many layers of the retina, confirming the hypothesis of their role in acting as a survival factor. In retinae with PVR, the highly expressed levels of Otx2 suggest the role of this gene in the proliferation of retinal stem cells as replacement for dead cells. Further studies are needed to better comprehend the genetic mechanism subtended to PVR exogenesis.

A better understanding of cellular and molecular mechanisms that regulate growth-associated induced neurogenesis in the retina may lead to new approaches for enhancing or controlling the proliferative and regenerative capacity for future therapeutic uses.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Topical Nonsteroidal Anti-Inflammatory Drugs for Macular Edema

Andrea Russo,[1] Ciro Costagliola,[2] Luisa Delcassi,[1] Francesco Parmeggiani,[3] Mario R. Romano,[4] Roberto dell'Omo,[2] and Francesco Semeraro[1]

[1] *Eye Clinic, Department of Neurological and Vision Sciences, University of Brescia, Piazzale Spedale Civili 1, 25123 Brescia, Italy*
[2] *Eye Clinic, Department of Health Sciences, University of Molise, Via de Santis, 86100 Campobasso, Italy*
[3] *Department of Ophthalmology, University of Ferrara, Corso Giovecca 203, 44121 Ferrara, Italy*
[4] *Eye Clinic, Istituto Clinico Humanitas, Via Manzoni 56, 20089 Milano, Italy*

Correspondence should be addressed to Andrea Russo; dott.andrea.russo@gmail.com

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are nowadays widely used in ophthalmology to reduce eye inflammation, pain, and cystoid macular edema associated with cataract surgery. Recently, new topical NSAIDs have been approved for topical ophthalmic use, allowing for greater drug penetration into the vitreous. Hence, new therapeutic effects can be achieved, such as reduction of exudation secondary to age-related macular degeneration or diabetic maculopathy. We provide an updated review on the clinical use of NSAIDs for retinal diseases, with a focus on the potential future applications.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed classes of medication, and they are routinely employed for their analgesic, antipyretic, and antiinflammatory properties. Because they are potent inhibitors of cyclooxygenase (COX) enzymes, they reduce the synthesis of pro-inflammatory prostaglandins (PGs). NSAIDs have been widely used systemically for many decades and have more recently become available in the form of topical ophthalmic formulations [1]. In ophthalmology, topical NSAIDs are mostly used to stabilize pupillary dilation during intraocular surgery, to control postoperative pain and inflammation (particularly after refractive surgery), and to treat allergic conjunctivitis and pseudophakic cystoid macular edema (CME) [2, 3]. A growing body of evidence suggests that NSAIDs may also be beneficial in diabetic retinopathy (DR), ocular tumors, and age-related macular degeneration [1, 4–8]. This review focuses on the potential application of NSAIDs to treat retinal disease.

2. NSAIDs and Cyclooxygenases

COX enzymes are an active component of the inflammatory process. They catalyze the biosynthesis of eicosanoids from arachidonic acid to produce 5 classes of PGs: PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 [1]. Ocular actions of PGs are manifested in three ways [9]. Firstly, they act on intraocular pressure (IOP). PGE_2 increases the IOP by local vasodilation and increased permeability of blood aqueous barrier. On the other hand $PGF_{2\alpha}$ lowers the IOP, which is attributed to increased uveoscleral outflow. Secondly it acts on iris smooth muscle to cause miosis. Thirdly, PGs cause vasodilation and increase the vascular permeability with the disruption of the blood-ocular barrier with leukocyte migration and therefore edema formation [10]. By definition, NSAIDs lack a steroid nucleus.

COX-1 and COX-2 are the main COX isoforms, although there is a third isoform, COX-3. COX-3 is an acetaminophen-sensitive alternatively spliced variant of COX-1, and it has not been well defined [11–13]. COX-1 regulates normal physiological processes and is mainly expressed in the gastrointestinal

Table 1: Commercially available topical NSAIDs.

Molecule	Class	Administration
Indomethacin 0.5%	Indole acetic acid derivative	TID, QID
Ketorolac tromethamine 0.5%	Aryl acetic acid derivative	TID, QID
Bromfenac 0.09%	Aryl acetic acid derivative	BID
Nepafenac 0.1% (prodrug converted to amfenac)	Aryl acetic acid derivative	TID
Diclofenac 0.1%	Aryl acetic acid derivative	QID
Flurbiprofen 0.03%	Aryl propionic acid derivatives	QID
Pranoprofen 0.1%	Aryl propionic acid derivatives	TID
Piroxicam 0.5%	Enolic acid derivatives	TID, QID

BID: 2 times a day; TID: 3 times a day; QID: 4 times a day.

tract, kidneys, platelets, and vascular endothelium. COX-2 is the predominant isoform in the retinal pigment epithelium (RPE) [14] and is upregulated during inflammatory processes, pain, and fever, but it is also expressed under normal conditions in sites such as the brain and kidneys [15]. COX-2 has also been found in choroidal neovascularization (CNV) and in DR [4, 5, 7, 8, 16–19]. PGs act by upregulating a number of soluble mediators responsible for the expression of vascular endothelial growth factor (VEGF), which plays a key role in the CNV and in the DR [20–22]. In a number of experimental models COX-2 inhibition has been found to inhibit angiogenesis [23–26], CNV, and DR [17, 18, 27, 28].

Commercially Available Formulations. NSAIDs are a chemically heterogeneous group of molecules, described in detail elsewhere [29]. There are six major classes: salicylates, indole acetic acid derivatives, enolic acid derivatives, fenamates, aryl acetic acid derivatives, and aryl propionic acid derivatives. However, the topical NSAIDs available for ophthalmic usage are mostly limited to the soluble forms: indole acetic, aryl acetic, and aryl propionic acid derivatives [9, 16]. A list of commercially available NSAID eyedrops is provided in Table 1.

Most of the NSAIDs are weakly acidic drugs, which ionize at the pH of the lacrimal fluid and therefore have limited permeability through the anionic cornea which has an isoelectric point (pI) of 3.2 [9]. Reducing the pH of the formulation increases the unionized fraction of the drug which enhances permeation. Because of their acidic nature, NSAIDs are inherently irritating [30]; reducing the pH further increases their irritability and decreases their aqueous solubility. In addition, the anionic nature of NSAIDs leads to the formation of insoluble complexes with cationic quaternary ammonium preservatives, such as benzalkonium chloride [31]. Hence, a NSAID formulation that is comfortable when topically applied is somewhat difficult to formulate.

3. Pharmacokinetics and Pharmacodynamics

NSAIDs are adsorbed by the gastrointestinal tract, reaching a peak serum concentration after 1–3 hours. They are metabolized by the liver and excreted in the urine and bile; they are highly protein bound in the plasma (>95%), normally to albumin; thus their volume of distribution approaches that of plasma [16]. Topically administered NSAIDs follow this distribution, since they are systemically adsorbed by the nasolacrimal outflow system and the mucosal surfaces.

Nepafenac is a prodrug that is rapidly converted to the more potent amfenac by intraocular hydrolases. Since nepafenac is a noncharged molecule, it exhibits greater corneal permeability than the other NSAIDs. This was demonstrated in an *in vitro* study that showed sixfold greater corneal penetration by nepafenac than by diclofenac [34]. Bromfenac has a similar structure to amfenac, with the exception of a bromine atom at the C_4 position. This modification increases the penetration of bromfenac into ocular tissues, increasing its anti-inflammatory activity.

Ketorolac is reportedly the most potent inhibitor of COX-1, while bromfenac and nepafenac/amfenac are the most potent inhibitors of COX-2 [9, 35, 36]. However, ketorolac 0.45% inhibited PGE_2 more strongly than bromfenac 0.09% and nepafenac 0.1%, reaching significantly greater aqueous concentrations [37, 38]. Bromfenac has been reported to be a 3- to 18-fold more potent inhibitor of COX-2 than diclofenac, ketorolac, and nepafenac/amfenac, although these data remain to be confirmed in randomized controlled clinical trials [1, 9, 39]. It is possible that COX-1 may also play a role in inflammation [1, 16] therefore the specific roles of COX-1 and COX-2 in this context require further investigation.

A number of studies have measured intraocular NSAID levels after topical administration. After a single eye-drop, peak aqueous drug levels are detectable for diclofenac 0.1% (82 ng/mL; 2.4 h peak), flurbiprofen 0.03% (60 ng/mL; 2.0 h peak), nepafenac 0.1% (205.3 ng/mL; 30 min peak), amfenac (following administration of the prodrug nepafenac 0.1%; 70.1 ng/mL), ketorolac 0.4% (57.5 ng/mL; 60 min peak), and bromfenac 0.09% (25.9 ng/mL) [35, 40].

More prolonged and more frequent administration of NSAIDs leads to higher aqueous levels. Twelve doses of ketorolac 0.4% over 2 days reportedly result in an aqueous level of 1079 ng/mL, and the same dosing regimen of nepafenac 0.1% results in 353 ng/mL; both concentrations far exceed that is reportedly required to inhibit COX-1 and COX-2, which is 50 ng/mL [41].

While topical administration of NSAIDs achieves therapeutic levels in the aqueous humor, thereby reducing the synthesis of PGs in the ciliary body and the iris, such a therapeutic effect is less evident in the retina and the choroid. Few studies have measured NSAID levels in the human vitreous cavity after topical administration. Heier et al. [42]. measured vitreous drug levels in patients who received ketorolac 0.4% QID, bromfenac 0.09% BID, or nepafenac 0.1% TID for 3 days before vitrectomy. Vitreous levels of ketorolac, bromfenac, and amfenac were reportedly 2.8 ng/mL, 0.96 ng/mL, and 2.0 ng/mL, respectively, but only

ketorolac resulted in significantly lower vitreous PGE_2 levels compared to placebo.

NSAIDs inhibit the expression of COX enzymes, thereby reducing the endogenous PGs that act on the iris and ciliary body to induce vasodilation, blood-ocular barrier disruption, leukocyte migration, pain stimulation, IOP control, and miosis [2, 3, 16, 43]. Commercially available $PGF_{2\alpha}$ analogues act by increasing uveoscleral outflow in the ciliary body, while PGE_2 reportedly increases IOP via vasodilation and partial disruption of the blood-ocular barrier [43]. The administration of topical NSAIDs does not have any effect on IOP, as it is not selective with regard to PG class. However, NSAIDs may have a slight additive effect when administered together with $PGF_{2\alpha}$ analogous [44, 45]. A pivotal difference between NSAIDs and corticosteroids is the effect of the latter on both IOP and lipoxygenase, which facilitates a greater anti-inflammatory effect, albeit with an associated increase in the likelihood of IOP elevation.

4. Macular Edema after Cataract Surgery

There is convincing clinical evidence in the peer-reviewed literature attesting to the capacity of topical NSAIDs to reduce postoperative inflammation after eye surgery [1, 2, 16, 46]. In randomized controlled clinical trials, bromfenac 0.09%, nepafenac 0.1%, diclofenac 0.1%, ketorolac 0.5%, flurbiprofen 0.03%, and indomethacin 1% have been shown to decrease postoperative inflammation following cataract surgery [34, 41, 47–58]. Corticosteroids are also widely used postoperatively to reduce inflammation; therefore studies comparing the 2 drug classes have been conducted. While significant differences in the reduction of intraocular inflammation after cataract surgery were not observed [55, 56, 59], NSAIDs were more effective at reestablishing the blood-aqueous barrier as indicated by flare, which was measured via either slit-lamp examination or fluorophotometry [16, 46, 52, 59]. Thus, the collective evidence suggests that topical NSAIDs may be used in place of topical corticosteroids after cataract surgery or, perhaps preferably, in addition to them; a number of clinical trials have reported a synergistic effect when NSAIDs and corticosteroids are administered together [49, 50, 58, 60, 61].

Despite advances in technique and surgical materials, cystoid macular edema (CME) is the most frequent cause of reduced vision following uneventful modern cataract surgery, with a seemingly rare incidence of 0.1–2.35% for clinically significant CME [62–64]. Also known as Irvine-Gass syndrome, it is mainly caused by the accumulation of extracellular fluid within the retina due to leakage from dilated capillaries [1, 16, 63]. The pathogenesis of it is not fully understood, but the main trigger is thought to be surgical trauma of the intraocular tissues, involving rupture of the blood-aqueous barrier; this may cause diffusion of PGs and other inflammatory mediators into the vitreous cavity, inducing a cascade of inflammatory events with subsequent rupture of the blood-retinal barrier, resulting in CME in some patients [64]. Therefore it seems reasonable to take strong measures to minimize the inflammatory process, possibly including the administration of both corticosteroids and NSAIDs together. A recent study by Ersoy et al. [65] that quantitatively assessed aqueous flare after phacoemulsification reported that patients who developed CME had significantly higher flare values than those who did not, suggesting that inflammatory pathogenesis and a breakdown of the blood-ocular barrier may be involved.

CME can be diagnosed and classified clinically, on fluorescein angiography and by optical coherence tomography (OCT). The range of the reported incidence rates is wide (0.10–2.35% for clinically important CME, defined as a retinal thickening within 500 microns of the center of the macula causing a significant vision impairment) [62, 64], which may be due to the different patient populations, cataract stages, surgical techniques, and, particularly, diagnostic methods utilized by the relevant studies. Notably, after small-incision cataract surgery the reported rates of CME range from 9 to 19% based on fluorescein angiography and are as high as 41% as determined by OCT [66–68], although clinically important CME is far less common [1, 69].

A number of studies report the effectiveness of topical NSAIDs in the prophylaxis of CME following cataract surgery [2, 16, 63, 70–74], although the angiographic reduction of CME is reportedly most evident in the first postoperative month and is no longer statistically significant a year after surgery. However, interpretation of the independent effects of NSAIDs based on the results of the available studies is difficult, due to the common concomitant administration of corticosteroids. One trial by Flach et al. [73] reported that prophylactic use of ketorolac 0.5% was effective in reducing CME without the use of corticosteroids. Miyake et al. [75] prospectively compared the effects of topical diclofenac 0.1% versus fluorometholone 0.1% (a corticosteroid with limited intraocular penetration that therefore could be reasonably approximated to a placebo) in the prophylaxis of CME and reported that 5 weeks after surgery, angiographic CME was present in 5.7% of diclofenac-treated eyes and 54.7% of fluorometholone-treated eyes.

A randomized comparison of topical ketorolac 0.4% plus corticosteroid versus corticosteroid alone showed a significantly reduced rate of CME with combination treatment after phacoemulsification [58]. However, the incidence of definite or probable CME (definite CME is intended as the presence of cystoid changes associated with $\geq 40\ \mu$m retinal thickening evident on OCT, while probable CME is intended as the presence of changes in retinal contour and increased macular thickness relative to preoperative baseline, but without definite cystoid changes) was low in both groups (2.4% in the corticosteroid group and 0% in the ketorolac/corticosteroid group) and there was no difference in visual outcomes. Such results raise the issue of the cost effectiveness of routine administration of CME prophylactic treatment with both corticosteroid and NSAIDs for patients at low risk of CME. However, cost effectiveness ratio is certainly lower in diabetic and uveitic patients who are at higher risk of CME and are reported to benefit from routine concomitant use of NSAIDs and corticosteroids [76].

CME following phacoemulsification may be treated early (less than 6 months) or late (6 months or more) following its diagnosis, respectively, defining acute and chronic CME

[1, 16]. The treatment of chronic CME following cataract surgery has been assessed in a number of studies [16, 77–80] which have shown an overall beneficial effect of NSAID treatment, although a recent meta-analysis [81] reported that for acute CME, the evidence is not yet conclusive. This finding is consistent with a recent study by Almeida et al. [82]. assessing the efficacy of ketorolac and nepafenac with regard to the prevention of postoperative CME after uneventful phacoemulsification. The authors concluded that prophylactic topical NSAIDs are not recommended for routine surgery patients without risk factors.

In chronic disease, trials by Flach et al. [63, 79, 80] suggest that topical ketorolac 0.5% is effective and that treatment for a duration of 3 months provides a longer lasting benefit than treatment for 2 months. However, there are few published trials and they tend to have small sample sizes; therefore, further controlled studies are required.

Four topical NSAIDs (diclofenac 0.1%, ketorolac 0.4%, nepafenac 0.1%, and bromfenac 0.09%) have been evaluated in combination with intravitreal corticosteroid and bevacizumab injections for the treatment of chronic pseudophakic CME [83]. Results suggested that while NSAIDs apparently provide additional benefit to that produced by corticosteroids and anti-VEGF, only nepafenac- and bromfenac-treated eyes showed reduced retinal thickness at 12 and 16 weeks, and only nepafenac led to a significant improvement in vision. Similarly, in a retrospective and uncontrolled study, nepafenac 0.1% improved retinal thickness and visual acuity in patients with chronic CME [84].

In all reported studies NSAIDs are administered using the traditional BID, TID, and QID regimens. Further studies are needed to enlighten if varying the dosing regimen affects the efficacy of NSAIDs in CME resolution.

In conclusion, although there is no specific approved treatment for pseudophakic CME, overall evidence supports the administration of topical NSAIDs and also suggests that combining them with topical corticosteroids yields a synergistic effect. However, the advisability of NSAIDs for CME prophylaxis in patients with low risk factors is debatable, given the low clinically significant incidence and the cost effectiveness ratio.

5. Age-Related Macular Degeneration

In developed countries, age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in patients over 60 years of age [85]. Typical features of neovascular AMD include choroidal neovascularization (CNV) beneath the macula, with associated retinal hemorrhages and swelling. Involution of the new vessels is accompanied by fibrous metaplasia, permanent loss of photoreceptors, and disciform scarring, which often result in loss of central vision [86]. Large-scale clinical trials have demonstrated that monthly intravitreal injection of anti-VEGF prevents vision loss and may even improve visual acuity in patients with neovascular AMD [87, 88].

VEGF is not the sole cause of CNV. Inflammation plays an important role and some patients exhibit an inadequate response to anti-VEGF treatment, along with persistent exudation [89]. In particular, a multitude of recent genetic analyses in human AMD patients supports the role of complement factor H in the pathogenesis of it in up to 50% of cases [90–93].

The complement system is a major contributor to innate immunity. There are several complement components (C3, C5, the C5b-9 membrane attack complex (MAC), and CD46) found in drusen. This indicates that complement components and regulators may contribute to the formation of drusen and upregulate VEGF expression [94–96]. Although AMD is not a classic inflammatory disease, inflammatory cells have an important role in AMD pathogenesis and progression [94, 97]. Autoimmunity has also been suggested to have a role in drusen formation and AMD pathogenesis. It has been suggested that the presence of a number of antiretinal autoantibodies such as anticarboxyethylpyrrole and antiastrocyte antibodies is an early feature of AMD pathogenesis [98, 99]. Recently, Morohoshi et al. [100] demonstrated that 94% of patients with early-stage AMD and 83% of patients with late-stage AMD had elevated levels of serum retinal autoantibodies, compared with only 9% of normal controls.

NSAIDs may have a protective effect with regard to Alzheimer's disease, reducing its prevalence [101, 102], and similarly a prospectively followed group of patients under long-term anti-inflammatory treatment for rheumatoid arthritis showed a very low prevalence of AMD [103]. Moreover, a larger retrospective study reported reduced rates of CNV among AMD patients undergoing aspirin treatment [104]. Even the anecdotal use of loxoprofen sodium for toe cellulitis has been reported to improve CNV [105]. However, a more recent Australian population-based study reported that regular aspirin use is associated with increased risk of incident neovascular AMD [106]. This is consistent with a report emerging from the European Eye Study [107] that frequent aspirin use is associated with early AMD and late wet AMD and the odds ratio rises with increasing frequency of consumption. Nevertheless, evidence supports the additive role of NSAIDs in the treatment of CNV, with a protective effect that is probably due to the control of both inflammation, and COX-2 which is a known promoter of angiogenesis and can be found in CNV [8, 19, 24, 108]. Pharmacological inhibition of COX seems to reduce VEGF expression in cultured human RPE cells [8, 109]. Kim et al. [17, 18] have demonstrated that both topical and intravitreal ketorolac significantly reduce angiographic leakage and retinal levels of PGE_2 and VEGF in an animal model of CNV.

Therefore, the addition of an anti-inflammatory agent could be a valid option for controlling CNV, as simply inhibiting VEGF addresses neither the multifactorial pathogenesis of CNV nor the underlying cause of VEGF production.

Although the evidence coming from human clinical trials is less consistent than that arising from animal models, a favorable effect of additive topical NSAID therapy with regard to anti-VEGF for the control of exudative AMD has recently been reported in 3 prospective, randomized, and controlled clinical studies (Table 2) [4, 5, 7].

Russo et al. [7] demonstrated that topical ketorolac acts in conjunction with intravitreal anti-VEGF treatment; central

Table 2: Studies investigating NSAIDs in combination with anti-VEGF.

Study	Design, sample size, and study duration	NSAID	Treatment arms	Results	Author conclusions
Russo et al. (2013) [7]	Randomized, prospective, controlled, 56 eyes 6 months	Ketorolac 0.45% TID	Ketorolac plus IVR versus IVR alone for new exudative AMD	37.1 μm greater CMT reduction at 6 months in ketorolac arm. No differences for VA or no. of injections	Topical ketorolac supplements the activity of intravitreal ranibizumab in reducing CMT in CNV
Gomi et al. (2012) [5]	Randomized, prospective, placebo-controlled, 30 eyes 6 months	Bromfenac 0.1% BID	Bromfenac plus IVR versus IVR alone for exudative AMD	Reduced CMT and fewer injections in bromfenac group, but similar VA	Bromfenac may reduce the frequency of IVR over 6 months in eyes with small CNV
Flaxel et al. (2012) [4]	Randomized, prospective, controlled, 30 eyes 12 months	Bromfenac 0.09% BID	Bromfenac plus IVR versus IVR alone for exudative AMD	63.3 μm greater CMT reduction at 12 months in bromfenac arm. No differences for VA or no. of injections	Combination is efficacious for the treatment of exudative AMD
Chen et al. (2010) [32]	Retrospective, uncontrolled 25 eyes 3 months	Nepafenac 0.1% TID	Nepafenac plus IVR/IVB for recalcitrant exudative AMD	No changes in VA or CMT	No significant changes in VA or CMR, but a mild trend towards improved anatomy
Zweifel et al. (2009) [33]	Retrospective, uncontrolled 22 eyes 2 months	Bromfenac 0.09% BID	Bromfenac plus IVR/IVB for persistent exudative AMD	No changes in VA or CMT	No beneficial effect of adding bromfenac for persistent exudative AMD

AMD: age-related macular degeneration; CNV: choroidal neovascularization; VA: visual acuity; CMT: central macular thickness; IVR: intravitreal ranibizumab; IVB: intravitreal bevacizumab.

macular thickness (CMT) is significantly lower (−37.1 μm) after 6 months in patients receiving combination therapy, although there were no differences in either visual acuity or the number of injections between the 2 groups. Such results are partially in contrast with the findings of Gomi et al. [5], in which the authors also reported a reduction in the frequency of ranibizumab injections over 6 months when topical bromfenac was used as an adjunctive treatment with ranibizumab. However, in addition to the differences in the pharmacological properties of bromfenac and ketorolac, another point of difference was that Gomi et al. [5] administered just one ranibizumab injection and then treated the patients on an as-needed basis; therefore the number of injections administered was not consistent. Similar results were reported in another recently published trial [4] evaluating the use of topical bromfenac in combination with ranibizumab versus ranibizumab alone. A significantly greater reduction in CMT was found after 12 months in the combination group (−28.3% versus −18.9%), without concomitant differences in visual acuity changes between the 2 arms [4].

Such findings are in contrast with 2 previous retrospective studies [32, 33] that did not detect any improvement in visual acuity or in CMT, with the addition of bromfenac or nepafenac in conjunction with anti-VEGF administration. However, these inconsistent results may be due to differences in study design (shorter retrospective design and smaller sample sizes) and the presence of recalcitrant and persistent exudative AMD in the examined cohorts, which render direct comparisons problematic.

Overall the literature supports the concomitant off-label administration of topical NSAIDs with on-label anti-VEGF intravitreal therapy, as NSAIDs act synergistically to reduce CMT in CNV. It will be important to evaluate the long-term efficacy of NSAIDs, as AMD is a chronic disorder. In particular, careful attention should be paid to the corneal complications associated with long-term use of topical NSAIDs.

6. Diabetic Retinopathy

DR is the most frequent cause of legal blindness in working-age individuals in developed countries [110]. In addition to DR, diabetic patients can suffer from diabetic macular edema (DME), which is caused by breakdown of the blood-retinal barrier resulting in leakage of plasma and water from small vessels [111]. These leakages result in swelling and thickening of the retina around the macula, the central part of the retina in which fine visual discrimination occurs. In patients with type 2 diabetes, DME is the primary cause of moderate and legal blindness [112].

Growing scientific evidence shows that an immunological cascade has a major role in the pathogenesis of DR [113]. Increased levels of inflammation mediators and PGs in DR have been found in the vitreous cavity in both animal and human studies [22, 114, 115], and the level of PGE_2 correlates significantly with vitreous levels of VEGF [116]. The role of inflammation in the progression of DR has also

been indirectly supported in a recent study [117] by the Diabetic Retinopathy Clinical Research Network, in which authors concluded that intravitreal triamcinolone appears to be associated with a reduced risk of worsening of proliferative DR.

In animal models PGs stimulate VEGF expression [17], and in cultured Muller cells agonism and antagonism of the PGE_2 receptor increase and decreases VEGF production, respectively, in a dose-dependent manner [118]. In fact, NSAID treatments have been shown to prevent or delay DR progression in animal models [21, 27, 28, 119].

While no benefit was found in advanced DR in the Early Treatment Diabetic Retinopathy Study [120] examining the effect of 650 mg aspirin, the incidence of DR is reduced in human patients taking salicylates for rheumatoid arthritis [121], just as previously reported with exudative AMD, attesting to the contribution of COX to the development of DR. Such findings were confirmed in the Dipyridamole Aspirin Microangiopathy Diabetes Study (DAMAD) [122] that assessed the effect of 990 mg aspirin in early DR; a significant protective effect was associated with high doses of aspirin, which slowed the development of retinal microaneurisms. Subsequently, either 2 prospective randomized studies confirmed these findings with the administration of sulindac and celecoxib [123, 124].

The benefits of topical NSAID therapy for DR control are mainly reported anecdotally or in uncontrolled retrospective case studies. Pseudophakic DME showed improvement in retinal thickness and visual acuity after treatment with nepafenac 0.1% for 6 months in a case report [84]. Similarly, in a case series of 6 eyes with DME that were treated with nepafenac 0.1%, the average foveal thickness decreased significantly from 417 μm to 267 μm after a mean of 178 days. Authors moreover reported that four eyes gained vision and two eyes maintained vision, with a statistically significant mean visual acuity improvement from 0.78 logMAR to 0.67 logMAR [125]. Such results suggest that nepafenac 0.1% may exhibit activity against diabetic macular edema and warrant further investigation in larger, controlled studies, possibly with and without associated anti-VEGF therapy. In this regard a placebo-controlled study to assess the effect of nepafenac 0.1% on macular retinal volume in eyes with noncentral DME is being conducted (ClinicalTrials.gov Identifier: NCT01331005).

The intravitreal route is a privileged route for the delivery of drugs to the posterior eye, and it has been proposed as the route of administration for NSAIDs to treat DME. Evidence emerging from published case reports collectively suggests an increase in visual acuity without significant changes in the CMT. Soheilian et al. [126] evaluated the effect of a single dose of intravitreal diclofenac (500 μg/0.1 mL) on 5 eyes with clinically significant diabetic macular edema and reported prominent improvements in visual acuity with no significant decrease in CMT. A similar result was reported by do Ceu Afonso Reis et al. [127] in a study involving 20 patients with DME refractory to retinal photocoagulation, who were treated with intravitreal ketorolac (500 μg/0.1 mL) in one eye only. These findings are consistent with a study by Maldonado et al. [128] who treated 25 patients with ketorolac at a dose of 3000 μg. On the other hand, Elbendary and Shahin [129] randomized 32 eyes in a 1 : 1 ratio to treatment with either 500 μg/0.1 mL of diclofenac or 4 mg/0.1 mL of triamcinolone and reported a significant reduction in CMT with both treatments, but improvements in visual acuity were only evident in the triamcinolone group.

7. Conclusion

The initial pathological changes in macular edema appear in macular photoreceptors, RPE, Bruch's membrane, and choriocapillaris [97]. While their etiology is not fully understood, it is incontrovertible that inflammation has a critical role in the various manifestations of macular edema and its progression. The fact that inflammation is a common denominator in pseudophakic, exudative AMD and diabetic macular edema may explain why anti-inflammatory agents are beneficial as preventive or adjunctive therapies.

Considering our growing understanding of the underlying role of PGs, complement, and inflammation in eye diseases, the clinical use of topical NSAIDs will likely continue to expand. The newer and more potent topical formulations emerging are also likely to contribute to this expansion.

In summary, topical NSAIDs could be used alone for pseudophakic CME or as a favorable adjunct together with anti-VEGF for exudative AMD. Cost effectiveness ratio must be considered given the low incidence of pseudophakic CME in low-risk patients; however, the heavy economic burden of anti-VEGF injections that could potentially be reduced if future studies support the use of NSAIDs should also be considered.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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Review Article

Proliferative Vitreoretinopathy after Eye Injuries: An Overexpression of Growth Factors and Cytokines Leading to a Retinal Keloid

Francesco Morescalchi,[1] Sarah Duse,[1] Elena Gambicorti,[1] Mario R. Romano,[2,3] Ciro Costagliola,[2] and Francesco Semeraro[1]

[1] *Ophthalmology Clinic, Spedali Civili di Brescia, Department of Medical and Surgical Specialties, Radiological Specialties and Public Health, University of Brescia, 1 Piazzale Spedali Civili, Brescia 25123, Italy*
[2] *Ophthalmology Clinic, Department of Health Science, University of Molise, Campobasso 86100, Italy*
[3] *Ophthalmology Clinic, Istituto Clinico e di Ricerca Humanitas, Rozzano 86100, Milan, Italy*

Correspondence should be addressed to Sarah Duse; sarah.duse@alice.it

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Eye injury is a significant disabling worldwide health problem. Proliferative Vitreoretinopathy (PVR) is a common complication that develops in up to 40–60% of patients with an open-globe injury. Our knowledge about the pathogenesis of PVR has improved in the last decades. It seems that the introduction of immune cells into the vitreous, like in penetrating ocular trauma, triggers the production of growth factors and cytokines that come in contact with intra-retinal cells, like Müller cells and RPE cells. Growth factors and cytokines drive the cellular responses leading to PVR's development. Knowledge of the pathobiological and pathophysiological mechanisms involved in posttraumatic PVR is increasing the possibilities of management, and it is hoped that in the future our treatment strategies will evolve, in particular adopting a multidrug approach, and become even more effective in vision recovery. This paper reviews the current literature and clinical trial data on the pathogenesis of PVR and its correlation with ocular trauma and describes the biochemical/molecular events that will be fundamental for the development of novel treatment strategies. This literature review included PubMed articles published from 1979 through 2013. Only studies written in English were included.

1. Introduction

Eye injury is a significant health problem worldwide that often results in disability; the National Research Council reported eye injury as the most underrecognized major health problem affecting those living in industrialized countries. There are approximately 203,000 cases of open-globe injury each year [1]. Such ocular trauma is the major cause of vision loss in young adults and children [2].

Up to 14% of ocular traumatic injuries result in severe vision loss or permanent blindness. It has been estimated that up to 19 million people are unilaterally blind as a result of ocular trauma. The high incidence of ocular trauma has extensive socioeconomic costs [2, 3]. Trauma can involve open- or closed-globe injuries, due to damage from sharp or blunt objects. Open injuries are classified in 4 subgroups on the basis of the type of trauma: rupture, penetration, perforation, and intraocular foreign body (IOFB). Closed-globe injuries are divided into 2 subgroups: contusion and laceration [4, 5].

Penetrating trauma is the most common cause of ocular morbidity; it is estimated that as many as 40% of globe penetration injuries are associated with retained IOFB [6–9]. The risk of visual loss is increased if the force that caused a closed-globe injury was sufficient to rupture the globe. Retinal detachment (RD) is a frequent sequel of severe ocular trauma, and RD often leads to proliferative vitreoretinopathy (PVR) [10, 11]. PVR is a complex cellular

process characterized by the proliferation of membranes on or beneath the retina, intraretinal degeneration, gliosis, and contraction [12, 13]. By a mechanism not yet fully understood, excessive inflammation interferes with physiologic wound healing. This gives rise to an abnormal, protracted course of wound healing. Contraction of these proliferative membranes over the ultraspecialized tissue of the retina has disastrous consequences for vision.

PVR develops as a relatively rare complication in about 8–10% of patients with primary retinal detachment. The condition is much more frequent after trauma, occurring in 40–60% of patients with open-globe injury [14]. The frequency of PVR following perforation, rupture, penetration, persistence of an intraocular foreign body, and contusion is estimated to be 43%, 21%, 15%, 11%, and 1%, respectively [15].

The high incidence of PVR after ocular trauma is thought to be due to the inflammatory reaction that follows injury, which may have involved the direct introduction of cells from outside the eye. Those eyes that develop PVR after a trauma have worse visual outcomes, with PVR considered as the primary reason for the loss of vision [16].

In this review, we have summarized current knowledge on the pathogenesis of PVR and its correlation with ocular trauma and discussed how a fundamental understanding of the biochemical/molecular events involved is instrumental in developing novel treatment strategies.

2. Etiopathogenesis of PVR

Trauma to the retina gives rise to inflammation, which involves breakdown of the blood-retinal barrier (BRB). This process allows the body to heal and repair any tissue damage. Physiologic ocular wound healing involves inflammation, scar proliferation and modulation, tissue remodeling, and restoration of retinal integrity. This healing process includes the chemotaxis of inflammatory cells such as macrophages, lymphocytes, and polymorphonuclear cells and rarely evolves to PVR. However, when certain pathological events occur simultaneously, the stimulus to protracted wound healing triggers PVR. The most important of such events are retinal break, RD, and intravitreal hemorrhage.

A retinal break is likely necessary for PVR; protracted exudative RD and hemivitreal detachments without holes are insufficient to trigger PVR [17]. The formation of a retinal break exposes the RPE to the vitreous cavity and its components, which leads to RD. The dimensions of the retinal break are directly and strongly correlated to the probability of PVR; giant retinal tears (width > 1 quadrant) are almost invariably followed by PVR. Rhegmatogenous RD occurs when the tractional forces of the vitreous on the retinal tear permit the fluid from the vitreous humor to enter the subretinal space (SRS). Vitreous fluid contains a large amount of cytokines and growth factors that stimulate the activation and the proliferation RPE and retinal glial cells [12, 18].

Once the retina has separated from the RPE, the increased distance to the choroidal blood supply and the reduced oxygen flux from the choriocapillaris to the photoreceptors lead to hypoxia. The resulting ischemia further compromises the BRB. Photoreceptors consume almost 100% of the oxygen provided to the retina by the choroid. An RD of only 1 mm creates sufficient hypoxia [19] to recruit proinflammatory cytokines to the RPE monolayer. Separation of the sensory retina from the underlying RPE violates the integrity of the tight junctions that form the BRB, which results in a loss of contact inhibition between RPE cells. These cells then grow in an uncontrolled manner into the vitreous.

The formation of a retinal tear or ocular injury can also trigger an intraocular hemorrhage. The direct influx of blood, serum proteins, and vitreal cells through the retinal break further stimulates PVR development. Research in animal models has shown that a single injection of fibroblasts was sufficient to induce PVR. Notably, the introduction of a sufficient amount of any cell type (whether macrophages, dermal cells, fibroblasts, or RPE cells) to the vitreous cavity results in pathology that mimics PVR. After a penetrating trauma, cells introduced from outside the eye (e.g., Tenon's layer or dermal tissue) may directly initiate PVR formation [20].

Inflammation, ischemia, and blood activate inflammatory cells (mainly macrophages, lymphocytes, and polymorphonuclear cells), which trigger the development of PVR through the formation of cytokines and growth factors [13]. Growth factors, cytokines, and proteins entering the SRS from the circulation come in direct contact with the RPE and glial or Müller cells, stimulating their proliferation.

2.1. Predisposing Factors. Several risk factors for developing PVR have been identified: size of the retinal hole or tear (cumulative break area > 3 optic discs), detachment involving > 2 quadrants, intraocular inflammation, vitreal hemorrhage, and preoperative choroidal detachment.

Other predisposing factors are grade A or B preoperative PVR, the duration of RD before corrective surgery, high levels of vitreal proteins, repeated intraocular surgeries, aphakia, previous cryotherapy and photocoagulation, and the use of intraocular gas and silicone [21–23].

Kuhn and colleagues identified and stratified rupture, endophthalmitis, perforating injury, retinal detachment, and afferent pupillary defects as key risk factors predictive of a worse visual prognosis [24]. Additional risk factors for worse final best-corrected visual acuity (<20/40) are age (young patients, especially <5 years old), injuries with retrolimbal involvement, wound length ≥ 6 mm, and blunt injuries [25]. More posterior or longer wounds are also more likely to result in PVR. Vitreous hemorrhage is also strongly linked to less favorable outcomes [25–31].

The time from injury to the onset of PVR ranges from 1 to 6 months. A shorter interval between injury and PVR onset is observed for perforated globes (median, 1.3 months) followed by rupture (2.1 months), IOFB (3.1 months), penetration (3.2 months), and contusion (5.7 months) [15, 17].

2.2. Histopathology of RD and the Implications for PVR and Visual Outcomes. Adhesion of the neurosensory retina to the RPE is weak owing to the existence of a specialized extracellular SRS, in which the apical processes of the RPE

interdigitate with the rod outer segments and specialized projections from the RPE ensheath the cone outer segments. This physiology stems from events during ontogenesis, when invagination of the optic vesicle into 2 layers forms the optic cup. The inner layer of the optic cup will eventually form the neuroretina, and the outer layer of the optic cup will form the RPE. Only pressure keeps the 2 layers apposed; the virtual space between them may be readily widened under the influence of weak tractional vitreous forces. Each RPE cell makes contact with 30–40 photoreceptors, forming a functional unit; survival of the photoreceptors is dependent on the RPE and vice versa [31, 32].

The RPE also contributes to the formation of the BRB, which in addition to maintaining ionic homeostasis of the SRS prevents proteins and blood components from penetrating neurosensory retina.

Anatomically, the neuroretina is usually considered to consist of 2 parts: the outer retina (which is avascular) and the inner retina (which is supplied with blood). The outer part is mainly nourished by diffusion from the choroid, while the inner half is supplied by the retinal circulation. Separation of the sensory retina from the underlying RPE deprives the outer retina of nutrients, with disruptive metabolic and neurochemical consequences for the entire retina. Most of detachment-induced retinal damage appears to be directly related to the reduced supply of oxygen and, to some extent, also to low levels of other substances, such as glucose [33–35]. The photoreceptor layer is by far the most vulnerable area, probably because the inner segments of the photoreceptors account for almost all oxygen consumption by the outer retina and because the outer retina is mainly supplied with oxygen and nutrients via diffusion from the choroid [36].

An RD alters the RPE-photoreceptor relationship [37, 38]. The outer retina becomes hypoxic [34]; the photoreceptors are stressed, and some die by apoptosis [39]. This is followed by programmed deconstruction of the surviving photoreceptor cells. A few hours or days after the RD, important cellular remodeling may be observed [40]. In the detached retina, the light-sensitive outer segments of rod photoreceptors degenerate and the synaptic terminals retract from the outer plexiform layer (OPL), so that rod synapses now occur deep in the outer nuclear layer (ONL). After a few days, up to 20% of photoreceptors (mainly rods) are apoptotic, while the other photoreceptors may have survived through changes in shape and/or metabolism but risk engulfment by the hypertrophic lateral branches of Müller cells. Müller glial cells, with their main stalk of cytoplasm extending across the width of the entire retina, undergo several changes in morphology during their lifespan [40]. Their nucleus often migrates into the ONL, at which point their main process and fine lateral branches increase in size and fill with glial fibrillary acidic proteins (GFAP) (intermediate filaments that play a role in mitosis).

Müller cells proliferate as part of an inflammatory response designed to heal the retina to protect neurosensory retina from mechanical stimuli (i.e., passive movement of the detached retina) and to protect photoreceptors from apoptosis. Müller cell proliferation is evident even in portions of the retina that are not yet detached, which suggests that RD involves a general reaction of the entire retina. Recent research seems to suggest that the release of diffusible growth factors such as PDGF from the site of retinal detachment induces the activation of Müller and glial cells, even in parts of the retina that remain attached [41].

However, hypertrophic Müller cells tend to fill all the empty spaces previously occupied by neurons that have degenerated, thus irreversibly altering retinal structure and function. In detached retina, the main stalk of the Müller cell often grows onto the surface of the ONL, along the outer limiting membrane and into the SRS where it can form a "glial scar." Microglial cell proliferation and immune cell invasion may be detected in both detached and attached retinal areas. This proliferation contributes to retinal gliotic remodeling and to neuronal retinal degeneration, which could explain the impaired recovery of vision after reattachment surgery, particularly in patients with PVR [42].

Reattachment allows for the regrowth of outer segments and rod axons, although some of these now grow past the OPL, their normal target layer, and penetrate the inner retina. Reattachment inhibits the hypertrophy of Müller cells within the retina and in the SRS but appears to allow the growth of these cells onto the vitreal surface of the ganglion cell layer (GCL), where they form epiretinal membranes. Neuritic sprouts from the GCL often intermingle with the Müller cell processes that form epiretinal membranes. These protracted remodeling events, associated with photoreceptor cell death, often prevent complete functional recovery after surgical retinal reattachment.

Early reattachment probably halts and partially reverses the remodeling process and may stimulate withdrawal of many of the neurites that grow from these cells during detachment. However, prolonged detachment may stimulate further growth of Müller cells [41–43].

Restoration of the blood supply to the outer retina via reconnection with RPE microvilli stimulates the regrowth of outer segments and thus restores the retina's structural integrity [44]. It is reasonable to think that retinal reattachment represents a return of the retina to its "normal" state, but data from animal models suggests otherwise.

Reattachment has the ability to stop the growth of Müller cell processes into the SRS [42] but cannot stop growth in the opposite direction, which stimulates the formation of epiretinal membranes [41–43]. Müller cell changes allow for the formation of a scaffold that permits the adhesion and subsequent proliferation of other glial cells, leading to subretinal fibrosis and PVR.

Research performed in animal models suggests that one of the mechanisms by which Müller cells play a role in PVR is by upregulating the expression of PDGFR-α and GFAP, thus starting a process of dedifferentiation in cells whose behavior resembles that of fibroblasts [45]. Moreover, Müller cells in peripheral retina, where PVR most often occurs, have been shown to express stem cell markers indicative of active proliferation and dedifferentiation [46]. In addition, as yet unidentified cytokines and cofactors produced by migrated RPE cells may stimulate Müller cells to transform into cells with fibroblastic behavior, which then contribute to membrane formation and contraction. A thorough understanding of the molecular mechanisms underlying RD will be critical

to controlling conditions such as PVR and may also elucidate associated rod axon outgrowth.

3. Pathobiology and Pathophysiology of PVR

Five distinct stages appear to be important in PVR development. These include breakdown of the BRB, chemotaxis and cellular migration, cellular proliferation, membrane formation with remodeling of the extracellular matrix, and contraction [47].

Soon after an RD, macrophages enter the vitreous cavity through the retinal injury [48, 49] and release inflammatory cytokines that stimulate cell migration and proliferation. However, immunohistochemical studies of PVR membranes show the presence of various subtypes of immune cells: macrophages, monocytes, T lymphocytes, B lymphocytes, glial cells, and cells expressing HLA-DR and DQ [50]. Macrophages and other inflammatory cells likely initiate the central event in the pathogenesis of PVR: the vigorous proliferation of RPE. Notably, the RPE is a monolayer of differentiated cells located between the neural retina and the choroidal vasculature essential for the survival of retinal neurons and visual function. The RPE contributes to the BRB, which, in addition to maintaining the ionic homeostasis of the SRS, prevents proteins and blood components from penetrating neural retina. The RPE is necessary for the preservation of normal photoreceptors and choriocapillaris and also plays an important role in the intraocular wound-healing response [51].

RPE cells are mitotically inactive under physiological conditions. Contact between the RPE and vitreous cytokines triggers dedifferentiation and epithelial-to-mesenchymal transformations.

Various signals have been found to trigger the migration and proliferation of RPE cells: the loss of contact, factors present in the vitreous, and signals from photoreceptors and inflammatory cells. Although RPE cells express receptors for hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), tumor necrosis factor (TNF), and other growth factors [52, 53], the interactions between RPE and Müller cells are likely the primary force regulating membrane formation and contraction [45].

Müller and RPE cell interaction can lead to the upregulation of PDGF-receptor α and increase Müller cell pathogenicity. Müller cells may also play a more active role than previously thought in the development of PVR membranes, especially when stimulated by an environment rich in RPE cells [46]. Depending on the size and age of the detachment as well as the size and location of the retinal tear, RPE cells are more or less likely to abandon their natural monolayer and migrate into the subretinal and preretinal space. These cells often attach to the vitreous, which acts as a scaffold, then migrate and secrete cytokines and cofactors that can alter Müller cell phenotype in ways that increase fibroblastic behavior and pathogenicity.

BRB breakdown and blood coagulation over a wound site expose the RPE to various serum components, including thrombin, fibrin, and plasmin. Thrombin and fibrin have been shown to promote growth factor secretion, neural cell survival and apoptosis, cytoskeletal rearrangement, and cell proliferation [54]. Plasmin is a serine protease that dissolves fibrin blood clots. Plasmin has also been identified as the major PDGF-C processing protease in the vitreous of animal models of PVR as well as patients undergoing retinal surgery. Blocking plasmin may prevent the generation of active PDGF-C, the PDGF isoform most relevant to PVR. For this reason, plasmin was identified as a novel therapeutic target for patients with PVR [55].

RPE cells undergo an epithelial-mesenchymal transition [55–57] and develop the ability to migrate out into the vitreous, producing a provisional extracellular matrix containing collagen, fibronectin, thrombospondin, and other matrix proteins [58]. During this process, subretinal RPE cells may lose their connection to the RPE extracellular matrix [59–62] and migrate through the retinal break to enter the vitreous cavity.

Kiilgaard et al. [63] used 5-bromo-2-deoxyuridine (BrdU) to detect proliferating RPE cells and found that posterior pole injury in the porcine eye results in RPE proliferation in the anterior part of the RPE but not in the vicinity of the lesion. This suggests that a population of RPE progenitor cells exists in the vicinity of the ora serrata [64]. These cells as well as the neural progenitors of Müller cells could supply the cells necessary for proliferation in PVR [46]. Notably, most PVR membranes are formed by fibroblasts. Animal models of PVR are typically created by injecting fibroblasts directly into the vitreous. The intravitreal fibroblasts observed in PVR derive ontologically from transdifferentiated RPE or Müller cells in the case of a primary rhegmatogenous RD and from fibroblasts that originated extraocularly in the case of ocular injury.

The mechanisms of induction of posttraumatic PVR are probably the same implied in experimental PVR, obtained by injection of extraocular cells into the vitreous of animal models.

When a wound is created, membranes are often seen to extend intraocularly from the wound edge; the fibroblasts that constitute these membranes may be derived from Tenon's layer [10]. Fibroblasts and transdifferentiated cells give rise to myofibroblasts that bestow PVR membrane contractility. The contraction of these cells is responsible for the most deleterious effects of PVR, including retinal wrinkling and distortion, formation of new retinal breaks, and reopening of previously sealed breaks [65].

Two mechanisms have been proposed to explain the membrane contraction that can lead to a secondary RD. One is the active contraction of myofibroblastic cells; the second is the motile activity of myofibroblasts, which remodel the surrounding extracellular matrix [66]. The second mechanism is supported more strongly by scientific evidence. According to this theory, TGF-β secreted by macrophages induces the transformation of fibroblasts into smooth muscle- (SM-) actin-positive myofibroblasts [67].

3.1. Cytokines Involved in PVR. The emerging hypotheses regarding the pathogenesis of PVR have focused on abnormal

local concentrations of growth factors and cytokines in the vitreous. This environment is conducive to transdifferentiation, migration, proliferation, survival, and extracellular matrix formation [68]. The growth factors likely to be involved are PDGF, TNF-α and TNF-β, HGF, transforming growth factor beta 2 (TGFβ_2), epidermal growth factor (EGF), and fibroblast growth factor (FGF). Cytokines such as interleukin- (IL-) 1, IL-6, IL-8, IL-10, and interferon gamma (INF-γ) are also thought to play a role. Recent experiments have focused attention on the activation of a receptor for PDGF (PDGFR-α), which seems to play a crucial role in PVR. Both PDGF and PDGFR-α are gaining more attention as novel therapeutic targets.

3.2. The Role of PDGF and PDGFR in the Pathogenesis of PVR. In recent decades, vitreous samples from patients undergoing vitrectomy for PVR were found to have elevated concentrations of FGF and PDGF when compared to patients with RD uncomplicated by PVR [69]. PDGF is an abundant regulator of cell growth and division. It plays a central role in blood vessel formation (angiogenesis) [70] and is produced by a plethora of cells, including SM cells, activated macrophages, endothelial cells, and RPE. PDGF is also synthesized, stored, and released by platelets upon activation.

PDGF exists as a dimeric glycoprotein composed of 2 A (-AA) or 2 B (-BB) chains or a combination of the two (-AB). PDGF acts as a chemoattractant and mediator of cellular contraction in RPE cells [71, 72]; it is a potent mitogen for cells of mesenchymal origin, such as smooth muscle and glial cells.

The PDGF signaling network consists of 4 ligands (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) and 2 receptors (PDGFR-α and PDGFR-β). PDGFRs are classified as tyrosine kinase receptors and are encoded by 2 genes that can homodimerize or heterodimerize to form PDGFR-$\alpha\alpha$, PDGF-$\beta\beta$, and PDGFR-$\alpha\beta$. PDGF is mitogenic during early development; during later maturation stages, it has been implicated in cellular differentiation, tissue remodeling, and morphogenesis. PDGF has been shown to direct the proliferation, migration, division, differentiation, and function of a variety of specialized mesenchymal and migratory cell types, especially fibroblasts, during development as well as adulthood [72]. In essence, PDGF allows a cell to skip the G1 (growth) phase in order to divide. Lei et al. found that the presence of PDGF, mainly PDGF-C, in the vitreous cavity was tightly associated with PVR, present in 8/9 PVR patients versus 1/16 patients with other types of retinal disease [73].

The analysis of epiretinal membranes from eyes with PVR showed RPE and Müller cell overexpression of PDGF and PDGFR-α [45, 53, 74]. PDGF, with PDGF-C as the predominant isoform, is highly expressed in the vitreous of humans and animals with PVR [75]. PDGF-C is secreted as a latent protein that requires proteolytic processing for activation. Plasmin has been identified as the major PDGF-C processing protease in the vitreous of PVR animals and patients undergoing retinal vitrectomy. The blockade of plasmin prevents the generation of active PDGF-C [55].

PDGF-C, together with its receptor PDGFR-α, is currently considered as the main contributor to PVR pathology in ocular trauma. PDGFR-α has been shown to be more readily activated than PDGFR-β and more likely to contribute to PVR [74]. Increased expression of PDGFR-α in the retina is associated with the formation of epiretinal membranes and the proliferation of RPE cells and Müller cells [45, 76, 77]. Furthermore, the expression of functional PDGFRs in either RPE or fibroblasts is an essential step for experimental PVR [53, 75, 78]. However, in animal models, cells with no PDGFR-α carried a low risk of developing PVR and were able to revert to PVR reexpression upon reestablishment of the wild-type PDGFR genotype. Similarly, blocking PDGFR reduced the potential for PVR development [78]. Nonetheless, recent investigations have shown that blocking PDGF was not sufficient to block PDGFR-α activity [79].

Various PDGF isoforms are abundant in the vitreous of patients and experimental animals with PVR but make only a minor contribution to activating PDGFR-α and driving experimental PVR. Experimental PVR was found to be dependent on PDGFR-α activation, rather than the concentration of PDGF. PEGFR-α is also activated by EGF, FGF, insulin, and HGF [75, 78, 79]. Probably indirect activation of PDGFR-α by non-PDGF agents is the most important way to activate PVR also by other growth factors.

Vascular endothelial growth factor A (VEGF-A), which mediates neovascularization, competitively blocks PDGF-dependent binding and PDGFR-α activation [80]. However, a recent study showed that intravitreal agents that neutralize VEGF-A also inhibit non-PDGF-mediated activation, which protects against PVR [81]. PDGFR-α is a tyrosine kinase receptor that requires high levels of intracellular reactive oxygen species. Activation by non-PDGF agents increases intracellular levels of reactive oxygen species (ROS), which in turn activate Src kinase and PDGFR, promoting PVR [81].

Clinical researchers are currently evaluating drugs that target PDGFR-α or signaling events required for indirectly activating PDGFR-α rather than directly activating PDGF. Antioxidant-directed approaches such as those using N-acetylcysteine or tyrosine kinase inhibitors such as AG1295 or SU9518 could protect against PVR in humans [81–84].

3.3. Other Growth Factors and Cytokines. TGF-β is another growth factor implicated in PVR progression. TGF-β_2 is the most predominant isoform in the posterior segment [85] and is secreted as a latent inactive peptide into the vitreous by epithelial cells of the ciliary body and the lens epithelium. TGF-β_2 is also produced by RPE and Müller cells, fibroblasts, platelets, and macrophages [58]. Similar to PDGF, TGF-β_2 is 3 times more abundant in eyes affected by PVR versus normal eyes [86, 87].

TGF-β_2 is a potent chemoattractant secreted by RPE cells that plays a key role in transforming RPE cells into mesenchymal fibroblastic cells and in inducing type I collagen and extracellular matrix synthesis in RPE cells [88, 89]. Like PDGF, TGF-β_2 was found to increase RPE-mediated retinal contraction. Antibodies against TGF-β_2 and IL-10, an antagonist of TGF-β, inhibit the contractility of RPE cells

on epiretinal membranes [90]. In vivo experiments have shown that decorin, a naturally occurring TGF-β inhibitor, and fasudil, a potent inhibitor of a key downstream mediator of TGF-β called Rho-kinase, may reduce fibrosis and RD development [91–94].

Another factor that has been implicated in inflammation and is considered to promote PVR is TNF-α, a monocyte-derived cytotoxin. The presence of active TNF-α increases serum concentrations of the soluble form of its receptor (sTNF-RI and sTNF-RII), which can be used as a marker of active inflammation [95]. Genetic analysis has identified a single nucleotide polymorphism of the TNF locus that predisposes the eye to PVR [96].

HGF stimulates RPE cell migration and is present at high levels in retinal membranes. It is secreted by macrophages and acts as a multifunctional cytokine on cells of epithelial origin. HGF is also a potent chemoattractant for cultured human RPE cells. Its ability to stimulate cell motility, mitogenesis, and matrix invasion makes it a central player in tissue regeneration and in RPE-related diseases such as PVR [52, 97].

Mounting evidence suggests that chemokines play a role in the inflammatory pathways involved in PVR. Those named most commonly are IL-1β, IL-6, IFN-γ, and monocyte chemoattractant protein- (MCP-) 1. IL-6 is secreted by T cells and macrophages to stimulate the immune response after trauma, especially burns or other tissue damage leading to inflammation. IL-6 stimulates the proliferation of glial cells and fibroblasts and promotes the synthesis of collagen during wound healing [98]. IL-6 levels are significantly higher in the vitreous and subretinal fluid (SRF) in PVR, particularly posttraumatic PVR [52, 98]. In a recent study, IL-6 levels in the vitreous were found to be predictive for the development of PVR [87].

MMPs are proteolytic enzymes involved in MEC homeostasis; their expression is largely modulated by IL-6. IL6, MMP, and TIMP1 are expressed at high levels in grade B PVR, which involves intense MEC remodeling, [99].

Another cytokine involved in PVR is IFN-γ, a dimerized soluble cytokine that is the only member of the type II class of interferons. IFN-γ has a variable capacity to stimulate the immune response; this cytokine appears to activate macrophages during the development of PVR. IFN-γ levels are 6 times higher in eyes with PVR as compared with control eyes [100].

As mentioned above, the molecular events leading to epiretinal membrane formation in PVR are similar to those occurring in normal wound healing and scar formation [101]. Mononuclear phagocytes play a central role. MCP-1 is implicated in recruiting and directing leukocyte movement [102]. Abu El-Asrar et al. found that MCP-1 is present in the vast majority (76%) of eyes affected by PVR [103].

3.4. Emerging Therapeutic Opportunities. Few published studies have investigated the prevention of posttraumatic PVR; surgical management remains the primary mode of therapy. However, it is possible to extend the findings about emerging therapies for the prophylaxis of PVR, the prevention of posttraumatic PVR, on the basis of the molecular mechanisms described above. The most important therapeutic targets in efforts to control the immune response after trauma are Müller and EPR cell proliferation and epiretinal membrane formation.

A recent research on a feline model of RD reported that hyperoxic conditions reduced glutamate cycling dysregulation as well as Müller cell proliferation and transformation [3]. Similar experiments were then conducted in the ground squirrel retina, which is cone-dominated, in contrast to the rod-dominated feline retina [35]. The squirrel study showed a similarly protective effect of oxygen supplementation on photoreceptor degeneration. Providing supplemental oxygen after a diagnosis of RD may help to improve VA recovery after surgery and may reduce the incidence and severity of glial-based complications, such as PVR. Clinical trials with corticosteroids and antiproliferative agents have demonstrated clear success in preventing PVR.

The compounds tested for their ability to prevent PVR include antineoplastic agents, antiproliferative agents, anti-inflammatory agents, antioxidant agents, and anti-growth-factor agents. Current pharmacologic intervention to prevent PVR is principally focused on the use of antiproliferative and anti-inflammatory agents [104]. A number of antiproliferative drugs such as colchicine, daunomycin, alkylphosphocholines, and 5-FU have been tested due to their ability to inhibit the proliferation of human retinal glial cells in vitro. These antiproliferative compounds inhibit non-neural retinal cells, including Müller cells, which can form subretinal membranes that block photoreceptor outer segment regeneration after successful reattachment surgery [105]. One of the most promising antiproliferative candidates is 5-FU; it has been tested in combination with heparin in recent clinical trials. 5-FU acts on DNA synthesis by inhibiting thymidine formation, which inhibits cell proliferation, particularly in fibroblasts. This appears to improve the prognosis for long-term retinal reattachment following the development of PVR in animal models [106, 107].

Because 5-FU and low molecular weight heparin (LMWH) are involved in two different aspects of PVR pathogenesis, the two compounds are used together to exert a synergistic effect. Heparin is a naturally occurring complex polysaccharide that is able to bind fibronectin and a range of growth factors involved in the pathogenesis of PVR, such as FGF and PDGF [108].

One randomized clinical trial included 174 high-risk patients undergoing primary vitrectomy for RRD who were randomized to receive either 200 μg/mL 5-FU and 5 IU/mL LMWH or placebo. The results showed a significant reduction in the incidence of postoperative PVR and reoperation rates in the patients who received 5-FU and LMWH therapy [109]. Wickham et al. performed a prospective randomized clinical trial that included 641 patients who presented with primary retinal detachment. Patients were treated by either vitrectomy and adjuvant therapy of 5 IU/mL of LMWH and 200 mg/mL of 5-FU or vitrectomy and placebo [110]. These results showed that the use of 5-FU and LMWH did not improve anatomic or visual success rates after 6 months. This discrepancy may stem from the inclusion criteria used for each study: the first study included high-risk patients,

the latter included patients with primary RD. Although the efficacy of LMWH with 5-FU infusion during vitrectomy in preventing PVR remains controversial, this combined therapy may be used in the future to treat high-risk patients [111].

Another drug that has been used to inhibit the uncontrolled mitogenic activity of cells at the vitreoretinal interface is daunomycin; it is an anthracycline antibiotic, a topoisomerase inhibitor of DNA and RNA synthesis that arrests cell proliferation and cell migration. This antiproliferative compound inhibits fibroblast and RPE cell proliferation in vitro [112]. Since 1984, daunorubicin has been used for the prophylaxis of idiopathic and traumatic PVR [113]. In a multicenter, prospective, randomized and controlled study that used daunomycin to treat PVR, use of this compound during the vitrectomy increased the rate of reattachment. The evidence for any impact on anatomical success rate and/or visual outcomes was inconclusive [114].

In the early nineties, Campochiaro et al. were the first to put in evidence the ability of retinoic acids (RA) in inhibiting RPE cell growth in vitro [115]; subsequently also retrospective and prospective in vivo studies have been conducted [116].

Encouraging results from the use of retinoic acid were published by Chang et al. from a prospective controlled interventional case series of 35 patients affected by retinal detachment complicated with PVR who were randomized to receive either 10 mg oral RA twice daily for 8 weeks postoperatively or placebo. At a one-year postoperative follow-up, the treated group had significantly lower rates of macular pucker formation with higher rates of retinal reattachment [117].

Efforts to inhibit growth factor activity have focused on the tyrosine kinase receptor. Umazume et al. found that dasatinib prevents RPE sheet growth, cell migration, cell proliferation, the epithelial-mesenchymal transition (EMT), and extracellular matrix contraction in a concentration-dependent manner and prevents tractional retinal detachment (TRD) without any detectable toxicity [118].

PDGFR-α can be activated by PDGF, VEGF, and various other growth factors [78, 119]. VEGF binding to the receptor prevented PVR development in an animal model.

The apparent mechanism of action of ranibizumab involves the depression of PDGFs, which, at the concentrations present in PVR vitreous, inhibits non-PDGF-mediated activation of PDGF receptor alpha. The inhibition of the receptor by the way of non-PDGF results in a protection for the development of PVR in rabbit models. These preclinical findings suggest that the approaches to neutralize VEGF-A seem to be prophylactic for PVR, but more investigations are needed [81].

Because PVR is thought to be caused by the inflammatory healing process, intravitreal corticosteroids may be of use for treatment. These compounds exert their therapeutic action by limiting BRB breakdown, reducing neutrophil transmigration, inhibiting fibroblast proliferation, suppressing macrophage recruitment, limiting leucocyte migration, decreasing cytokine production, and reducing the formation of granulation tissue [120].

Corticosteroids inhibit the proliferation of fibroblasts, RPE cells, and RPE-transformed myofibroblasts that are responsible for the contractile properties of PVR membranes [121, 122]. Steroids also seem to interfere with the recruitment of macrophages to the site of a lesion and may block the action of monocyte/migration inhibitory factors (MIFs) [123].

These drugs are applied topically as eye drops, locally by subconjunctival, peribulbar, or retrobulbar injection, and systemically via oral, intravenous, and intramuscular routes. Numerous experimental studies conducted on animal models have demonstrated the benefits of the intravitreal administration of triamcinolone [124]. Despite this success in animal models, the same positive results have not been achieved in human studies.

Encouraging results regarding the use of triamcinolone acetonide emerged from a study conducted by Jonas et al. The authors demonstrated that the intravitreal injection of crystalline cortisone reduces postoperative intraocular inflammation. However, the mean follow-up period adopted in this study was less than 2 months, which reduces the validity of the results [125]. Despite the potential benefits, the intravitreal injection of triamcinolone acetonide is associated with side effects, including glaucoma and cataract, so recent research in this area has focused on the use of dexamethasone. A recent study conducted by Bali et al. showed that the subconjunctival injection of dexamethasone prior to surgery decreased the extent of postoperative BRB breakdown as measured by laser flare photometry 1 week postoperatively [122].

In this regard, we take the opportunity to report an important recent study in which Hoerster et al. evaluated the anterior chamber aqueous flare with laser flare photometry and found that it is a strong preoperative predictor for PVR in eyes with RD [126].

The disadvantage of using dexamethasone is the compound's short half-life, which has led to the development of long-acting intravitreal dexamethasone implants [127].

The antioxidant compounds represent one last class of drugs under investigation. As demonstrated by Lei and Kazlauskas, the indirect activation of PDGFR triggers signaling events leading to PVR [83]. Non-PDGF growth factors can increase intracellular concentrations of reactive oxygen species (ROS), leading to PDGFR activation. Lei et al. tested whether an antioxidant such as N-acetylcysteine (NAC) was able to prevent the accumulation of ROS and thereby block PDGFR activation. A 10 mmol/L-dose of NAC suppressed PDGFR-α activation and protected against RD in a rabbit model. Although NAC did not prevent the formation of an epiretinal membrane, the compound did limit the extent of vitreous-driven contraction [128]. Antioxidants may prevent detachments after retinal surgery and should be considered for use in combination with other therapeutic approaches.

4. Conclusions

Although the exact impetus for proliferation remains unknown, there is compelling evidence that posttraumatic PVR is similar to wound healing in terms of the inflammation, proliferation, and remodeling involved. The greatest challenge is to identify a pharmacological approach and adjuvant surgery that could be truly prophylactic for the development of PVR.

Various pharmacological agents have demonstrated potential in reducing postoperative PVR risks, including intravitreal LMWH, 5-FU, daunomycin, and anti-VEGF drugs. Clinical reports have suggested that either systemic or intravitreal corticosteroids may be useful in attenuating PVR gravity by limiting BRB breakdown. However, many clinical trials have shown inconclusive results; none of these agents has been shown to be decisive in preventing PVR after surgery.

Our knowledge about the pathogenesis of PVR has improved over recent decades. The introduction of immune cells into the vitreous cavity, as is the case in penetrating ocular trauma, triggers the production of growth factors and cytokines that come in contact with intraretinal Müller and RPE cells. It is widely accepted that growth factors and cytokines, including PDGFs, HGF, TNFα, and bFGF, drive the cellular responses intrinsic to PVR. These cytokines and growth factors promote an environment of cell transdifferentiation, migration, and proliferation that allows for expansion of the extracellular matrix. As this scaffold forms, it may physically attach to the retina. Subsequent contraction causes wrinkling, shortening, and tearing of the retinal tissue, otherwise known as PVR.

The process involves a host of cytokines and growth factors. To our knowledge, none seem to be indispensable for disease onset or progression. However, these pathways appear to converge at the steps necessary for the expression and activation of PDGFR-α, which seem to be crucial in the development of PVR.

In addition to the PDGFs, all of the other growth factors mentioned above stimulate the expression and activation of PDGFR-α on the surface of RPE cells, Müller cells, glial cells, and fibroblasts. The activity of this receptor promotes transdifferentiation, migration, proliferation, survival, the formation of extracellular matrix, membrane formation, and contraction. A combination therapy that could block all of these agents would be an ideal addition to the arsenal currently used to prevent PVR.

When used in combination with other tyrosine kinase inhibitors, the antioxidant NAC prevents tractional RD in animal models by blocking non-PDGF growth factor-mediated PDGFR-α activation. A recent study showed that a cocktail of neutralizing reagents targeted to multiple growth factors and cytokines was able to reduce PVR development. Antibodies against PDGF, EGF, FGF-2, IFN-γ, IL-8, TGF-α, VEGF, TGF-β, HGF, and IGF-1 to IGF-12 were effective in preventing RD in a rabbit model [129].

In the future, novel therapeutic agents could enhance functional recovery after RD by limiting cellular proliferation. A combined therapy involving oxygen supplementation, a cocktail of neutralizing reagents, and tyrosine kinase inhibitors would target intracellular and extracellular activation of PDFGR-α, thereby protecting against PVR. Current investigations into the pathobiological and pathophysiological mechanisms involved are increasing the possibilities for management. It is hoped that our treatment strategies will evolve and become even more effective in achieving complete vision recovery.

Conflict of Interests

The authors report no conflict of interests with this work.

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Review Article

Drug Delivery Implants in the Treatment of Vitreous Inflammation

Jillian Wang,[1] Angela Jiang,[1] Malav Joshi,[2] and John Christoforidis[2]

[1] *Department of Ophthalmology, The Ohio State University Wexner Medical Center, 915 Olentangy River Road, Suite 5000, Columbus, OH 43212, USA*
[2] *Department of Ophthalmology & Visual Science, The University of Arizona Medical Center, 655 N. Alvernon Way, Suite 108, Tucson, AZ 85711, USA*

Correspondence should be addressed to John Christoforidis; jbchristo@hotmail.com

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The eye is a model organ for the local delivery of therapeutics. This proves beneficial when treating vitreous inflammation and other ophthalmic pathologies. The chronicity of certain diseases, however, limits the effectiveness of locally administered drugs. To maintain such treatments often requires frequent office visits and can result in increased risk of infection and toxicity to the patient. This paper focuses on the implantable devices and particulate drug delivery systems that are currently being implemented and investigated to overcome these challenges. Implants currently on the market or undergoing clinical trials include those made of nonbiodegradable polymers, containing ganciclovir, fluocinolone acetonide, triamcinolone acetonide, and ranibizumab, and biodegradable polymers, containing dexamethasone, triamcinolone acetonide, and ranibizumab. Investigational intravitreal implants and particulate drug delivery systems, such as nanoparticles, microparticles, and liposomes, are also explored in this review article.

1. Introduction

Posterior uveitis and vitreous inflammation can have devastating effects on vision. Treatment usually involves a long course of medication for adequate control of symptoms. Posterior subtenon or intravitreal injection of immunomodulators and steroids is short lasting, requiring frequent administration. Penetration of the posterior segment with topical and systemic agents can also prove challenging and can be associated with significant side effects [1]. With these limitations, efforts are being made to develop and implement implantable devices that slowly release drug into the vitreous. The anatomy of the eye makes it an excellent organ for such a drug delivery system.

Intravitreal drug delivery systems are coated with biodegradable or nonbiodegradable polymers. The main advantage of biodegradable implants is that they do not require removal. Properties of these polymers have been previously discussed in "Intravitreal devices for the treatment of vitreous inflammation [2]." This review describes current and investigational intravitreal drug delivery devices with a primary focus on their use in vitreous inflammation. Studies in other eye diseases such as macular edema, infection, and neovascularization are also included to illustrate the versatility of these devices and technologies and their potential application to vitreous inflammation.

2. Clinically Used Intravitreal Implants

2.1. Nonbiodegradable Devices. The nonbiodegradable devices are implanted into the vitreous surgically due to their large size, with the exception of Iluvien (Alimera Sciences Inc., Alpharetta, GA; pSivida Inc., Watertown, MA) which is smaller than the others. They usually require removal and reimplantation of a new device following depletion of the drug. These devices are made of a permeable membrane and include a drug-containing reservoir. Permeable and impermeable polymers can be layered to slow release of contents [3]. Thickness and surface area can also be manipulated to change the diffusion rate [4]. The first implantable device for clinical use in the eye was composed of a nonbiodegradable polymer containing ganciclovir. This drug delivery system,

Vitrasert (Bausch & Lomb, Rochester, NY), was released to the market in 1996 for the treatment of acquired immunodeficiency syndrome- (AIDS-) related cytomegalovirus (CMV) retinitis [5, 6]. Also on the market is Retisert (Bausch & Lomb, Rochester, NY). Iluvien, I-vation (SurModics, Eden Prairie, MN), and the ranibizumab (Genentech, San Francisco, CA) port delivery system are still in the clinical trial phases for approval in the United States.

2.1.1. Vitrasert. Vitrasert is a ganciclovir pellet coated in polyvinyl alcohol (PVA), a permeable polymer that allows drug diffusion, and ethylene vinyl acetate (EVA), an impermeable, a hydrophobic polymer that restricts release. It contains at least 4.5 mg of ganciclovir and 0.25% magnesium stearate as an inactive ingredient. The device is composed of outer and inner permeable PVA layers sandwiching a discontinuous layer of impermeable EVA. This combination of polymers results in a ganciclovir release rate of 1 mcg/hour that lasts 5 to 8 months before reimplantation is necessary [7].

Vitrasert has shown to be effective in treating CMV retinitis in AIDS patients, increasing median time to progression when compared to groups receiving intravenous ganciclovir [7]. More recent studies suggest that, in the era of highly active antiretroviral therapy, a ganciclovir implant device is less effective than systemic therapy at improving survival and decreasing dissemination [8].

Postoperative complications directly associated with Vitrasert implantation include cataract, vitreous hemorrhage, retinal detachment, endophthalmitis, and epiretinal membrane formation [9, 10].

2.1.2. Retisert. Retisert is a sustained-release intravitreal implant containing 0.59 mg of fluocinolone acetonide coated with PVA and silicon laminate. Receiving FDA approval in 2005, it became the first intravitreal device for the treatment of chronic noninfectious uveitis. It is 5 mm long, 2 mm wide, and 1.5 mm thick with a release rate of 0.3-0.4 mcg/day for approximately 3 years. The device is inserted into the vitreous cavity and sutured to the sclera through a pars plana incision, a technique similar to the implantation of Vitrasert. Besides chronic noninfectious uveitis, studies have also shown the implant to be effective in edema from diabetes and central retinal vein occlusion (CRVO) [11, 12].

The clinical studies that resulted in the approval of Retisert found significant reduction of recurrence of uveitis, determined by anterior chamber cell number and vitreous haze, in patients treated for noninfectious posterior uveitis. In this three-year study, recurrence rate was significantly decreased from 62% in the year prior to implantation to 4%, 10%, and 20% in the 1st, 2nd, and 3rd year after implantation, respectively. Ocular complications encountered in implanted eyes, namely, lens opacification and increased intraocular pressure (IOP), were significant. Nearly 11% of implanted eyes required cataract extraction within the period of the study. In the same trial, 2-year safety data indicated that almost 100% of phakic patients would require cataract removal. This is a percentage much greater than that in patients with uveitis treated by steroids alone and indicates that the implant itself is contributing to increased lens opacity. By the end of the 3-year trial, 67% of implanted eyes had IOP elevated by 10 mmHg or more from baseline. Additionally, 49% required antihypertensive medication as compared to 13.6% at baseline. Other postoperative adverse events encountered included eye pain (52%), conjunctival hyperemia (31%), conjunctival hemorrhage (29%), hypotony (11%), retinal detachment (4%), and endophthalmitis (1%) [13, 14].

A recent randomized, controlled, phase 2b/3 trial demonstrated a lower rate of recurrence of uveitis in fluocinolone acetonide implanted eyes (18.2%) compared with those receiving standard of care, or systemic prednisolone or corticosteroid, treatment (63.5%). Observed adverse effects were similar to those in prior clinical trials. The systemically treated group, however, encountered nonocular adverse events (most commonly arthralgia and hypertension) of 25.7% compared to 0% in the implant group [15].

A large study enrolling 255 patients (479 eyes with uveitis), the multicenter uveitis steroid treatment (MUST) trial, also compared relative effectiveness of systemic therapy and fluocinolone acetonide implant in uveitis. It was shown that both approaches adequately controlled inflammation, but the implant group did so more often and earlier. Visual acuity was equally improved in both groups at the conclusion of the two-year study. As demonstrated in previous studies, eyes in the implant group had high complication rates with 80% requiring cataract surgery, 61% requiring treatment for IOP, and 16% with transient vitreous hemorrhage. Systemic treatment was well tolerated with no significant adverse events [16].

Another potential problem with the Retisert implant is the dissociation of the 2 main components (the suture strut and drug reservoir), which complicates removal and is potentially vision threatening. A retrospective study including 27 eyes found that 40.7% of the implants were dissociated at the time of removal or exchange [17].

2.1.3. Iluvien. Iluvien is an intravitreal implant for the treatment of chronic diabetic macular edema (DME), defined as equal to or greater than 3 years of disease. It is 3.5 mm long by 0.37 mm wide and contains 190 mcg of fluocinolone acetonide. Due to its small size, it can be injected through a 25-gauge needle, creating a self-closing hole. The material is nonerodible and does not require removal, potentially resulting in multiple devices in the eye if subsequent implants are required. Besides use in DME, phase 2 studies in wet age-related macular degeneration (AMD), dry AMD, and retinal vein occlusion (RVO) are also in process. Iluvien is currently awaiting FDA approval following the recent completion of phase 3 clinical trials, also known as the fluocinolone acetonide for diabetic macular edema (FAME) trials.

The FAME study evaluated fluocinolone acetonide implants with release rates of 0.5 mcg/day and 0.2 mcg/day for 24–36 months. At 36 months, the percentage of patients who had gained at least 15 points in best-corrected visual acuity (BCVA) score was 28.7% in the low dose and 27.8% in the high dose implant groups compared to 16% in the sham group. Improvement of at least 2 lines in the early treatment of diabetic retinopathy study (ETDRS) acuity score was seen in a higher percentage of patients in the low dose group (13.7%) than in the sham group (8.9%). There was no significant

difference in acuity between the high dose implant and sham. As with the Retisert implant, there was a high rate of increased IOP and cataract formation. Cataract surgery was performed in 80%, 87.2%, and 27.3% in the low dose, high dose, and sham groups, respectively. Adverse events related to IOP were more frequent in implant groups (low dose, 37.1%; high dose, 45.5%) compared to the shame (11.9%) [18]. These results indicate that the Iluvien implant, although with associated complications, is effective in DME, a disease that currently only has one FDA approved treatment.

2.1.4. I-vation. I-vation is a helical sustained-release implant containing 0.925 mcg triamcinolone acetonide coated in titanium, PVA, and EVA. The implant elutes drug for up to 2 years. It measures 0.4 mm long by 0.21 mm wide and is implanted through a pars plana sclerotomy less than 0.5 mm in diameter. The helical shape is designed to increase surface area available for drug diffusion and anchor the device to the sclera, while the flat cap is meant to sit just beneath the conjunctiva. This facilitates removal of the implant if necessary.

Twenty-four-month interim results for phase 1 clinical trials of I-vation showed its effectiveness in treating DME. Macular thickness, measured by optical coherence tomography, was decreased and visual acuity improved. Major complications included increased IOP and cataract development [19]. Phase 2b trials were terminated, and no further clinical trials have been completed [20].

2.1.5. Ranibizumab Port Delivery System. A novel port delivery system (PDS) with ranibizumab designed to release 10 mg/mL over an extended period of time is currently being investigated. A unique feature of this system is the ability to refill the device. A phase 1 uncontrolled clinical trial on neovascular age-related macular degeneration was recently completed in Latvia. The PDS, initially filled with 150 mcg of ranibizumab, resulted in improved visual acuity, sustained decrease in macular thickness, and evidence of decreased choroidal neovascular leakage comparable to monthly injections. Although final study data is pending, this technology shows promise in providing a long-term alternative to monthly ranibizumab injections [21].

2.2. Biodegradable Devices. Implants undergoing clinical trials in the United States for use in ocular disease include Surodex (Oculex Pharmaceuticals, Sunnyvale, CA) and Verisome (Icon Biosciences Inc., Sunnyvale, CA). A third, Ozurdex (Allergan Inc., Irvine, CA), is already approved for several indications. These devices are composed of biodegradable polymers that allow dissolution of the implant, eliminating the need for extraction and decreasing risks associated with surgery. There are currently two such devices on the market, both containing dexamethasone as the active ingredient.

2.2.1. Ozurdex. Ozurdex is a dexamethasone-containing intravitreal implant coated in biodegradable poly(lactic-co-glycolic acid) (PLGA). The implant is a 6.5 mm by 0.45 mm rod placed in the vitreous through the pars plana with a 22-gauge needle device. It contains 0.7 mg of dexamethasone and releases peak doses for 2 months followed by a lower dose for up to 4 additional months. When compared to triamcinolone and fluocinolone, dexamethasone is 5 and 20 times more potent, respectively, but has a shorter half-life than either [22]. Ozurdex is approved for macular edema following BRVO or CRVO and noninfectious posterior uveitis.

In the HURON study, a 26-week multicenter, randomized clinical trial, 229 patients with noninfectious intermediate or posterior uveitis were randomized into groups receiving implants with 0.70 mg dexamethasone, 0.35 mg dexamethasone, or sham. Fifteen-letter improvement in BCVA was achieved in the dexamethasone groups at a rate 2- to 6-fold greater than that achieved in the sham. In addition to improvement in visual acuity, the mean decrease from baseline central macular thickness was also found to be greater in implant groups compared to sham at 8 weeks. They were not significantly different at 26 weeks. Percent of subjects with vitreous haze score of 0 at 8 weeks was 47%, 36%, and 12%, for those receiving high dose, low dose, and sham, respectively. This effect was maintained at 26 weeks. Adverse events included increased IOP with 23% of the 0.7 mg treatment group requiring medication to lower pressure and one patient requiring laser iridotomy. Cataract development was greater in treatment groups compared to the sham, but differences were not significant [23, 24]. When the device was implanted in patients with macular edema due to retinal vein occlusion in the GENEVA study, similar improvements in visual acuity were found. Unlike in the HURON study, occurrence of elevated IOP was not found to be significantly different between implanted and sham eyes by day 180 [25, 26].

The SOLO study, a retrospective chart study designed to compare results from the clinical setting to those of the GENEVA study, also showed improvement in visual acuity and reduction of macular edema. Early retreatment, defined as reinjection within the labeled 6-month interval, was performed in 40.7% and 50% of CRVO and BRVO eyes, respectively [27].

Several studies comparing the Ozurdex implant with intravitreal ranibizumab in retinal vein occlusion are ongoing [28–30]. Additionally, favorable outcomes have been demonstrated in small case series in patients with persistent uveitic cystoid macular edema with history of pars plana vitrectomy, radiation macular edema, and macular edema from retinitis pigmentosa [31–33].

2.2.2. Surodex. Surodex is a 60 mcg dexamethasone pellet coated in PLGA and hydroxypropyl methylcellulose. It measures 1.0 mm by 0.4 mm and provides sustained release for 7–10 days following insertion into the anterior chamber. Surodex has completed phase 3 clinical trials in the United States and has been approved in China, Singapore, and several other countries. It has primarily been investigated as a treatment for postcataract surgery inflammation. One study showed that, over 7 days, the insert achieved higher concentration in the eye than the maximum peak concentrations reached with topical dexamethasone drops following cataract extraction [34]. A randomized clinical trial of Surodex as a steroid drug delivery system for cataract surgery showed the implant to be safe and effective in reducing postoperative inflammation. Their study included a group of subjects

receiving two pellets in the anterior chamber, two pellets in the ciliary sulcus, and a control group that only received conventional topical 0.1% dexamethasone. Lower flare scores were experienced in both implant groups compared to the control without any difference between the two placements. No complications were encountered [35]. In a more recent study, Surodex was shown to be just as effective as topical 0.1% dexamethasone postcataract surgery without any significant improvement in decreasing flare. This study also reported no adverse events [36].

2.2.3. Verisome. Verisome is an injectable drug delivery system that provides long-lasting intravitreal therapy. Once injected with a 30-gauge needle, the material coalesces to form a spherule that sits in the posterior chamber and slowly degrades as medication is released. According to the manufacturer, its versatility allows it to deliver small molecules, peptides, proteins, and monoclonal antibodies. It can, furthermore, be formulated as a gel, liquid, or solid [37].

A phase 1 multicenter study showed triamcinolone acetonide formulated with Verisome to be well tolerated and without injection-related complications such as endophthalmitis or uveitis. It was also demonstrated to be effective in improving chronic cystoid macular edema (CME) due to retinal vein occlusion [38]. A phase 2 clinical study for neovascular AMD with ranibizumab formulated with Verisome has also demonstrated its efficacy. Results of the study indicated that frequency of ranibizumab injections might be decreased with this drug delivery system [39].

3. Investigational Implants

3.1. Cyclosporine. Several animal studies have shown that intravitreal delivery of cyclosporine can help control inflammation of the posterior chamber. A PLGA cyclosporine microsphere delivery system significantly decreased severity of cellular infiltrate, leukocyte number, and protein levels in eyes of rabbits with uveitis without long-term toxicity [40]. A separate rabbit study demonstrated that cyclosporine A conjugated to a polycaprolactone (PCL)/PLGA copolymer was more effective in treating chronic uveitis when compared to oral cyclosporine [41]. Cyclosporine A contained in a 6 mm diameter suprachoroidal implant placed in the deep sclera has also been found to be effective in controlling inflammation and maintaining vision in an equine recurrent uveitis model [42].

3.2. Indomethacin. PLGA implants containing 7 mg of indomethacin released over 3 weeks were evaluated in a postoperative model in rabbits. Inflammation following capsulorhexis, phacoemulsification, and intraocular lens placement was significantly decreased, although the rate of posterior capsule opacification was unchanged from the control [43]. More recent studies focus on surface indomethacin implants in treating inflammation in the anterior chamber [44, 45].

3.3. Particulate Drug Delivery. Particulate drug delivery systems utilize small biodegradable colloidal particles for long-term delivery of medication. These systems also provide targeted therapy with improved bioavailability and decreased systemic toxicity. They include liposomes, microparticles, and nanoparticles. Microparticles and nanoparticles can further be subdivided into micro- or nanospheres, in which the drug is homogenously dispersed within a polymeric matrix, and micro- or nanocapsules, in which the drug is encased in a polymeric membrane. Distinction is based on particulate size with microparticles generally accepted as 1 to 1000 microns in diameter and nanoparticles between 10 and 1,000 nanometers [46].

3.3.1. Liposomes. Liposomes are colloidal spheres made up of phospholipids, such as lecithin and phosphatidylcholine, which encapsulate therapeutic agents. Hydrophilic drugs are tucked away within the lipid core of the sphere, whereas hydrophobic drugs remain soluble between the bilayer. Because the phospholipids that compose these bilayers are naturally occurring, they are biocompatible with little toxicity and are capable of crossing hydrophobic membranes. Limitations of this delivery method include short half-life, instability, and minimal control of drug release over time [47]. Size of liposomes can also be engineered based on application. Those injected intravitreally are typically 100 nm to 400 nm in diameter according to the literature [48].

Use of liposomes as ocular drug delivery systems was first evaluated in superficial disease through topical instillation [49]. In more recent years, intravitreal administration has been under investigation. Currently, verteporfin (Visudyne, QLT Inc., Vancouver, BC, Canada), a benzoporphyrin derivative, is the only ophthalmic liposomal therapeutic agent approved. It is indicated in the treatment of neovascularization due to AMD, pathologic myopia, or presumed ocular histoplasmosis [50, 51]. Liposomal amphotericin or AmBisome (Gilead Sciences, Foster City, CA) is indicated for the treatment of leishmaniasis and various fungal infections in immunocompromised individuals and patients with renal impairment. It is also used off label for fungal endophthalmitis. The liposomal formulation has fewer toxic effects than the native form thus allowing delivery of higher dosages intravitreally [52]. Improvement of drug pharmacokinetic properties by intravitreal injection of liposomal therapeutics has also been observed in amikacin [53], amphotericin B [54], bevacizumab [55], cidofovir [56], ganciclovir [57], ciprofloxacin [58], clindamycin [59], gentamicin [60], and tobramycin [61].

3.3.2. Microparticles. Microparticles are similar to liposomes in shape and size but have greater stability and capacity for carrying the drug. They are often composed of biodegradable polymers such as PLGA and polylactic acid (PLA). Surface polymer modification can also enhance specific cell targeting and decrease degradation by the mononuclear phagocytic system. The microparticles themselves, however, are not without risk. Unlike liposomes, the components of microparticles are not naturally found in the body and their exact interactions with living cells and tissue are not clearly understood [62]. To date, there are no microparticle drug delivery systems on the market. There are, however, many microparticle and nanoparticle therapeutic agents under investigation for improvement in long-term drug delivery.

Microcapsules containing TG-0054, a water soluble anti-angiogenic drug in phase 2 clinical trials, were shown to sustain *in vivo* release for 3–6 months when injected in the vitreous. These PLA microparticles are, thus, a potentially useful formulation for long-term treatment of neovascular disorders of the eye [63].

Microspheres composed of PLGA and triamcinolone acetonide have shown potential utility in the treatment of DME in a nine-patient preliminary study. One mg triamcinolone acetonide in a controlled-release microsphere system (called RETAAC in the study) was well tolerated and demonstrated superior long-term pharmacological performance compared to a 4 mg injection of triamcinolone acetonide. No drug- or procedure-related side effects were noted in either group [64]. Other microspheres developed for sustained ocular delivery of agents include those containing adriamycin, pegaptanib, and cyclosporine [65–67]. Microspheres composed of chitosan, a natural biodegradable polymer, for transcorneal delivery of acyclovir have also demonstrated prolonged drug release [68].

3.3.3. Nanoparticles. Nanoparticles have been used, experimentally, with several therapeutic agents for intraocular drug delivery. Injection of tamoxifen incorporated into polyethylene glycol- (PEG-) coated nanoparticles was found to be effective in the treatment of autoimmune uveoretinitis induced experimentally in rats. Injection of free tamoxifen, however, did not alter the course of disease [69]. Triamcinolone acetonide formulated in PLGA nanoparticles was recently studied in a rabbit model of endotoxin-induced uveitis. No significant difference existed between the effectiveness of triamcinolone acetonide injection and nanoparticles. Sustained-release nanoparticles, however, could potentially require fewer administrations and better patient compliance [70]. Intravitreally injected polyethylcyanoacrylate nanoparticles containing acyclovir and ganciclovir together showed sustained levels in rabbits but were also associated with cataracts and flare [71].

Nanoparticle technology has also been implemented in experimental gene transfer therapy. Periocularly injected recombinant pigment epithelium-derived factor (PEDF) particles significantly reduced choroidal neovascularization in mouse and pig models by increasing retinal PEDF levels [72, 73]. A similar technology was used in a phase 1 clinical trial of adenoviral vector-delivered PEDF in neovascular AMD with promising results. No serious adverse events were noted, but mild, transient intraocular inflammation did occur in 25% of patients and increased IOP in 21% [74]. Bevacizumab nanospheres composed of PLGA also demonstrated long-term release (over 90 days). Rate of release was adjusted by changing the drug to polymer ratio [75]. Topical cyclodextrin nanoparticles for the treatment of DME are currently being studied in a phase 2 clinical study in comparison to dexamethasone [76].

4. Summary

The structure of the eye makes it an organ that is well suited for local delivery of therapeutic agents. Several intravitreal devices are approved for inflammatory processes as well as other pathologic conditions of the eye. Intraocular nanoparticles and microparticles are also being developed and show great promise in sustained and targeted delivery of therapeutics. A multidisciplinary approach involving biomedical engineering, pharmacology, and molecular biology will continue to be critical in the design of implants for the treatment of ocular inflammation. Many of the discussed drug delivery devices have varying benefits and limitations. As knowledge of these delivery systems and implants broadens, a safe and efficacious device that does not necessitate removal or surgical implantation may, in the future, be available as standard treatment for many ocular diseases.

Conflict of Interests

The authors declare that there is no competing/conflict of interests related to any topic in this paper.

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