NEURAL REGENERATION RESEARCH
www.nrronline.org

Review

Inflammation in diabetic retinopathy: possible roles in pathogenesis and potential implications for therapy

Lei Tang[1], Guo-Tong Xu[1, *], Jing-Fa Zhang[2, 3, *]

https://doi.org/10.4103/1673-5374.355743

Date of submission: April 7, 2022

Date of decision: May 25, 2022

Date of acceptance: August 16, 2022

Date of web publication: October 10, 2022

From the Contents

Abstract

Diabetic retinopathy, characterized as a microangiopathy and neurodegenerative disease, is the leading cause of visual impairment in diabetic patients. Many clinical features observed in diabetic retinopathy, such as capillary occlusion, acellular capillaries and retinal non-perfusion, aggregate retinal ischemia and represent relatively late events in diabetic retinopathy. In fact, retinal microvascular injury is an early event in diabetic retinopathy involving multiple biochemical alterations, and is manifested by changes to the retinal neurovascular unit and its cellular components. Currently, intravitreal anti-vascular endothelial growth factor therapy is the first-line treatment for diabetic macular edema, and benefits the patient by decreasing the edema and improving visual acuity. However, a significant proportion of patients respond poorly to anti-vascular endothelial growth factor treatments, indicating that factors other than vascular endothelial growth factor are involved in the pathogenesis of diabetic macular edema. Accumulating evidence confirms that low-grade inflammation plays a critical role in the pathogenesis and development of diabetic retinopathy as multiple inflammatory factors, such as interleukin-1β, monocyte chemotactic protein-1 and tumor necrosis factor-α, are increased in the vitreous and retina of diabetic retinopathy patients. These inflammatory factors, together with growth factors such as vascular endothelial growth factor, contribute to blood-retinal barrier breakdown, vascular damage and neuroinflammation, as well as pathological angiogenesis in diabetic retinopathy, complicated by diabetic macular edema and proliferative diabetic retinopathy. In addition, retinal cell types including microglia, Müller glia, astrocytes, retinal pigment epithelial cells, and others are activated, to secrete inflammatory mediators, aggravating cell apoptosis and subsequent vascular leakage. New therapies, targeting these inflammatory molecules or related signaling pathways, have the potential to inhibit retinal inflammation and prevent diabetic retinopathy progression. Here, we review the relevant literature to date, summarize the inflammatory mechanisms underlying the pathogenesis of diabetic retinopathy, and propose inflammation-based treatments for diabetic retinopathy and diabetic macular edema.

Key Words: anti-inflammation therapy; anti-vascular endothelial growth factor; diabetic retinopathy; hyperreflectivity foci; inflammation; inflammatory cells; inflammatory cytokines; leukostasis; microglia; Müller cells

Introduction

Diabetic retinopathy (DR) is a neurodegenerative disease featuring microvascular retinal lesions and causes acquired blindness in the working-age population worldwide (Altmann and Schmidt, 2018). The metabolic abnormalities of DR caused by chronic hyperglycemia include the activation of the polyol pathway, the hexosamine biosynthetic pathway, and the protein kinase C pathway, and advanced glycation end-products (AGEs) accumulation, resulting in increased reactive oxygen species in cells, and aggravation of retinal oxidative stress and inflammation (Whitehead et al., 2018).

Low-grade inflammation triggers a series of cellular abnormalities and tissue injury culminating in the retina (Altmann and Schmidt, 2018; Rübsam et al., 2018; Wang and Lo, 2018; Whitehead et al., 2018), as pro-inflammatory mediators, adhesion molecules, chemokines, and growth factors (Altmann and Schmidt, 2018; Lu et al., 2018; Rübsam et al., 2018; Wang and Lo, 2018; Whitehead et al., 2018), are increased and extensively involved in the pathogenesis of DR. Inflammatory cells in the retina also respond to injury and stress. Noxious stimuli activate endothelial cells and pericytes to secrete pro-inflammatory factors, recruiting leukocyte which adhere to the vascular endothelium, causing leukostasis and subsequent capillary non-perfusion (Spencer et al., 2020). Glial cells, which provide structural support in the normal retina, are also activated in early DR and participate in microenvironmental homeostasis regulation (Sorrentino et al., 2016; Rübsam et al., 2018). In DR, microglia proliferate and migrate from the inner to outer retina, and secrete multiple inflammatory cytokines. These may increase vascular permeability and induce intraretinal fluid accumulation by disrupting tight junction proteins and triggering breakdown of the blood-retinal barrier (BRB) (Tang et al., 2022). Inflammation also aggravates retinal neurodegeneration in the early stage of DR (Wang and Lo, 2018).

Recently, anti-vascular endothelial growth factor (anti-VEGF) agents have been regarded as a promising treatment for patients with proliferative DR (PDR) or diabetic macular edema (DME), but there remains a significant proportion of patients who show poor or incomplete response to anti-VEGF treatment, indicating that DR is a multi-factorial disease. Indeed, the increased levels of inflammation-related chemokines and cytokines have been evidenced in serum, vitreous, aqueous humor and retina in DR patients, and antagonization of these inflammation-related molecules may delay or prevent the angiogenesis and neurodegeneration in DR (Semeraro et al., 2015), suggesting inflammation as a novel therapeutic target for DR treatment. Thus, the present review describes the pathogenetic role of inflammation in DR and proposes potential mechanism-based therapies targeting inflammation in DR.

Database Search Strategy

Literature review was performed using the PubMed database. The following combinations of key words were used to initially select articles for evaluation: diabetic retinopathy AND inflammation; microglia AND diabetic retinopathy; anti-inflammation therapy AND diabetic retinopathy; and hyperreflectivity foci AND diabetic retinopathy. Most of the selected studies (65% of all references) were published since 2016. An article published in 1993 was included due to its topic (macrophages) with relevance to the search.

[1]Department of Ophthalmology of Tongji Hospital, Tongji Eye Institute, Department of Regenerative Medicine, and Department of Pharmacology, Tongji University School of Medicine, Shanghai, China; [2]Department of Ophthalmology, Shanghai General Hospital (Shanghai First People's Hospital), Shanghai Jiao Tong University, Shanghai, China; [3]National Clinical Research Center for Eye Diseases; Shanghai Key Laboratory of Ocular Fundus Diseases; Shanghai Engineering Center for Visual Science and Photomedicine; Shanghai Engineering Center for Precise Diagnosis and Treatment of Eye Diseases, Shanghai, China

*__Correspondence to:__ Guo-Tong Xu, MD, PhD, gtxu@tongji.edu.cn; Jing-Fa Zhang, MD, PhD, 13917311571@139.com.

https://orcid.org/0000-0002-0541-7214 (Guo-Tong Xu); https://orcid.org/0000-0003-0601-4342 (Jing-Fa Zhang)

Funding: *This work was supported by the National Natural Science Foundation of China, No. 82171062 (to JFZ).*

How to cite this article: *Tang L, Xu GT, Zhang JF (2023) Inflammation in diabetic retinopathy: possible roles in pathogenesis and potential implications for therapy. Neural Regen Res 18(5):976-982.*

Role of Inflammatory Cytokines in Diabetic Retinopathy

Data increasingly implicate chronic inflammation in DR pathogenesis, especially in the development of pathological vascularization and macular edema (Rangasamy et al., 2012; Trotta et al., 2022). In hyperglycemic conditions, upregulated pro-inflammatory molecules promote the synthesis of chemokines, inflammatory cytokines and other factors (Semeraro et al., 2015), aggregating leukostasis, cell apoptosis, and capillary leakage in the retina. The upregulated cytokines which mediate inflammation in DR patients are listed in **Table 1**.

Inflammatory cytokines

Serum and vitreous levels of interleukin-1β (IL-1β), mainly produced by macrophages, are raised in DR patients (Demircan et al., 2006). As the potent up-regulators of adhesion molecules, both IL-1β and tumor necrosis factor-α (TNF-α) prompt nuclear factor κappa-B (NF-κB) transcription by binding its receptor, and result in increased expressions of IL-6 and IL-8, as well as activating caspase-1. Increased IL-1β is triggered by cleaved caspase-1 and promotes neovascularization in diabetes-induced mice (reviewed by Boss et al 2017). TNF-α, another inflammatory initiator mediating NF-κB activation, mainly induces leukocyte-capillary adhesion, and subsequent pericyte loss and capillary degeneration in diabetic rat retinas (Behl et al., 2009; Joussen et al., 2009).

Similarly, other inflammatory factors, such as IL-6, IL-8, and IL-12, are also found in high concentrations in the vitreous of patients with progressive PDR (Doganay et al., 2002; Zorena et al., 2008; Gverović Antunica et al., 2012). Upon pro-inflammatory cytokine stimulation, endothelial cells produce intercellular adhesion molecules, recruiting leukocyte adhesion to capillaries. Once attached, these leukocytes induce capillary blockage and disrupt tight junctions between endothelial cells, accompanied by acellular capillary formation, vascular leakage and diabetic macular edema (DME) (Lutty, 2013). Several studies have revealed significant positive correlations between vitreous IL-6 and the subtype of DME with serous retinal detachment (Coscas et al., 2013; Chung et al., 2019). The *in vitro* studies demonstrated that inflammatory cytokines, rather than hyperglycemic stimuli, are largely responsible for endothelial apoptosis (Doganay et al., 2002).

Adhesion molecules and integrins

In addition, adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are indirectly involved in the inflammatory response (Chibber et al., 2007). Hyperglycemia and oxidative stress upregulate the expressions of ICAM-1 and VCAM-1, which further induces leukocyte-capillary endothelium adhesion at an early stage of DR. Mice deficient in ICAM-1 are significantly prevented from vascular injury in experimental DR, typically in pericyte ghost and capillary degeneration (Joussen et al., 2004).

Integrins, such as cluster of differentiation 11 (CD11)/CD18, have been found to be responsible for mediating leukocyte adhesion in early DR. Leukocyte activation, via integrins binding to the adhesion molecule, such as ICAM-1, on the endothelial surface, amplifies the leukostasis and retinal non-perfusion. Blockade of integrins attenuates the diabetes-induced leukostasis and vascular leakage in the retina (Van Hove et al., 2021). In addition, administration of leukocyte antagonist has been confirmed to significantly reduce retinal vasculopathy and inflammatory factors such as VEGF and TNF-α in diabetic rats (Rao et al., 2010).

VEGF and placental growth factor

VEGF is considered the predominant factor in the progression of DR. Accumulation of VEGF in the retina can trigger glial activation, which produces VEGF and numerous other vascular permeable factors associated with retinal neovascularization and macular edema. Elevated VEGF, binding to its receptors, induces endothelial cell proliferation, migration and neovascularization with increasing leakage (Ishida et al., 2003). In addition, VEGF has been shown to exacerbate cytokine release and leukostasis by promoting ICAM-1 expression of endothelial cells, which further amplifies the inflammatory response. Moreover, as a pro-inflammatory molecule, VEGF itself also promotes the expressions of other pro-inflammatory cytokines such as macrophage inflammatory protein-1α, monocyte chemotactic protein-1 (MCP-1) and IL-8. Specific blockade of VEGF decreased the levels of TNF-α, ICAM-1 and NF-κB in diabetic mice, alleviating retinal leukostasis and thus BRB breakdown (Ishida et al., 2003; Wang et al., 2010).

Placental growth factor (PlGF), a member of the VEGF family, is also detected at high levels in the vitreous of PDR patients (Mitamura et al., 2002). PlGF plays a physiological role in cell survival, and its dysregulation may correlate with DR development (Sensenbrenner, 1993). In contrast, PlGF deficiency in diabetic mice prevents BRB breakdown and retinal cell death (Huang et al., 2015).

Chemokines and other inflammatory mediators

Under inflammatory conditions, chemokines, such as CXC motif chemokine ligand 10 (CXCL10), C-C motif ligand-5 (CCL-5), and CCL-2 (also known as MCP-1), are upregulated in the vitreous and serum of DR patients (Demircan et al., 2006; Maier et al., 2008; Murugeswari et al., 2008). These chemokines facilitate leukocyte activation and recruitment, causing leakage of fluids and neutrophils from the vessels into retinal tissue. Inducible nitric oxide synthase (iNOS), together with cyclooxygenase-2 (Tang and Kern, 2011) participate in inflammation during DR progression.

Matrix metalloproteinases (MMPs) are major regulators in the initial phase of acute inflammation and the late phase of tissue remodeling (Kuno and Fujii, 2010), and play an important role in chemokine activation (Schwartz and Flynn, 2011). Studies have reported that increased MMP-2 and MMP-9 levels in the vitreous of DR patients contribute to retinal neovascularization (Kompella et al., 2010) and the level of angiopoietin-2, an important modulator of angiogenesis, is significantly upregulated in patients with DME (Campochiaro et al., 2012).

Table 1 | **Cytokines mediated inflammation in patients with diabetic retinopathy**

Category	Mediators	Mechanism	Involved in DR stage	References
Inflammatory cytokines	TNF-α	Mediating leukocyte adhesion; inducing ROS formation, iNOS expression and NF-κB activation	NPDR, and increased with DR progression	Joussen et al., 2009; Huang et al., 2011
	IL-1β	Increasing ICAM-1 expression	NPDR, and increased with DR progression	Zorena et al., 2008; Gverović Antunica et al., 2012; Boss et al., 2017
	IL-6	Increasing vascular permeability; angiogenesis	Upregulated in PDR, DME	Coscas et al., 2013; Chung et al., 2019
	IL-8	Activating the chemotaxis of neutrophils and monocytes; inducing angiogenesis	Upregulated in PDR, DME	Gverović Antunica et al., 2012; Boss et al., 2017; Chung et al., 2019
	IL-12	Neurotoxic effect	Upregulated in PDR	Gverović Antunica et al., 2012; Cheng et al., 2015; Al-Rashed et al., 2020
Chemokines	CCL-5	Immuno-stimulation	Upregulated in PDR, DME	Demircan et al., 2006; Murugeswari et al., 2008
	CCL-2/ MCP-1	Recruiting leukocytes; activating microglia; fibrosis and angiogenesis	Upregulated in PDR	Maier et al., 2008; Murugeswari et al., 2008
	CXCL10	Immuno-stimulation	Upregulated in PDR	Maier et al., 2008; Murugeswari et al., 2008
Adhesion molecules	ICAM-1	Increasing leukocyte adhesion, endothelial cell damages, and capillary nonperfusion	NPDR, and increased with DR progression	Joussen et al., 2004; Chibber et al., 2007; Rao et al., 2010; Lutty, 2013
	VCAM-1	Increasing pro-inflammatory factors such as TNF-α and IL-1β	NPDR, and increased with DR progression	Chibber et al., 2007
Growth factors	VEGF	Increasing vascular permeability and angiogenesis	Upregulated in PDR, DME	Wang et al., 2010; Coscas et al., 2013; Chung et al., 2019
	PEDF	Anti-angiogenic and anti-inflammatory factor	Decreased in PDR	Zhang et al., 2006; Calado et al., 2016
	TGF-β	Regulating cell growth, differentiation, proliferation, and apoptosis	Upregulated in PDR	Bhatt and Addepalli, 2010; Praidou et al., 2011; Hirsch et al., 2015
	PlGF	Cell survival and vascularization	Upregulated in PDR	Sensenbrenner, 1993; Mitamura et al., 2002; Huang et al., 2015
Others	COX-2	Angiogenesis	Upregulated in PDR	Psaltis and Simari, 2015; Madonna et al., 2016
	iNOS	Cellular toxicity	Upregulated in PDR	Tang and Kern, 2011; Cheng et al., 2015; Al-Rashed et al., 2020
	MMP-9	Immuno-stimulation; increasing chemokines and neurotoxic inflammation	Upregulated in NPDR, and PDR	Kompella et al., 2010; Kuno and Fujii, 2010; Schwartz and Flynn, 2011
	Ang-2	Angiogenesis	Upregulated in PDR	Campochiaro et al., 2012

Ang-2: Angiopoietin-2; CCL-5: chemokine ligand-5; COX-2: cyclooxygenase-2; CXCL10: CXC motif chemokine ligand 10; DME: diabetic macular edema; DR: diabetic retinopathy; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; iNOS: inducible nitric oxide synthase; MCP-1: monocyte chemotactic protein-1; MMP-9: matrix metalloproteinase-9; NPDR: non-proliferative DR; PEDF: pigment epithelium-derived factor; PlGF: placental growth factor; ROS: reactive oxygen species; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor.

Inflammation-Related Pathways in Diabetic Retinopathy Using Bioinformatics Analysis

We searched datasets in the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database using the keyword "diabetic retinopathy", and restricted to human species and retinal tissue. The GSE160306 dataset was the most suitable for this review, and was used for bioinformatics analysis. To identify the possible involvement of inflammation in DR, we analyzed the retinal mRNA expression profiles between DR patients and healthy individuals by downloading the retina gene profiles. Through bioinformatic analysis of GSE160306, 574 up-regulated genes and 225 down-regulated genes were enriched with the fold change > 1 and $P < 0.05$. Among these genes, the inflammation-related factors, such as TNF-α, NF-κB, IL-1β, IL-17, and transforming growth factor-β (TGF-β) were significantly increased (**Figure 1A** and **B**). The gene ontology (GO) terms were primarily enriched in inflammatory response, angiogenesis, signal transduction, and other processes (**Figure 1C**), and the Kyoto encyclopedia of genes and genomes (KEGG) database indicated that the significant genes were largely enriched in cell adhesion molecules, leukocyte trans-endothelial migration, and NF-κB signaling pathway (**Figure 1D**). Using Metascape's functional enrichment analysis, the enriched terms mainly included human TYRO protein tyrosine kinase-binding protein (TYROBP) causal network in microglia, cell migration, regulation of response to external stimulus, glial cell differentiation, and response to growth factor (**Figure 1E**). Results of the above bioinformatic analysis suggested the involvement of inflammatory signaling pathways and processes in DR (from our unpublished data).

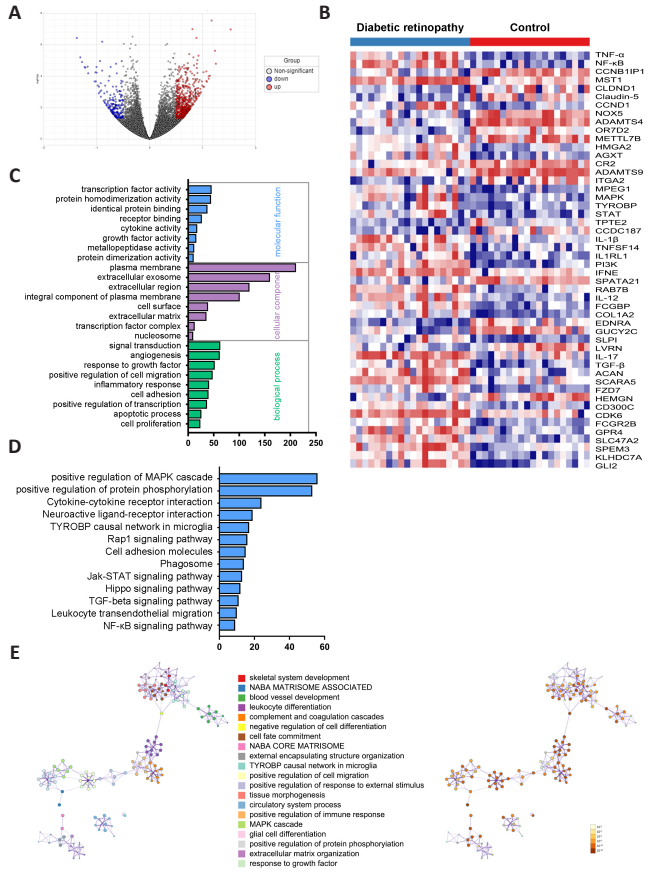

Figure 1 | **mRNA transcriptomes analysis in diabetic patient retinas.**
(A) Volcano plot of differentially expressed genes (DEGs) in the diabetic patient compared with those of healthy controls from the Gene Expression Omnibus (GEO) dataset. Red and blue indicate up-regulated and down-regulated genes, respectively. (B) The heatmap shows the top 50 genes from DEGs of diabetic retinas. The red and blue represents up- and down-regulated genes respectively. (C) Differentially expressed genes of diabetic retinas were enriched by gene ontology (GO) analysis including biological process, molecular function, and cellular component. (D) Kyoto encyclopedia of genes and genomes (KEGG) pathways enrichment for up-regulated genes. (E) Functional annotation of significantly expressed gene using Metascape. Orange denotes the enrichment terms color coded by *p*-values and an interactive network of the enrichment terms color coded by cluster ID. The retina gene profiles (GSE160306) were downloaded from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) and modified from our unpublished data.

Role of Inflammatory Cells in Diabetic Retinopathy

Retinal cell dysfunction may also amplify retinal inflammation at initial stages in DR. Retinal cell dysfunction, including microglial activation, Müller cell gliosis, retinal pigment epithelium (RPE) secretion, endothelial cell proliferation, and pericyte ghost, all involve in inner BRB disruption and macular edema in the progression of DR (Tang and Kern, 2011).

Müller cells

In the healthy retina, Müller cells span the entire neuronal retinal from the internal to external limiting membrane, and are responsible for retinal homeostasis, including structural support, neuronal metabolism, and blood vessel function (Coughlin et al., 2017). In DR, proliferation and reactive gliosis of Müller cells are indicated by increased expression of glial fibrillary acidic protein (GFAP) and increased secretion of IL-1β, IL-6, IL-8, VEGF, as well as ICAM-1 during diabetes progression, confirming their participation in the inflammatory process (Rangasamy et al., 2012; Nagayach et al., 2014). Other inflammatory cytokines, including iNOS, MCP-1, and prostaglandin E, are also produced by Müller cells when exposure to high glucose (Carpi-Santos et al., 2022), confirming that Müller cells are the main source of the inflammatory factors in DR. In addition, Müller cells may also directly influence microglia, enhancing microglial activation and migratory mobilization (Portillo et al., 2017).

RPE

Altered RPE secretion of growth factors and inflammatory cytokines also plays a role in inflammation and angiogenesis during DR (Ponnalagu et al., 2017). One of the most relevant molecules is pigment epithelium-derived factor, a potent anti-angiogenic and anti-inflammatory factor, which is decreased in PDR patients (Zhang et al., 2006). One study showed that sustained pigment epithelium-derived factor expression was able to downregulate VEGF and inflammatory molecules both in diabetic mice retinas and in human RPE cells *in vitro* (Calado et al., 2016).

TGF-β plays a role in regulating cell growth, proliferation, apoptosis and differentiation (Hirsch et al., 2015) and platelet derived growth factor (PDGF), secreted by RPE, is angiogenic (Lefevere et al., 2022). Both TGF-β and PDGF promote the proliferation of endothelial cells and induce angiogenesis (Bhatt and Addepalli, 2010), and have been detected at high concentrations in the vitreous of PDR patients (Praidou et al., 2011).

Endothelial cells

Under hyperglycemia, endothelial adhesion molecules stimulate leukocyte adherence to the endothelium (Chibber et al., 2007), which in turn induces endothelial activation and leukostasis. Leukocytes release inflammatory cytokines and vascular permeability factors, further disrupting endothelial junctional proteins, prompting leukocytic diapedesis into the retina (Zhang et al., 2011), and aggravating BRB breakdown. In addition to BRB disruption, increased VEGF promotes endothelium sprouting and angiogenesis (Chibber et al., 2007). Many studies have reported that ICAM-1 and VCAM-1 are increased in endothelial cells, mediating leukocyte adhesion in diabetic animals and patients (Kasza et al., 2017), while ICAM-1 deficiency results in significant reduction of adherent leukocytes (Joussen et al., 2004). Cyclooxygenase-2 and MMPs have been shown to co-localize in endothelial cells, and play an essential role in angiogenesis and vascularization. High glucose and cytokines strongly induce the expression of MMP-2, MMP-9, and cyclooxygenase-2 in human endothelial cells (Psaltis and Simari, 2015; Madonna et al., 2016).

Microglia

In view of numerous pro-inflammatory cytokines and chemokines found in the vitreous of diabetic patients, it is evident that microglia play a crucial role in triggering inflammatory response in DR.

In the normal retina, ramified microglia are scattered in the inner retina, while microglia are markedly activated and increased in number at different stages of DR (Zeng et al., 2008). An initial stimulus triggers the ameboid microglia to release anti-inflammatory cytokines, such as IL-4, IL-10, and IL-13, which resolve inflammation and enhance survival of neurons (Tang and Le, 2016). However, sustained hyperglycemia and harmful insults induce the overproduction of pro-inflammatory factors from microglia, such as TNF-α, IL-1β, and iNOS (Scholz et al., 2015), resulting in phagocytosis, neuronal death, and BRB breakdown. These inflammatory molecules also lead to activation of other glial cells such as astrocytes which respond by amplifying neuroinflammation.

Studies have confirmed that activated microglia proliferate and migrate into the plexiform layers in non-proliferative DR (NPDR) patients, whereas microglia gather at ischemic regions in PDR (Zeng et al., 2008; Okunuki et al., 2019; Kinuthia et al., 2020). High numbers of microglia are found throughout the retina, especially in the subretinal space, in DME patients (Zeng et al., 2008).

Similarly, microglial activation has been confirmed in animal models (Zeng et al., 2008; Xie et al., 2021). In our studies, the microglia in diabetic rat retina were activated compared with a normal control, characterized by enhanced migration and proliferation, as well as changed morphologies from ramified to amoeboid (**Figure 2**). Microglial activation occurs at the beginning of DR, producing plenty of proinflammatory cytokines, such as IL-1β, TNF-α, VEGF, and MMPs, which induce adhesion molecules (ICAM-1 and VCAM-1) secretion, cell apoptosis, leukocytes infiltration and BRB breakdown (Ibrahim et al., 2011).

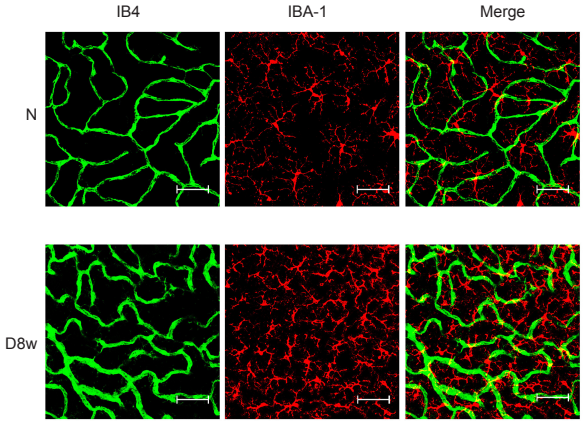

Figure 2 | **Representative images showing the relationship between blood vessels (IB4, green) and microglia (IBA-1, red) in deep capillary plexus of the retinal flatmounts in normal control (N) and 8-week diabetic (D8w) rat retinas.**
In normal controls, the microglia show ramified morphology and are distributed evenly in retina; while they became activated in diabetic group, showing characteristic amoeboid morphology and close proximity to retinal capillaries. N: Normal control group; D8w: 8-week diabetes group. Scale bars: 50 μm. Modified from our unpublished data.

Astrocytes

Astrocytes, mainly distributed in the nerve fiber layer in the healthy retina, surround the blood vessels and ganglion cells, and are responsible for the integrity of inner BRB and neurotrophic protection. In DR, the astrocytes decrease in number and reduce the expressions of connexin-26/43, glial cell line-derived neurotrophic factor (GDNF), and GFAP with disease progression. In addition, astrocytes amplify the inflammatory process by producing pro-inflammatory cytokines such as IL-1β, MCP-1 and VEGF, aggravating damage to the retinal neurovascular unit and resulting in neuroinflammation (Nagayach et al., 2014).

Macrophages

The breakdown of the BRB at an early stage of DR facilities blood monocytes entering the retina in significant numbers, and the inflammatory-immune response mediated by macrophages plays an important role in the development of DR.

Multiple studies have suggested macrophages as the primary driving force in the pathogenesis of PDR (Pavlou et al., 2018; Al-Rashed et al., 2020; Jia and Zhou, 2020). Macrophages induce chemotaxis and fibroplasia through secretion of leukotrienes and fibronectin. They also influence cellular proliferation via the synthesis of VEGF, PDGF, fibroblast growth factor (FGF), and TGF-β (Pavlou et al., 2018). Torres-Castro et al. (2016) showed that human monocytes and macrophages were activated both *in vitro* and *in vivo*, as the levels of CD11c and iNOS were upregulated in high glucose-treated macrophages, and in circulating monocytes of DR patients. Also, activated macrophages secrete excessive inflammatory factors such as IL-1β, TNF-α, IL-6, and IL-12 via the NF-κB signaling pathway (Cheng et al., 2015; Al-Rashed et al., 2020), while their phagocytosis function was damaged, suggesting the dysfunction of macrophages may aggravate the inflammation in DR.

Hyperreflective Foci on Optical Coherence Tomography Images Indicate Macrophage/Microglia Activation

Recently, retinal hyperreflective foci (HRF) on optical coherence tomography (OCT) have been considered to indicate active inflammatory cells, involved in the pathogenesis of many retinal diseases (Zur et al., 2018; Chung et al., 2019). HRF were first described by Coscas et al. (2013) as hyperreflective dots (HRD) by spectral-domain OCT in age-related macular degeneration patients. Subsequently, HRF have been identified in DR, DME, retinal vein occlusion, as well as retinal degenerative diseases (Romano et al., 2020). Although there is still no consensus on their origin, HRF may correlate with inflammatory microenvironments and reflect a damaged retinal state.

Typically, HRF on OCT angiography appear as discrete intraretinal spots distinct from hard exudates, and may represent activated microglia or macrophages in DR (Vujosevic et al., 2017). According to OCT angiography images from our research, HRF are mainly distributed in the inner retina of DME patients compared with controls, while HRF were also detected around the subretinal fluid (SRF) in DME with serous retinal detachment (**Figure 3**). In one study, DME patients with diffuse edema exhibited high level of sCD14 in the aqueous and the inner retina showed increased number of HRF which correlated with level of sCD14, a biomarker of microglia and macrophage, suggesting that the HRF observed on OCT may reflect the activated microglia in DME (Lee et al., 2018).

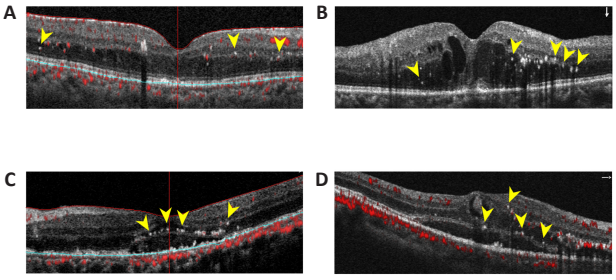

Figure 3 | **Representative optical coherence tomography angiography (OCTA) images showing the subtypes of diabetes macular edema (DME) with hyperreflective foci (HRF).**
(A) Diffuse macular edema, (B) cystoid macular edema, (C) DME with subretinal fluid (DME-SRF), and (D) mixed type. The HRF are indicated by arrowheads. Modified from our unpublished data.

Current and Novel Therapeutic Strategies in Diabetic Retinopathy

Inflammation-related treatments

Since inflammation has been recognized as an important mechanism for DR pathogenesis, significant effort has been made to target the inflammatory process (**Table 2**).

Corticosteroids

Corticosteroids, such as triamcinolone acetonide (TA), dexamethasone, and fluocinolone acetonide (FA), significantly reduce cellular swelling and prompt fluid reabsorption, thus reducing vascular permeability and restoring BRB integrity in DR (Himasa et al., 2022). As potent anti-inflammatory agents, corticosteroids also target a broad range of mediators, including adhesion molecules, chemokines, and inflammatory molecules (Jeong et al., 2017).

TA, as a potent glucocorticoid, has been shown to inhibit inflammatory factors under hypoxic conditions and decrease central macular thickness and neovascularization by downregulating ICAM-1, TNF-α, and IL-6 (Semeraro et al., 2019). Gillies et al. (2006) showed that intravitreal injection of TA in combination with laser was more effective in improving visual acuity and reducing macular edema than laser photocoagulation.

Several studies have demonstrated that intravitreal dexamethasone is effective in DME treatment, especially for patients unresponsive to anti-VEGF therapy (Lattanzio et al., 2017). Ozurdex, as a sterile, sustained-release implant of dexamethasone, has been implied in the treatment of macular edema. Two phase III pivotal trials of Ozurdex for diabetic macular edema (NCT00168337 and NCT00168389) showed that Ozurdex (0.35 and 0.7 mg) achieved significant improvement in vision and retinal thickness reduction in DME patients, without significant cataract or increased intraocular pressure complication.

Two FA implants have been applied in clinical trials for DME treatment. Retisert (0.59 mg) is superior at improving best-corrected visual acuity (BCVA) in DR patients especially with persistent DME (Wang and Lo, 2018). However, considering the high incidence of complications, such as intraocular pressure elevation and cataract, Iluvien (0.19 mg) has been designed as an alternative which successfully increases BCVA in persistent DME patients with a significantly low rate of intraocular pressure elevation in a two-year study (Studsgaard et al., 2022).

Antagonists of inflammatory factors

Several studies have demonstrated the role of inflammatory inhibitors in DR treatment. Canakinumab, a selective IL-1β antibody, can stabilize retinal neovascularization and reduce macular edema in PDR patients (Stahel et al., 2016). In addition, capillary degeneration is prevented in mice lacking the IL-1β receptor (Vincent and Mohr, 2007).

TNF-α, as an up-regulated proinflammatory factor in the vitreous of DR patients, has been identified as a promising target for DR (Demircan et al., 2006). Consistent with the role of IL-1β, mice genetically deficient in TNF-α show alleviated leukostasis, vascular leakage, and loss of pericyte and endothelial cells (Joussen et al., 2009; Huang et al., 2011). Infliximab, as a monoclonal antibody against TNF-α to treat Crohn's disease, was also applied in DME patients and showed positive results, including improvement in visual acuity and reduction in macular thickness (Markomichelakis et al., 2005). Infliximab also alleviates retinal capillary degeneration and pericyte loss (Behl et al., 2009), thus preventing BRB breakdown in diabetes.

Antagonism of the adhesion molecule and integrin is also under clinical investigation. Leucocyte function-associated antigen-1, which binds to ICAM-1, is responsible for leucocyte-endothelial cell interaction. One study showed that lifitegrast, an antagonist of leucocyte function-associated antigen-1, decreased leukostasis and retinal vascular leakage in diabetic rat retina (Rao et al., 2010). Similarly, anti-CD49 antibody, blocking interactions between VCAM-1 on endothelial cells and very late antigen-4 on leucocytes, significantly attenuates diabetes-induced leukostasis and vascular leakage (Iliaki et al., 2009). Moreover, the anti-CD49 antibody reduces levels of VEGF and TNF-α

by inhibiting NF-κB activity, indicating that leucocyte recruitment provides positive feedback to the DR inflammatory pathway (Zhang et al., 2011).

Luminate (ALG-1001), the integrin inhibitor, has promising effects on visual acuity and alleviating macula edema for DME by binding to the integrin receptors (Shaw et al., 2020).

Other treatments

As the VEGF level is significantly elevated in DR patients and increases with disease progression, anti-VEGF treatment becomes the first-line therapy in treatment of DR and DME. Meanwhile, anti-angiogenesis as well as other none-inflammatory treatment have been under development (**Table 3**).

Anti-VEGF agents

Currently, anti-VEGF agents, including brolucizumab, bevacizumab, ranibizumab, conbercept, and aflibercept, benefit millions of DME patients with improved visual function and decreasing macular edema.

Brolucizumab has a low molecular weight and is the first single-chain antibody fragment designed specifically to target VEGF-A via intraocular use in humans (Markham, 2019). Two phase III pivotal trials of brolucizumab for DME showed that at final visit, brolucizumab (6 mg) was not iinferior to aflibercept in BCVA improvement in patients with DME, while more subjects achieved anatomical improvements such as central subfield thickness (CSFT) < 280 μm, and decreased subretinal and intraretinal fluid compared with aflibercept (Brown et al., 2022).

Ranibizumab has been comprehensively evaluated in clinical trials, and the results show that it is effective in BCVA improvement (Spooner et al., 2020). In terms of OCT results, bevacizumab effectively reduced diffuse macular edema (Fazel et al., 2020). However, DME patients with subretinal fluid (DME-SRF) may not respond well to anti-VEGF treatments due to external limiting membrane and RPE impairment. The randomized controlled trial of DRCT. NET, Protocol T (NCT01627249) showed that aflibercept was superior to ranibizumab in improving BCVA of DME patients with poorer baseline visual acuity.

Anti-angiogenic therapy

Currently, several anti-angiogenic agents are undergoing clinical trials. Squalamine presented better visual improvement in DME patients by inhibiting multiple angiogenic factors, including VEGF, PDGF and bFGF (Wroblewski and Hu, 2016). AKB-9778, a small molecule targeting Ang/Tie2 signaling pathways, activates Tie2 by inhibiting VE-PTP and decreases vascular permeability (Campochiaro et al., 2016). Nesvacumab, an inhibitor of Ang-2, also decreases vascular permeability by activating Tie2. Faricimab, a bispecific antibody targeting both Ang-2 and VEGF-A, was comparable to aflibercept in DME treatment, with a longer treatment period (Sahni et al., 2019).

Targeting the renin-angiotensin system (RAS) and AGEs

RAS could be a promising target for DR treatment as it participates in diabetes or hypertension-induced retinal inflammation. Specific blockade of the RAS with angiotensin converting enzyme (ACE) inhibitor and angiotensin type-1 receptor blocker has been shown to prevent inflammation, oxidative stress, and vascular injury in diabetic animal models (Zhang et al., 2007).

AGEs and the receptor for AGEs, involved in inflammatory mechanisms, may also be promising targets for DR (Zhang et al., 2011). Studies have shown that inhibiting AGE formation improves neuronal function and prevents pericyte loss and acellular capillaries in diabetic mice (Barile et al., 2005).

Table 2 | **Mechanism-based treatments targeting inflammation in diabetic retinopathy and diabetic macular edem**

Classification	Drugs	Target	Mechanism	Clinical Trial ID and status	Clinical outcomes	Side effects
Corticosteroids	Triamcinolone acetonide	Glucocorticoid receptor	Blocking proinflammatory factors, leukostasis, and vascular leakage	NCT00367133; Off-label use	Greater BCVA improvements in combination with laser treatment in PDR	a. Cataract progression b. Increased intraocular pressure (IOP)
	Dexamethasone (Ozurdex)			NCT01309451; FDA approved	Greater BCVA improvement and greater reduction in CMT in DME patients	c. Vitreous hemorrhage
	Fluocinolone acetonide (Iluvien)			NCT02472366; FDA approved	Greater BCVA improvement in DME patients	d. Glaucoma
Antagonists of inflammation	Infliximab	TNF-α	Blocking TNF-α induced inflammation; reducing pro-angiogenetic stimuli	NCT00695682; Ongoing	Not provided	Not provided
	Lifitegrast (SAR 1118)	LFA-1	Decreasing leukostasis and retinal vascular leakage	NCT00936520; Terminated	Not provided	Not provided
	Luminate (ALG-1001)	Integrins	Blocking multiple integrin receptors	NCT02348918; Completed	Non-inferiority to bevacizumab in BCVA and CMT for the treatment of DME	Well-tolerated with no drug toxicity or intraocular inflammation noted
	Canakinumab (ILARIS®)	IL-1β	Stabilizing retinal neovascularization and reducing macular edema	NCT01589029; Terminated	Not provided	Not provided
	Tocilizumab	IL-6 receptor	Blocking pro-inflammation	NCT02511067; Withdraw	Not provided	Not provided
	EBI-031	IL-6	Blocking pro-inflammation	NCT02842541; Withdraw	Not provided	Not provided

BCVA: Best-corrected visual acuity; CMT: central macular thickness; DME: diabetic macular edema; FDA: U.S. Food and Drug Administration; IL: interleukin; LFA-1: lymphocyte function-associated antigen 1; PDR: proliferative diabetic retinopathy; TNF-α: tumor necrosis factor-α.

Table 3 | **Other treatments for diabetic retinopathy and diabetic macular edema**

Classification	Drugs	Target	Mechanism
Anti-VEGF	Brolucizumab	VEGF	Blocking VEGF-mediated inflammation, vascular permeability, and angiogenesis
	Ranibizumab		
	Conbercept		
	Aflibercept		
Anti-angiogenesis	Squalamine	Multiple angiogenic factors, including VEGF, PDGF, bFGF	Inhibiting angiogenesis
	AKB-9778	VE-PTP	Decreasing vascular permeability and angiogenesis
	Nesvacumab	Ang-2	
	Faricimab	Ang-2 and VEGF-A	
RAS blockage	Losartan	AT1R	Preventing oxidative stress, inflammation, and vascular damage
	Candesartan		
	Enalapril	ACE	
Others	Curcumin	VEGF, TNF-α, IL-8 and MCP-1	Preventing neurodegeneration and vascular leakage; inhibiting angiogenesis and inflammation
	Resveratrol	Caspase-3 and caspase-8	Suppressing inflammation and apoptosis; modulating neovascularization
	Palmitoylethanolamide	NF-κB	Preventing microglia activation; downregulating reactive gliosis; decreasing proinflammatory molecules and cytokines
	Melatonin	ICAM-1, IL-1β, TNF-α	Preventing microglia activation, stabilizing tight junction; decreasing inflammatory factors

ACE: Angiotensin converting enzyme; Ang-2: angiopoietin-2; AT1R: angiotensin type-1 receptor; bFGF: basic fibroblast growth factor; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; PDGF: platelet derived growth factor; RAS: renin-angiotensin system; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; VE-PTP: vascular endothelial protein tyrosine phosphatase.

Other potential treatments

Recently, increasing attention has been paid to supplemental therapies for DR. Agents, such as curcumin, resveratrol, palmitoylethanolamide and melatonin have regulated pathophysiological changes in DR via their anti-inflammatory and anti-proliferative properties.

Curcumin prevents neurodegeneration and vascular alterations, including restoration of blood vessels and repair of choroidal microvascular, by decreasing TNF-α and VEGF, and extracellular matrix production in DR (Yang et al., 2021).

Resveratrol, an antioxidant and neuroprotective agent, is particularly effective in the improvement in DR (Seong et al., 2017). Resveratrol suppresses apoptosis of retinal ganglion cells by downregulation of caspase-3 and caspase-8 expression in diabetes-induced mouse retinas (Chalke and Kale, 2021).

Palmitoylethanolamide, as an endogenous cell-protective lipid, inhibits oxidant stress and reactive gliosis between glial cells, thereby decreasing the levels of proinflammatory molecules and cytokines (Rajamani and Jialal, 2014).

Melatonin, produced in pineal gland and retina, regulates redox reactions and dopamine metabolism. Our previous work demonstrated that melatonin maintained the inner BRB by up-regulating the expressions of tight junction proteins and decreasing the production of inflammatory factors such as ICAM-1, IL-1β, and TNF-α (Tang et al., 2021).

Discussion

DR, characterized by microangiopathy and neuronal degeneration, is the main cause of visual impairment in diabetes patients. Increasing data confirm that chronic, low-grade inflammation plays an important role in the pathogenesis and development of DR. The inflammation-related cells, together with secreted inflammatory factors, promote microinflammation and aggravate destruction of the BRB. Thus, this review highlights the role of inflammatory cells and proinflammatory cytokines in the pathogenesis of DR, as well as anti-inflammatory treatments for DR and DME.

Although anti-VEGF therapies remain the first-line treatments for PDR and DME, their limitations including frequent injections, increasing financial burden, poor compliance of patients, and a lack of effect, are of major concern. The etiology of DR is multifactorial, and in addition to BRB breakdown pathogenesis involves inflammation. In DR patients, increased levels of serum C-reactive protein (Yang et al., 2016) and intraocular inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and MCP-1 (Tamura et al., 2012), have been detected. In addition, hyperglycemia-induced activation of retinal microglia, together with infiltration of immune cells such as neutrophils and macrophages, may contribute to the pathogenesis of DR (Xu and Chen, 2017). For example, patients with persistent DME who are not responsive to intravitreal bevacizumab, intravitreal methotrexate, synthetic folic acid analogue with anti-proliferative and anti-inflammatory properties, showed anatomical improvement in a significant proportion of eyes with a significant visual improvement in 16.6 % of eyes (Falavarjani et al., 2016). For this reason, it is important to explore immunosuppressive or immunomodulating therapies for the treatment of DR (Xu and Chen, 2017).

In this review, we explored the role of inflammatory factors and related signaling pathways with bioinformatics analysis in DR patients from public databases. Currently, in addition to anti-VEGF drugs and steroid hormones (such as dexamethasone, TA and FA), many drugs targeting inflammation-related molecules, such as infliximab, lifitegrast (SAR 1118), luminate (ALG-1001), canakinumab, tocilizumab and EBI-031, have already entered the clinical trial stage. However, most of the trials have been terminated or withdrawn, and have not achieved the primary endpoint. Limitations of the trials may include the fact that numerous cytokines are involved in inflammation, oxidative stress and apoptosis in DR pathogenesis, so it is difficult to treat DR by antagonizing a single molecule. The retina is a complex structure and DR involves interaction of all retinal cell types, so potential therapeutics targeting multiple molecules or pathways, instead of only one should be the focus of future research.

In conclusion, inflammation plays a pivotal role in the pathogenesis of DR and DME. Elucidation of the key inflammatory factors in DR would aid the development of mechanism-based therapies targeting inflammation as well as combination therapy (such as anti-VEGF combined with anti-inflammatory drugs) the latter having potential for comprehensive and personalized treatment in DR patients.

Author contributions: *LT, GTX and JFZ contributed to the conception, design and definition of intellectual content. LT contributed to the data analysis and manuscript preparation. GTX and JFZ revised the article critically for important intellectual content and are guarantors of this work, who had full access to all the data in this study and take responsibility for the integrity and accuracy of the data. All authors approved the final version of the manuscript.*

Conflicts of interest: *The authors declare that there is no conflict of interest regarding the publication of this paper.*

References

Al-Rashed F, Sindhu S, Arefanian H, Al Madhoun A, Kochumon S, Thomas R, Al-Kandari S, Alghaith A, Jacob T, Al-Mulla F, Ahmad R (2020) Repetitive intermittent hyperglycemia drives the M1 polarization and inflammatory responses in THP-1 macrophages through the mechanism involving the TLR4-IRF5 pathway. Cells 9:1892.

Altmann C, Schmidt MHH (2018) The role of microglia in diabetic retinopathy: inflammation, microvasculature defects and neurodegeneration. Int J Mol Sci 19:110.

Barile GR, Pachydaki SI, Tari SR, Lee SE, Donmoyer CM, Ma W, Rong LL, Buciarelli LG, Wendt T, Hörig H, Hudson BI, Qu W, Weinberg AD, Yan SF, Schmidt AM (2005) The RAGE axis in early diabetic retinopathy. Invest Ophthalmol Vis Sci 46:2916-2924.

Behl Y, Krothapalli P, Desta T, Roy S, Graves DT (2009) FOXO1 plays an important role in enhanced microvascular cell apoptosis and microvascular cell loss in type 1 and type 2 diabetic rats. Diabetes 58:917-925.

Bhatt LK, Addepalli V (2010) Attenuation of diabetic retinopathy by enhanced inhibition of MMP-2 and MMP-9 using aspirin and minocycline in streptozotocin-diabetic rats. Am J Transl Res 2:181-189.

Boss JD, Singh PK, Pandya HK, Tosi J, Kim C, Tewari A, Juzych MS, Abrams GW, Kumar A (2017) Assessment of neurotrophins and inflammatory mediators in vitreous of patients with diabetic retinopathy. Invest Ophthalmol Vis Sci 58:5594-5603.

Brown DM, Emanuelli A, Bandello F, Barranco JJE, Figueira J, Souied E, Wolf S, Gupta V, Ngah NF, Liew G, Tuli R, Tadayoni R, Dhoot D, Wang L, Bouillaud E, Wang Y, Kovacic L, Guerard N, Garweg JG (2022) KESTREL and KITE: 52-week results from two phase III pivotal trials of brolucizumab for diabetic macular edema. Am J Ophthalmol 238:157-172.

Calado SM, Diaz-Corrales F, Silva GA (2016) pEPito-driven PEDF expression ameliorates diabetic retinopathy hallmarks. Hum Gene Ther Methods 27:79-86.

Campochiaro PA, Brown DM, Pearson A, Chen S, Boyer D, Ruiz-Moreno J, Garretson B, Gupta A, Hariprasad SM, Bailey C, Reichel E, Soubrane G, Kapik B, Billman K, Kane FE, Green K (2012) Sustained delivery fluocinolone acetonide vitreous inserts provide benefit for at least 3 years in patients with diabetic macular edema. Ophthalmology 119:2125-2132.

Campochiaro PA, Khanani A, Singer M, Patel S, Boyer D, Dugel P, Kherani S, Withers B, Gambino L, Peters K, Brigell M; TIME-2 Study Group (2016) Enhanced benefit in diabetic macular edema from AKB-9778 Tie2 activation combined with vascular endothelial growth factor suppression. Ophthalmology 123:1722-1730.

Carpi-Santos R, de Melo Reis RA, Gomes FCA, Calaza KC (2022) Contribution of Müller cells in the diabetic retinopathy development: focus on oxidative stress and inflammation. Antioxidants (Basel) 11:617.

Chalke SD, Kale PP (2021) Combinational approaches targeting neurodegeneration, oxidative stress, and inflammation in the treatment of diabetic retinopathy. Curr Drug Targets 22:1810-1824.

Cheng CI, Chen PH, Lin YC, Kao YH (2015) High glucose activates Raw264.7 macrophages through RhoA kinase-mediated signaling pathway. Cell Signal 27:283-292.

Chibber R, Ben-Mahmud BM, Chibber S, Kohner EM (2007) Leukocytes in diabetic retinopathy. Curr Diabetes Rev 3:3-14.

Chung YR, Kim YH, Ha SJ, Byeon HE, Cho CH, Kim JH, Lee K (2019) Role of inflammation in classification of diabetic macular edema by optical coherence tomography. J Diabetes Res 2019:8164250.

Coscas G, De Benedetto U, Coscas F, Li Calzi CI, Vismara S, Roudot-Thoraval F, Bandello F, Souied E (2013) Hyperreflective dots: a new spectral-domain optical coherence tomography entity for follow-up and prognosis in exudative age-related macular degeneration. Ophthalmologica 229:32-37.

Coughlin BA, Feenstra DJ, Mohr S (2017) Muller cells and diabetic retinopathy. Vision Res 139:93-100.

Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S (2006) Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (London, England) 20:1366-1369.

Doganay S, Evereklioglu C, Er H, Türköz Y, Sevinç A, Mehmet N, Savli H (2002) Comparison of serum NO, TNF-alpha, IL-1beta, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. Eye (Lond) 16:163-170.

Falavarjani KG, Golabi S, Modarres M (2016) Intravitreal injection of methotrexate in persistent diabetic macular edema: a 6-month follow-up study. Graefes Arch Clin Exp Ophthalmol 254:2159-2164.

Fazel F, Nikpour H, Pourazizi M (2020) Combination of intravitreal bevacizumab and topical dorzolamide versus intravitreal bevacizumab alone for diabetic macular edema: a randomized contralateral clinical trial. Biomed Res Int 2020:6794391.

Gillies MC, Sutter FK, Simpson JM, Larsson J, Ali H, Zhu M (2006) Intravitreal triamcinolone for refractory diabetic macular edema: two-year results of a double-masked, placebo-controlled, randomized clinical trial. Ophthalmology 113:1533-1538.

Gverović Antunica A, Karaman K, Znaor L, Sapunar A, Buško V, Puzović V (2012) IL-12 concentrations in the aqueous humor and serum of diabetic retinopathy patients. Graefes Arch Clin Exp Ophthalmol 250:815-821.

Himasa FI, Singhal M, Ojha A, Kumar B (2022) Prospective for diagnosis and treatment of diabetic retinopathy. Curr Pharm Des 28:560-569.

Hirsch L, Nazari H, Sreekumar PG, Kannan R, Dustin L, Zhu D, Barron E, Hinton DR (2015) TGF-β2 secretion from RPE decreases with polarization and becomes apically oriented. Cytokine 71:394-396.

Huang H, Gandhi JK, Zhong X, Wei Y, Gong J, Duh EJ, Vinores SA (2011) TNFalpha is required for late BRB breakdown in diabetic retinopathy, and its inhibition prevents leukostasis and protects vessels and neurons from apoptosis. Invest Ophthalmol Vis Sci 52:1336-1344.

Huang H, He J, Johnson D, Wei Y, Liu Y, Wang S, Lutty GA, Duh EJ, Semba RD (2015) Deletion of placental growth factor prevents diabetic retinopathy and is associated with Akt activation and HIF1α-VEGF pathway inhibition. Diabetes 64:200-212.

Ibrahim AS, El-Remessy AB, Matragoon S, Zhang W, Patel Y, Khan S, Al-Gayyar MM, El-Shishtawy MM, Liou GI (2011) Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes. Diabetes 60:1122-1133.

Iliaki E, Poulaki V, Mitsiades N, Mitsiades CS, Miller JW, Gragoudas ES (2009) Role of alpha 4 integrin (CD49d) in the pathogenesis of diabetic retinopathy. Invest Ophthalmol Vis Sci 50:4898-4904.

Ishida S, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP (2003) VEGF164 is proinflammatory in the diabetic retina. Invest Ophthalmol Vis Sci 44:2155-2162.
Jeong S, Ku SK, Bae JS (2017) Anti-inflammatory effects of pelargonidin on TGFBIp-induced responses. Can J Physiol Pharmacol 95:372-381.
Jia Y, Zhou Y (2020) Involvement of lncRNAs and macrophages: potential regulatory link to angiogenesis. J Immunol Res 2020:1704631.
Joussen AM, Doehmen S, Le ML, Koizumi K, Radetzky S, Krohne TU, Poulaki V, Semkova I, Kociok N (2009) TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. Mol Vis 15:1418-1428.
Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP (2004) A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J 18:1450-1452.
Kasza M, Meleg J, Vardai J, Nagy B, Jr., Szalai E, Damjanovich J, Csutak A, Ujhelyi B, Nagy V (2017) Plasma E-selectin levels can play a role in the development of diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 255:25-30.
Kinuthia UM, Wolf A, Langmann T (2020) Microglia and inflammatory responses in diabetic retinopathy. Front Immunol 11:564077.
Kompella UB, Kadam RS, Lee VH (2010) Recent advances in ophthalmic drug delivery. Ther Deliv 1:435-456.
Kuno N, Fujii S (2010) Biodegradable intraocular therapies for retinal disorders: progress to date. Drugs Aging 27:117-134.
Lattanzio R, Cicinelli MV, Bandello F (2017) Intravitreal steroids in diabetic macular edema. Dev Ophthalmol 60:78-90.
Lee H, Jang H, Choi YA, Kim HC, Chung H (2018) Association between soluble CD14 in the aqueous humor and hyperreflective foci on optical coherence tomography in patients with diabetic macular edema. Invest Ophthalmol Vis Sci 59:715-721.
Lefevere E, Van Hove I, Sergeys J, Steel DHW, Schlingemann R, Moons L, Klaassen I (2022) PDGF as an important initiator for neurite outgrowth associated with fibrovascular membranes in proliferative diabetic retinopathy. Curr Eye Res 47:277-286.
Lu L, Jiang Y, Jaganathan R, Hao Y (2018) Current advances in pharmacotherapy and technology for diabetic retinopathy: a systematic review. J Ophthalmol 2018:1694187.
Lutty GA (2013) Effects of diabetes on the eye. Invest Ophthalmol Vis Sci 54:ORSF81-87.
Madonna R, Giovannelli G, Confalone P, Renna FV, Geng YJ, De Caterina R (2016) High glucose-induced hyperosmolarity contributes to COX-2 expression and angiogenesis: implications for diabetic retinopathy. Cardiovasc Diabetol 15:18.
Maier R, Weger M, Haller-Schober EM, El-Shabrawi Y, Wedrich A, Theisl A, Aigner R, Barth A, Haas A (2008) Multiplex bead analysis of vitreous and serum concentrations of inflammatory and proangiogenic factors in diabetic patients. Mol Vis 14:637-643.
Markham A (2019) Brolucizumab: first approval. Drugs 79:1997-2000.
Markomichelakis NN, Theodossiadis PG, Sfikakis PP (2005) Regression of neovascular age-related macular degeneration following infliximab therapy. Am J Ophthalmol 139:537-540.
Mitamura Y, Tashimo A, Nakamura Y, Tagawa H, Ohtsuka K, Mizue Y, Nishihira J (2002) Vitreous levels of placenta growth factor and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Diabetes Care 25:2352.
Murugeswari P, Shukla D, Rajendran A, Kim R, Namperumalsamy P, Muthukkaruppan V (2008) Proinflammatory cytokines and angiogenic and anti-angiogenic factors in vitreous of patients with proliferative diabetic retinopathy and eales' disease. Retina 28:817-824.
Nagayach A, Patro N, Patro I (2014) Astrocytic and microglial response in experimentally induced diabetic rat brain. Metab Brain Dis 29:747-761.
Okunuki Y, Mukai R, Nakao T, Tabor SJ, Butovsky O, Dana R, Ksander BR, Connor KM (2019) Retinal microglia initiate neuroinflammation in ocular autoimmunity. Proc Natl Acad Sci U S A 116:9989-9999.
Pavlou S, Lindsay J, Ingram R, Xu H, Chen M (2018) Sustained high glucose exposure sensitizes macrophage responses to cytokine stimuli but reduces their phagocytic activity. BMC Immunol 19:24.
Ponnalagu M, Subramani M, Jayadev C, Shetty R, Das D (2017) Retinal pigment epithelium-secretome: A diabetic retinopathy perspective. Cytokine 95:126-135.
Portillo JC, Lopez Corcino Y, Miao Y, Tang J, Sheibani N, Kern TS, Dubyak GR, Subauste CS (2017) CD40 in retinal müller cells induces P2X7-dependent cytokine expression in macrophages/microglia in diabetic mice and development of early experimental diabetic retinopathy. Diabetes 66:483-493.
Praidou A, Papakonstantinou E, Androudi S, Georgiadis N, Karakiulakis G, Dimitrakos S (2011) Vitreous and serum levels of vascular endothelial growth factor and platelet-derived growth factor and their correlation in patients with non-proliferative diabetic retinopathy and clinically significant macula oedema. Acta Ophthalmol 89:248-254.
Psaltis PJ, Simari RD (2015) Vascular wall progenitor cells in health and disease. Circ Res 116:1392-1412.
Rajamani U, Jialal I (2014) Hyperglycemia induces Toll-like receptor-2 and-4 expression and activity in human microvascular retinal endothelial cells: implications for diabetic retinopathy. J Diabetes Res 2014:790902.
Rangasamy S, McGuire PG, Das A (2012) Diabetic retinopathy and inflammation: novel therapeutic targets. Middle East Afr J Ophthalmol 19:52-59.
Rao VR, Prescott E, Shelke NB, Trivedi R, Thomas P, Struble C, Gadek T, O'Neill CA, Kompella UB (2010) Delivery of SAR 1118 to the retina via ophthalmic drops and its effectiveness in a rat streptozotocin (STZ) model of diabetic retinopathy (DR). Invest Ophthalmol Vis Sci 51:5198-5204.
Romano F, Arrigo A, MacLaren RE, Charbel Issa P, Birtel J, Bandello F, Battaglia Parodi M (2020) Hyperreflective foci as a pathogenetic biomarker in choroideremia. Retina 40:1634-1640.
Rübsam A, Parikh S, Fort PE (2018) Role of inflammation in diabetic retinopathy. Int J Mol Sci 19:942.
Sahni J, Patel SS, Dugel PU, Khanani AM, Jhaveri CD, Wykoff CC, Hershberger VS, Pauly-Evers M, Sadikhov S, Szczesny P, Schwab D, Nogoceke E, Osborne A, Weikert R, Fauser S (2019) Simultaneous inhibition of angiopoietin-2 and vascular endothelial growth factor-A with faricimab in diabetic macular edema: BOULEVARD phase 2 randomized trial. Ophthalmology 126:1155-1170.
Scholz R, Caramoy A, Bhuckory MB, Rashid K, Chen M, Xu H, Grimm C, Langmann T (2015) Targeting translocator protein (18 kDa) (TSPO) dampens pro-inflammatory microglia reactivity in the retina and protects from degeneration. J Neuroinflammation 12:201.
Schwartz SG, Flynn HW, Jr. (2011) Fluocinolone acetonide implantable device for diabetic retinopathy. Curr Pharm Biotechnol 12:347-351.
Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C (2015) Diabetic retinopathy: vascular and inflammatory disease. J Diabetes Res 2015:582060.
Semeraro F, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C (2019) Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. Diabetes Metab 45:517-527.
Sensenbrenner M (1993) The neurotrophic activity of fibroblast growth factors. Prog Neurobiol 41:683-704.
Seong H, Ryu J, Yoo WS, Kim SJ, Han YS, Park JM, Kang SS, Seo SW (2017) Resveratrol ameliorates retinal ischemia/reperfusion injury in C57BL/6J mice via downregulation of Caspase-3. Curr Eye Res 42:1650-1658.
Shaw LT, Mackin A, Shah R, Jain S, Jain P, Nayak R, Hariprasad SM (2020) Risuteganib-a novel integrin inhibitor for the treatment of non-exudative (dry) age-related macular degeneration and diabetic macular edema. Expert Opin Investig Drugs 29:547-554.
Sorrentino FS, Allkabes M, Salsini G, Bonifazzi C, Perri P (2016) The importance of glial cells in the homeostasis of the retinal microenvironment and their pivotal role in the course of diabetic retinopathy. Life Sci 162:54-59.
Spencer BG, Estevez JJ, Liu E, Craig JE, Finnie JW (2020) Pericytes, inflammation, and diabetic retinopathy. Inflammopharmacology 28:697-709.
Spooner K, Fraser-Bell S, Hong T, Chang A (2020) Prospective study of aflibercept for the treatment of persistent macular oedema secondary to retinal vein occlusions in eyes not responsive to long-term treatment with bevacizumab or ranibizumab. Clin Exp Ophthalmol 48:53-60.
Stahel M, Becker M, Graf N, Michels S (2016) Systemic interleukin 1β inhibition in proliferative diabetic retinopathy: A Prospective Open-Label Study Using Canakinumab. Retina 36:385-391.
Studsgaard A, Clemmensen K, Nielsen MS (2022) Intravitreal fluocinolone acetonide 0.19 mg (Iluvien®) for the treatment of uveitic macular edema: 2-year follow-up of 20 patients. Graefes Arch Clin Exp Ophthalmol 260:1633-1639.
Tamura K, Yokoyama T, Ebihara N, Murakami A (2012) Histopathologic analysis of the internal limiting membrane surgically peeled from eyes with diffuse diabetic macular edema. Jpn J Ophthalmol 56:280-287.
Tang J, Kern TS (2011) Inflammation in diabetic retinopathy. Prog Retin Eye Res 30:343-358.
Tang L, Zhang C, Yang Q, Xie H, Liu D, Tian H, Lu L, Xu JY, Li W, Xu G, Qiu Q, Liu K, Luo D, Xu GT, Zhang J (2021) Melatonin maintains inner blood-retinal barrier via inhibition of p38/TXNIP/NF-kappaB pathway in diabetic retinopathy. J Cell Physiol 236:5848-5864.
Tang L, Zhang C, Lu L, Tian H, Liu K, Luo D, Qiu Q, Xu GT, Zhang J (2022) Melatonin maintains inner blood-retinal barrier by regulating microglia via inhibition of PI3K/Akt/Stat3/NF-κB signaling pathways in experimental diabetic retinopathy. Front Immunol 13:831660.
Tang Y, Le W (2016) Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol 53:1181-1194.
Torres-Castro I, Arroyo-Camarena Ú D, Martínez-Reyes CP, Gómez-Arauz AY, Dueñas-Andrade Y, Hernández-Ruiz J, Béjar YL, Zaga-Clavellina V, Morales-Montor J, Terrazas LI, Kzhyshkowska J, Escobedo G (2016) Human monocytes and macrophages undergo M1-type inflammatory polarization in response to high levels of glucose. Immunol Lett 176:81-89.
Trotta MC, Gesualdo C, Petrillo F, Lepre CC, Della Corte A, Cavasso G, Maggiore G, Hermenean A, Simonelli F, D'Amico M, Rossi S (2022) Resolution of inflammation in retinal disorders: briefly the state. Int J Mol Sci 23:4501.
Van Hove I, Hu TT, Beets K, Van Bergen T, Etienne I, Stitt AW, Vermassen E, Feyen JHM (2021) Targeting RGD-binding integrins as an integrative therapy for diabetic retinopathy and neovascular age-related macular degeneration. Prog Retin Eye Res 85:100966.
Vincent JA, Mohr S (2007) Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. Diabetes 56:224-230.
Vujosevic S, Torresin T, Berton M, Bini S, Convento E, Midena E (2017) Diabetic macular edema with and without subfoveal neuroretinal detachment: two different morphologic and functional entities. Am J Ophthalmol 181:149-155.
Wang J, Xu X, Elliott MH, Zhu M, Le YZ (2010) Müller cell-derived VEGF is essential for diabetes-induced retinal inflammation and vascular leakage. Diabetes 59:2297-2305.
Wang W, Lo ACY (2018) Diabetic retinopathy: pathophysiology and treatments. Int J Mol Sci 19.
Whitehead M, Wickremasinghe S, Osborne A, Van Wijngaarden P, Martin KR (2018) Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. Expert Opin Biol Ther 18:1257-1270.
Wroblewski JJ, Hu AY (2016) Topical squalamine 0.2% and intravitreal ranibizumab 0.5 mg as combination therapy for macular edema due to branch and central retinal vein occlusion: an open-label, randomized study. Ophthalmic Surg Lasers Imaging Retina 47:914-923.
Xie H, Zhang C, Liu D, Yang Q, Tang L, Wang T, Tian H, Lu L, Xu JY, Gao F, Wang J, Jin C, Li W, Xu G, Xu GT, Zhang J (2021) Erythropoietin protects the inner blood-retinal barrier by inhibiting microglia phagocytosis via Src/Akt/cofilin signalling in experimental diabetic retinopathy. Diabetologia 64:211-225.
Xu H, Chen M (2017) Diabetic retinopathy and dysregulated innate immunity. Vision Res 139:39-46.
Yang J, Miao X, Yang FJ, Cao JF, Liu X, Fu JL, Su GF (2021) Therapeutic potential of curcumin in diabetic retinopathy (Review). Int J Mol Med 47:75.
Yang XF, Deng Y, Gu H, Lim A, Snellingen T, Liu XP, Wang NL, Domalpally A, Danis R, Liu NP (2016) C-reactive protein and diabetic retinopathy in Chinese patients with type 2 diabetes mellitus. Int J Ophthalmol 9:111-118.
Zeng HY, Green WR, Tso MO (2008) Microglial activation in human diabetic retinopathy. Arch Ophthalmol 126:227-232.
Zhang JZ, Xi X, Gao L, Kern TS (2007) Captopril inhibits capillary degeneration in the early stages of diabetic retinopathy. Curr Eye Res 32:883-889.
Zhang SX, Wang JJ, Gao G, Parke K, Ma JX (2006) Pigment epithelium-derived factor downregulates vascular endothelial growth factor (VEGF) expression and inhibits VEGF-VEGF receptor 2 binding in diabetic retinopathy. J Mol Endocrinol 37:1-12.
Zhang W, Liu H, Rojas M, Caldwell RW, Caldwell RB (2011) Anti-inflammatory therapy for diabetic retinopathy. Immunotherapy 3:609-628.
Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Lipowski P, Raczyńska K (2008) Interleukin-12 and tumour necrosis factor-alpha equilibrium is a prerequisite for clinical course free from late complications in children with type 1 diabetes mellitus. Scand J Immunol 67:204-208.
Zur D, Iglicki M, Busch C, Invernizzi A, Mariussi M, Loewenstein A (2018) OCT biomarkers as functional outcome predictors in diabetic macular edema treated with dexamethasone implant. Ophthalmology 125:267-275.

C-Editor: Zhao M; S-Editor: Li CH; L-Editors: Li CH, Song LP; T-Editor: Jia Y