Review Article

Inflammatory mediators in diabetic retinopathy: Deriving clinicopathological correlations for potential targeted therapy

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The role of inflammation in diabetic retinopathy (DR) is well-established and dysregulation of a large number of inflammatory mediators is known. These include cytokines, chemokines, growth factors, mediators of proteogenesis, and pro-apoptotic molecules. This para-inflammation as a response is not directed to a particular pathogen or antigen but is rather directed toward the by-products of the diabetic milieu. The inflammatory mediators take part in cascades that result in cellular level responses like neurodegeneration, pericyte loss, leakage, capillary drop out, neovascularization, etc. There are multiple overlaps between the inflammatory pathways occurring within the diabetic retina due to a large number of mediators, their varied sources, and cross-interactions. This makes understanding the role of inflammation in clinical manifestations of DR difficult. Currently, mediator-based therapy for DR is being evaluated for interventions that target a specific step of the inflammatory cascade. We reviewed the role of inflammation in DR and derived a simplified clinicopathological correlation between the sources and stimuli of inflammation, the inflammatory mediators and pathways, and the clinical manifestations of DR. By doing so, we deliberate mediator-specific therapy for DR. The cross-interactions between inflammatory mediators and the molecular cycles influencing the inflammatory cascades are crucial challenges to such an approach. Future research should be directed to assess the feasibility of the pathology-based therapy for DR.

Key words: Complications of diabetes mellitus, diabetic retinopathy, inflammation, treatment of diabetic retinopathy

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Diabetic retinopathy (DR) is often considered a sequela to chronic inflammatory stress that results from persistent and clinically unobtrusive activation of multiple harmful cascades in response to an aberrant metabolic memory.[1] The first indications of the involvement of inflammation in DR were provided more than half a century ago by retrospective studies analyzing the effects of rheumatoid arthritis and its therapy on DR.[2] Following multiple studies with experimental, *in vitro*, and *in vivo* designs, and scores of well-directed clinical trials which date back to the last millennium, inflammation is now seen as one of the essential elements driving various pathological pathways responsible for the clinical and pathological manifestations of DR.[3] Yet, there is a lapse in our understanding of the linkage between the inflammatory cascades and clinical DR, mainly due to a large number of the mediators and the pathways they result in. The understanding becomes further challenging for the clinician because of the multiple cross-interactions among these mediators.[4]

DR has multiple clinical manifestations, ranging from a staged non-proliferative DR (NPDR) to stages of proliferative DR (PDR) and diabetic macular edema (DME), and inflammation is known to be involved at all these stages.[5] Intravitreal steroid as an anti-inflammatory agent has been successfully employed for the management of chronic DME while other agents have been evaluated.[6,7] However, these agents, seen as "broad-spectrum," have not been developed for a pathology-based tailored use in the DR that matches specific clinical indicators, and are rather employed as second-line agents only. This is an important reason for the varied results obtained with such therapies.[8] Further, anti-inflammatory agents find minimal use in NPDR and PDR. This is despite the well-documented role of inflammation in these stages, and to date, we do not have any effective anti-inflammatory agent targeting the retardation of DR.

The literature has sufficient pre-clinical evidence favoring the utility of anti-inflammatory agents that target specific inflammatory cascades.[9] However, currently, most of these are not supported by a piece of sufficient clinical-level evidence, though their future use seems likely. As these therapeutic

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strategies will focus on specific and individual inflammatory cascades,[9] the clinician needs to understand if individual signs of DR can be associated with a particular inflammatory mediator or cascade. The current review evaluates the evidence linking various manifestations of DR with the corresponding inflammatory cascades. We explore whether a clinicopathological correlation can be developed, with a strength sufficient enough to guide pathology-driven therapy.

Methods

PubMed and Google Scholar databases were searched using the following keywords: "inflammation in diabetic retinopathy," "inflammatory mediators in diabetic retinopathy," and "pathogenesis of diabetic retinopathy." Cross-references were also screened from these articles wherever appropriate. Following the initial screening for duplicates and context, the articles published in the English language with available full text were evaluated for suitability, and 224 papers were included in this review. The articles were reviewed for the individual role of inflammatory mediators in DR, inflammatory mediators in association with pathological changes related to DR, correlation of inflammatory mediators with clinical lesions in DR, and individual treatment approaches if present. The data were organized into four sections that discuss the stimulus/source of inflammation in DR, the mediators of inflammation themselves, their role in pathology, and the linkage between inflammation and clinical DR. The data have been summarized in Table 1 and the pathogenesis has been depicted in Figs. 1-3.

Stimulus and Source of Inflammation

Chronic persistent hyperglycemia in type 2 diabetes mellitus (DM) leads to a pro-inflammatory diabetic milieu in the retina. This abnormal metabolic state is reflected by altered platelet physiology and function, hypercoagulable state, altered metabolic pathways, free radical accumulation, oxidative stress, and cellular hypoxia.[10,11] Sustained hyperglycemia acts as a key link in the development of the pro-inflammatory state where various pathways including polyol accumulation, advanced glycation end products (AGEs), oxidative stress, and activated protein kinase C (PKC) acts as a '*stimulus*' for the expression of various mediators of inflammation.[12] These stimuli subsequently affect the specific cells or pathways within the cellular and vascular domains of the retina to initiate a cascade of inflammation. Following the stimulus from these by-products of DM, the inner retina (mainly the inner nuclear layer), retinal ganglion cells, diversified microglial cells along with the components of the inner blood-retinal barrier (iBRB), Müller cells, retinal pigment epithelium, endothelial cells, and pericytes act as the prime source of inflammation.[12]

The retinal ganglion cells (RGCs) and astrocytes located in the inner retina act as the prominent cellular sites for the expression of monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α).[13] the accumulation of free radicals and hypoxia in the inner retina augments the expression of the MCP-1 and MIP-1α genes in these cells.[13] Hyperglycemia and associated hypertensive milieu increase the angiotensin II (Ang-II) production, which along with the increased oxidative stress and AGEs stimulates the expression of nuclear factor (NF)-κB.[14] Cells in the inner and outer nuclear layers, ganglion cell layer, and retinal pericytes are activated by these stimuli, leading to the phosphorylation of an inhibitory protein of NF-κB and its rapid degradation followed by the release of NF-κB.[15] NF-κB is present in nearly all cell types and has wide biological and inflammatory activity, including the promotion of gene transcription of several cytokines and chemokines.[16] the increased -glial cell, -caspase 1 and -caspase 3 activity along with the hypoxia stimulates the Müller glial cells, endothelial cells, macrophages, and neutrophils to increase the expression of Interleukin (IL)-1β [Figs. 1 and 2].[17]

The microglial cells, which traverse a wide portion of retinal thickness, get activated in response to varied diabetic stimuli. Hyperglycemia, AGEs, oxidative stress, and PKC lead to reactive gliosis with the activation of the microglial cells.[18] Once activated, the microglial cells can assume any of the several phenotypic states, some inflammatory, others anti-inflammatory. In the inflammatory context, they accelerate the production of various cytokines and chemokines including tumor necrosis factor-alpha (TNF-α), IL-1, IL-6, IL-8, and C-reactive protein (CRP). Moreover, elevated levels of IL-1, IL-6, interferon-gamma (IFN-Y), and TNF-α further activate more microglial cells and initiate a vicious circle of inflammation.[9] Hyperglycemia and dyslipidemia also lead to the deposition of C5b-9, a terminal product of complement activation within the retinal microcirculation which ultimately results in altered inflammatory signaling pathways, further exaggerating the inflammatory milieu. The Müller cells are the main source of increased vascular endothelial growth factor (VEGF) expression in the diabetic retina along with a significant contribution from the endothelial cells, astrocytes, and retinal pigment epithelial cells (RPEs).[19] Hyperglycemia, increased oxidative stress, hypoxia, and the synergistic proliferating effect of cytokines like IL-6 and IL-1β act as stimuli for the increased expression of the VEGF-A from these sources [Figs. 1 and 2].[19]

Several stimuli like oxidative stress, dyslipidemia, and poly- Adenosine diphosphate (ADP) ribose polymerase (PARP) activation lead to the upregulation of adhesion molecules (vascular cell adhesion molecule [VCAM-1],: Intercellular adhesion molecule [ICAM-1], integrins) from the endothelial cells and on the surface of the leukocytes,[20] whereas the endothelial cells and platelets act as a prime reservoir for the increased production of P-selectins and E-selectins, another class of adhesion molecules.[21] All these factors eventually culminate in leukostasis with a resultant inflammatory insult as detailed later [Figs. 1 and 2].

Inflammatory Mediators

A large number of inflammatory mediators have been identified. These are responsible for multiple pathologies that later manifest clinically too. Therapeutic avenues have been researched with varying levels of evidence to target these. These individual mediators are summarized in Table 1 and Figs. 1 and 2.

2a Interleukins

Interleukin 1β (IL-1β)

The role of IL-1β in DR is well identified.[22] It is produced as an inactive precursor pro-IL-1β which is cleaved by protease caspase 1 to its active form.[22,26-28] It also activates NF-κB which further increases the expression of other pro-inflammatory cytokines.[22] A high caspase 1 activity in the retina of diabetic

Table 1: Inflammatory mediators in diabetic retinopathy: Evidence, role, and avenues for intervention

Pathogen	References	Major model	Targeted intervention	Role in DR					
				Upregulated/ downregulated	Capillary dropout	Pericyte loss	Leakage	Neovascularization	Neurodegeneration
TNF-alpha	[35,64-68,83, 84,93,99]	Human serum, vitreous, mice	TNF-alpha Inhibitor (Etanercept, infliximab)	↑	+	+	+	+	-
IL-1ß	[22-32,100-107]	mice	IL-1ß Inhibitor - Anakinra, minocycline- caspase 1 inhibitor, canakinumab - anti-IL-1β monoclonal antibody	↑	+	+	+	+	+
IL-2	[33-35,43]	Human aqueous, ERM, mice	-	↑	-	-	-	+	-
IL-3	[44,45,108,109]	human aqueous, plasma	-	Lower levels noted in diabetics	-	-	-	-	-
IL-4	[35,46]	Human vitreous	-	↑	-	-	-	+	-
IL-5	[35]	Human aqueous	-	↑	-	-	-	+	-
IL-6	[35,47-53,63,87, 110-113,159, 161-169]\	HREC, vitreous	IL-6 Inhibitor (EBI-031 antibody)	↑	Endothelial apoptosis	-	+	+	-
IL-8	[54-57,94,108, 167,168,175-178,180,181]	Human vitreous, HUVEC	-	↑	-	-	+	+	-
IL-10	[35,58-62,79,80, 167,182]	Human aqueous, vitreous	-	Inconclusive	-	--	-	-	-
IL-13	[81,82]	Human vitreous	IL-13 act as anti-inflammatory agent	Lower levels noted in diabetics	-	-	-	-	-
INF-γ	[35,44,67,82]	Human vitreous	-	↑	-	-	-	-	-
Complement system	[69-73, 183,184]	Human vitreous, serum	-	↑	-	-	-	+	-
VEGF	[41,67,82,85,86,92-96,158,167,185-190]	Mice	Anti-VEGF	↑	-	+	+	+	+
CD 40	[128,129]	Human blood	-	↑	-	-	-	-	-
MCP-1	[44,84,117-123, 175, 191-198]	Human vitreous	-	↑	-	-	+	+	-
MCP-2	[44]	Human aqueous humor	-	↑	-	-	-	-	-
NF-κB	[124-127]	Mice	-	↑	+	+	+	+	+
Integrin	[74-77,129,170, 199-201]	Mice	-	↑	+	-	+	-	-
VCAM-1, ICAM-1, selectins	[39-42,92,141,142, 159,202-211]	Human vitreous, serum mice	SAR 1118	↑	+	+	+	+	-
Arachidonic acid metabolites	[27,36,38,64, 78,212,213]	Mice	COX inhibitors (rofecoxib, LOX inhibitor	↑	+	+	+	-	-
NO	[114,130-137]	Human, mice	Aminoguanidine	↑	+	-	+	+	+
PEDF	[214-218]	Human, mice	ACE inhibitor	↓	+	-	+	+	+

ERM - epiretinal membrane, HREC - human retinal endothelial cells, HUVEC - human umbilical vein endothelial cells, PEDF - pigment epithelial-derived factor

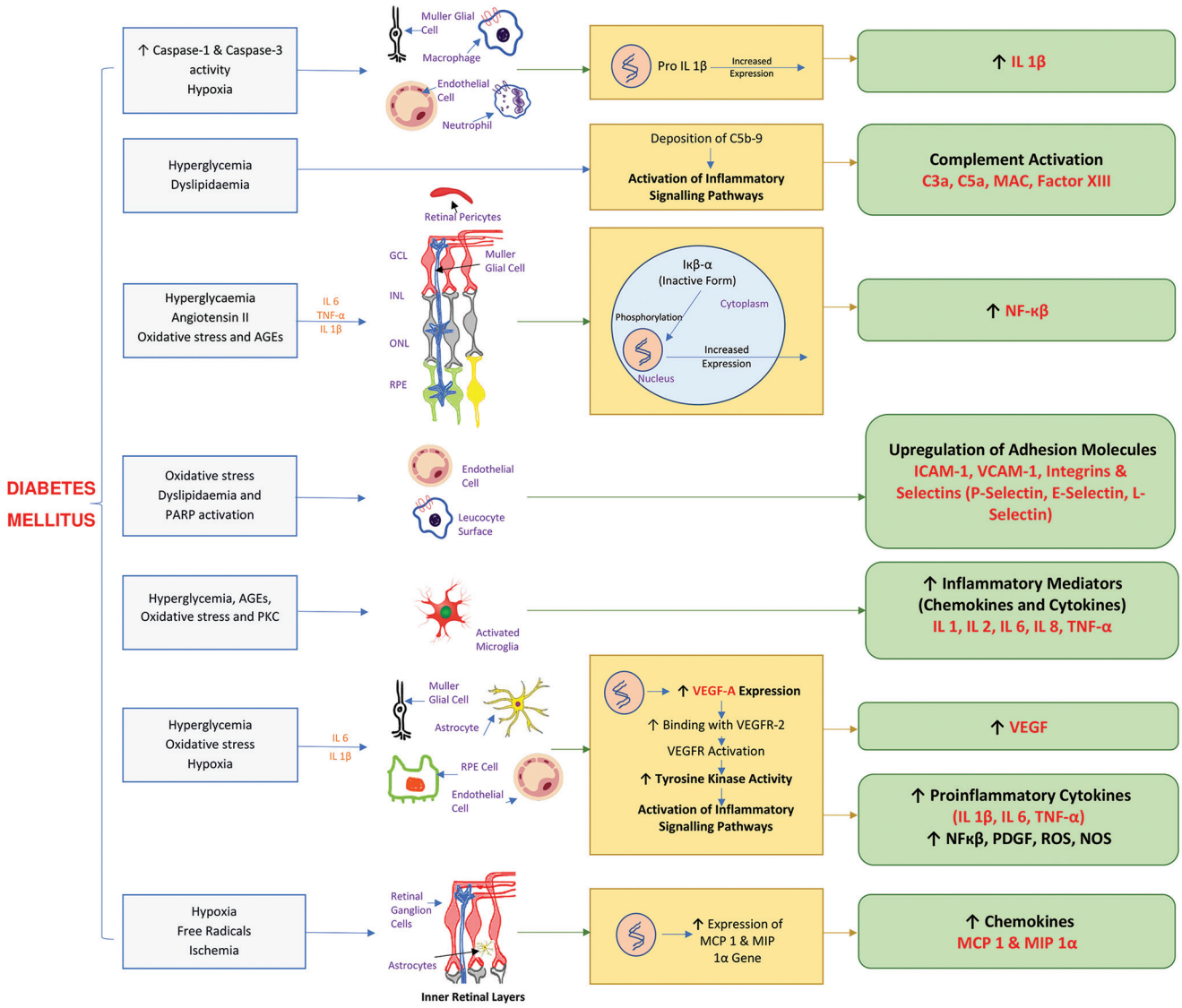

Figure 1: The figure depicts the stimulus of inflammation in diabetes mellitus, the cells (source) responding in the retina to produce the "retinal inflammation," and the consequent release of inflammatory mediators. AGE: Advanced glycation end products, PKC: Protein kinase C, PARP: Poly-ADP ribose polymerase, RPE: Retinal pigment epithelium, IL: Interleukin, VEGF: Vascular endothelial growth factor, VEGFR: VEGF receptor, MCP: Monocyte chemotactic protein, MIP: Macrophage inflammatory protein, MAC: Membrane attack complex, NF: Nuclear factor, ICAM: Intercellular adhesion molecule, VCAM: Vascular cell adhesion molecule, TNF: Tumor necrosis factor, PDGF: Platelet-derived growth factor, ROS: Reactive oxygen species, NOS: Nitric oxide synthase

animals, as well as humans, shows its role in the amplification of IL-1β activity.[23,24] Minocycline, an inhibitor of caspase 1 is known to blunt the diabetes-led increase of IL-1β in the retina.[25] the IL-1R antagonist Anakinra has also been shown to reduce the endothelial dysfunction in diabetic mice. Similarly, systemic IL-1β inhibition with canakinumab in diabetic patients resulted in decreased vessel leakage and resolution of macular edema.[29-32]

Interleukin 2 (IL-2)

The IL-2 activates T lymphocytes, NK cells, B lymphocytes, and monocytes.[33] Its role in DR has been correlated to its elevated levels. The IL-2 along with other inflammatory mediators leads to endothelial apoptosis and retinal leukostasis resulting in ischemia, thereby, culminating in vascular leakage and neovascularization [Fig. 2]. Johnsen-Soriano *et al*.[34] showed increased levels of IL-2 in diabetic mice as compared to controls suggesting a possible role in DR. the aqueous analysis of diabetic patients found IL-2 to be higher in the PDR group than in NPDR and controls.[35] Similarly, its levels were noted to be raised in a study of epiretinal membranes obtained from the PDR patients.[43]

Interleukins-3, 4, and 5 (IL-3, IL-4, IL-5)

The role of IL-3 in DR is inconclusive. Its levels were increased in the aqueous humor of diabetics without DR and lower in patients with DR.[44] Similarly, in a study by Hang *et al*.[45] in the plasma of diabetics, the IL-3 concentration was lower in the diabetics than in controls. The aqueous levels of IL-5 and vitreous levels of IL-4 were seen to be comparatively higher in

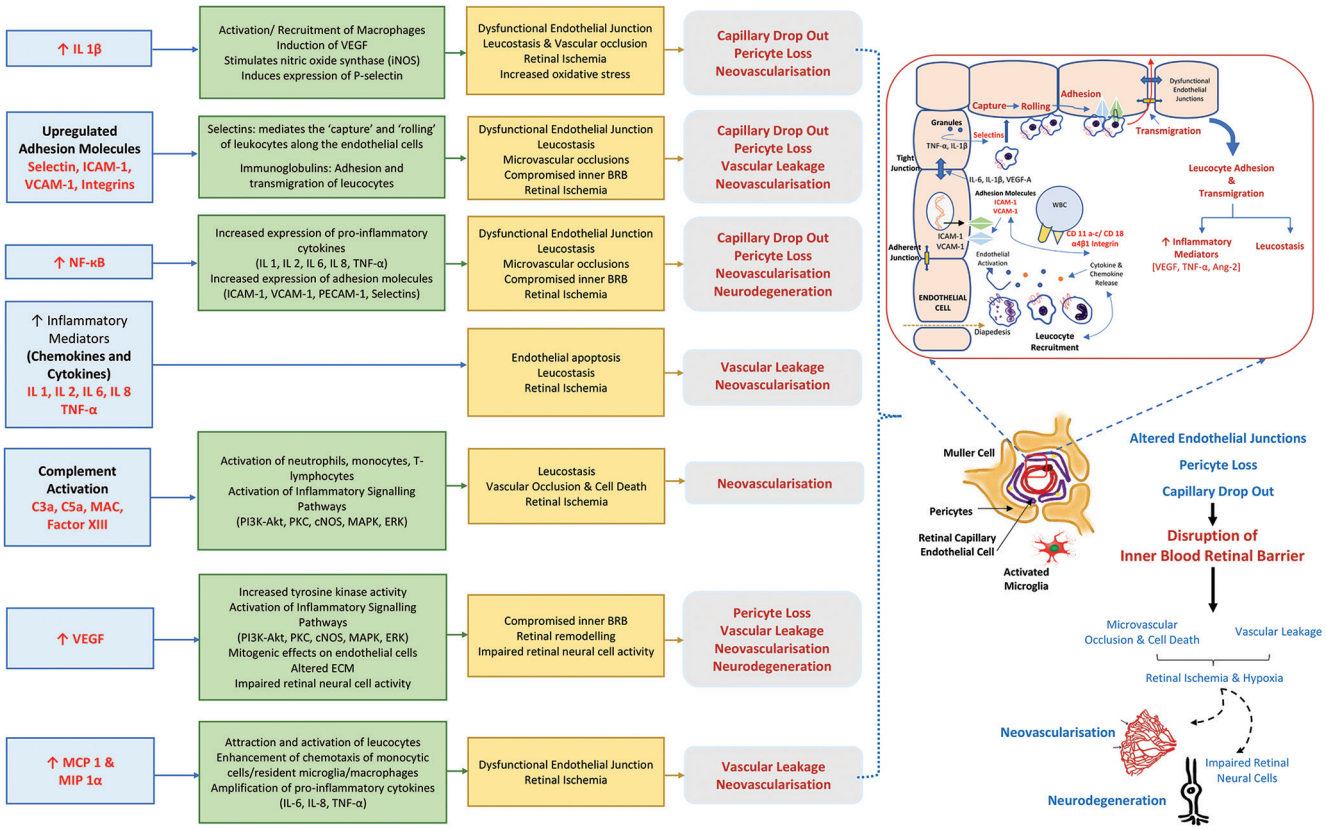

Figure 2: The figure depicts the actions of the individual inflammatory mediators and the resulting cascades causing the retinal pathology in diabetic retinopathy. NF: Nuclear factor, ICAM: Intercellular adhesion molecule, VCAM: Vascular cell adhesion molecule, IL: Interleukin, TNF: Tumor necrosis factor, MAC: Membrane attack complex, VEGF: Vascular endothelial growth factor, MCP: Monocyte chemotactic protein, MIP: Macrophage inflammatory protein PECAM: Platelet endothelial cell adhesion molecule, NOS: Nitric oxide synthase, MAPK: Mitogen-activated protein kinase, ERK: Extracellular signal-regulated protein, ECM: Extracellular matrix, BRB: Blood-retinal-barrier, ANG: Angiotensin

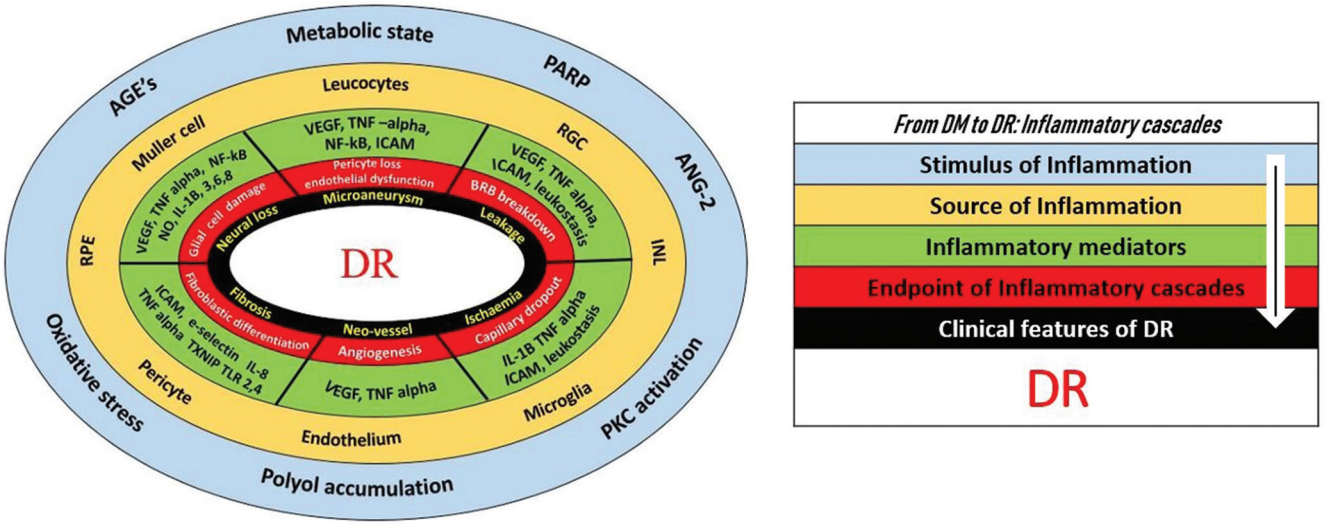

Figure 3: Doughnut chart representing the inflammatory pathogenesis of DR. The outermost blue circle represents the stimulus of inflammation developing outside the retina as a consequence of the metabolic state. The second circle shows the beginning of the retinal inflammation and the cells being stimulated as the source to produce the cytokines. The circles further in have been categorized to show how the inflammatory mediators are getting involved in the cascades to lead on to specific pathologic and cellular level changes in the retina that subsequently manifests as a clinical finding, completing the circle of diabetes mellitus (DM) to diabetic retinopathy (DR). There is tremendous overlapping at all levels of this process, horizontally as well as vertically. AGE: Advanced glycation end products, PKC: Protein kinase C, PARP: Poly-ADP ribose polymerase, ANG: Angiotensin, INL: Inner nuclear layer, RPE: Retinal pigment epithelium, RGC: Retinal ganglion cell

PDR, as compared to NPDR and non-DR patients, signifying a plausible association with PDR.[35,46]

Interleukin 6

IL-6 is produced acutely by macrophages and microglia on coming in contact with pathogen-associated molecular patterns (PAMP) and danger-associated molecular patterns.[47-49] PAMPs are microbial patterns that are recognized directly by the host immune system through cell receptors. The major PAMPs include nucleic acids, surface glycoproteins (GP), lipoproteins (LP), and membrane components. IL-6 is an important mediator in the pathogenesis of DR causing vascular inflammation and endothelial dysfunction.[50] It can directly affect the endothelial cells and disrupt the blood-retinal barrier (BRB) or even induce VEGF production.[50-53]

Interleukin 8 (IL-8)

IL-8 is a pro-inflammatory cytokine and serves as a chemoattractant for neutrophils and also activates the T cells.[54] It causes endothelial cell death and leukostasis along with other interleukins and TNF-alpha, resulting in ischemia, promoting leakage, and neovascularization [Fig. 2]. The high levels of IL-8 have been found in the vitreous of PDR patients and its levels correlated with PDR activity.[55] the aqueous levels of IL-8 were also significantly elevated in patients with DME refractory to anti-VEGF treatment suggesting its inflammatory role in leakage.[56] The effect of IL-8 on the endothelium was studied experimentally on the human vascular endothelial cell lines which showed that the IL-8 downregulated the tight junctions of the endothelium in a dose and time-dependent manner, thereby, altering its permeability.[57] It, therefore, is a promising target for managing refractory DME.

Interleukin 10 (IL-10)

It is an anti-inflammatory cytokine that decreases the synthesis of IL-1 and TNF-α.[58] It is also postulated to downregulate the VEGF expression, thereby, reducing angiogenesis.[59] Its concentration was low in the aqueous of patients with DR as compared to the controls with a significant negative correlation. Hence, the study concluded that low levels of IL-10 are pathogenetic in DR,[60] which has been noted by other studies too.[55,61,62] However, a significantly higher concentration of IL-10 was noted in the vitreous of PDR patients by Mao *et al*.[79] and a similar result was noted by Paine *et al*.[80] too. Thus at the moment, its utility in clinical manifestations and therapeutics of DR is debatable.

Interleukin 13 (IL-13)

IL-13 is produced by the T cells and dendritic cells. In a study by Sai *et al.*, the lower levels of IL-13 were observed in the vitreous of DR patients as compared to other inflammatory mediators, whereas other studies have noted higher levels in the vitreous of patients with PDR suggesting its role in the formation of fibrous membranes.[81,82] Therefore, its role in the management of DR is currently debatable just like IL-10.

2b TNF-α

TNF-α is a pro-inflammatory cytokine produced by microglia, Müller cells, macrophages, neutrophils, and T cells in response to various stimuli.[83] TNF-α is involved in various biological processes including the upregulation of adhesion molecules, proliferation, differentiation, and cell death, and is involved in the pathogenesis of DR and intraocular inflammation.[84] It can alter the distribution of tight junction proteins and increase the BRB permeability and leukocyte adhesion to the retinal endothelial cells.[64-66,84] A significantly higher level of TNF-α was found in the serum of the PDR patients suggesting its role in PDR.[67] In another study involving type 1 and type 2 DM mice, increased microvascular apoptotic cell and acellular capillaries were noted. On administration of Pegsunercept (a recombinant soluble TNF-receptor-1 which has been modified by the attachment of polyethylene glycol), significantly reduced the pericyte loss and the acellular capillaries were noted.[66] the administration of infliximab in the laser-refractory-DME patients led to a significant improvement, indicating the pathogenic role of TNF- α.[68] Therefore, therapy targeting the TNF-α cascade is a relevant option in at least the advanced cases of DR.

2c Interferon γ (INF-γ)

INF-γ is a cytokine released by helper T-cell class 1 (Th1) activating pro-inflammatory M1 macrophages or microglia and B cells. In independent studies, INF-γ was significantly elevated in the vitreous of DR patients as compared to controls and also in diabetic mice, suggesting the upregulation of Th1 cytokine response in DR.[34,44,82] Its application in the therapy for DR is yet to be proven.

2d Complement system

The initial studies showed complement deposition in the retinal choriocapillaris with decreased levels of complement inhibitors suggesting complement-induced diabetic damage.[69,70] Shahulhameed *et al*.[71] showed significantly elevated levels of C3bα in the vitreous of PDR patients and the involvement of alternate pathways was also deduced from the upregulation of complement factor H (CFH). Gao *et al*.[72] reported a similar observation in the vitreous of PDR patients where complement C3 and complement factor I were significantly elevated as compared to the controls. The role of the complement system in neovascularization in DR has been affirmed by other studies as well.[70,73]

2e Integrin

Integrins are cell adhesion receptors mediating cell-cell and cell-extracellular matrix interactions. They have also been associated with various pathological processes, such as vascular leakage, inflammation, neovascularization, and fibrosis.[74] The role of integrin in DME can be well-established from the study by Quiroz-Mercado *et al*.[75] in which non-inferiority of integrin inhibitor "ALG-1001" over bevacizumab was met with favorable results. In another experimental model, THR-687 a novel integrin receptor antagonist prevented neovascularization.[76] Its role in leukostasis and vascular leakage was confirmed in experimental mice where inhibition of alpha 4 integrin/CD49d significantly attenuated diabetes-induced leukocyte adhesion and vascular leakage.[77]

2f Arachidonic acid metabolites

Arachidonic acid is released from the phospholipids in the cell membranes in response to several stimuli where the cyclooxygenases (COX) and lipoxygenases convert arachidonic acid to leukotrienes and prostaglandins. Cyclooxygenase 2 activity (COX-2) has been noted in retinal endothelial cells of diabetic patients.[78] On administration of COX-2 inhibitor, the diabetes-led upregulation of retinal VEGF, retinal vessel permeability, and leukostasis was found to be inhibited suggesting that COX-2 induces pathological alteration in

DR.[36] In a study by Joussen *et al*.,[64] meloxicam (COX-2 inhibitor) reduced endothelial nitric oxide synthase (eNOS) levels, inhibited NF-κB activation in the diabetic retina, and partially reduced the TNFα levels. Similarly, nepafenac (COX inhibitor) inhibited leukocyte adhesion as well as apoptosis of the retinal capillary cells, and degeneration of retinal pericytes and capillaries.[27]

Arachidonic acid itself may act as a second messenger activator of protein kinase C—an important and established component in the pathogenesis of diabetic retinopathy (DR). Protein kinase C inhibitors have been used in DR. Protein kinase C b Inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS 2), a major clinical trial showed that oral ruboxistaurin led to significant visual improvement in the NPDR eyes more likely due to the reduction in macular edema.[37] the evidence also suggests that leukotrienes and other products of 5-lipoxygenase are involved in DR changes too. Lipoxygenase-derived 5-hydroxyeicosatetraenoic acid (5-HETE) was noted to be elevated in the vitreous of diabetic patients. In 5-lipoxygenase-deficient diabetic mice, degeneration of retinal capillaries, leukostasis, and superoxide generation was found to be inhibited indicating its potential role in these alterations.[38]

2g Cellular adhesion molecules

Adhesion molecules are required for leukocyte migration and adhesion to endothelial cells, leading to leukostasis and endothelial dysfunction. In a study by Joussen *et al*.,[39] the diabetic mice genetically deficient in ICAM-1 or its ligand (CD18) were protected from the development of lesions of early DR (including capillary degeneration, pericyte loss, and increased permeability) as well as leukostasis. Limb *et al*.[40] noted significantly elevated levels of soluble vascular cell adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) in the vitreous of PDR cases. Funatsu *et al*.[41] reported higher vitreous levels of sICAM-1 in patients with hyperfluorescent DME than in those with minimally fluorescent DME as well as its levels correlated with macular edema. Rao *et al*.[42] demonstrated that the topical administration of small molecule antagonists of the leukocyte function-associated antigen-1 (LFA-1) significantly reduced retinal leukostasis and blood-retinal-barrier breakdown in rats. Such topical mediators, thus, are very promising as they have a potential for use in both early and late DR, but their utility is yet to be proven.

2h Vascular endothelial growth factor

VEGF is a well-established mediator of DR induced by the ischemic-hypoxic drive of the retina, and its role in leakage and neovascularization is well-known. Various drugs like bevacizumab, ranibizumab, aflibercept, and brolucizumab have been extensively evaluated and found beneficial for advanced DR. However, it has been found that it is a pro-inflammatory molecule too and some patients on anti-VEGF therapy have shown regression of DR too. Its role in leukostasis and BRB breakdown can be judged from the fact that the blockade of endogenous VEGF resulted in significant suppression of these processes in DR.[85] A study by Wang *et al*.[86] in diabetic mice showed that the inhibition of Müller cell VEGF significantly decreased the expression of TNFα, ICAM-1, and NF-κB. the retinal leukostasis implicated in the DR pathogenesis too is mediated by VEGF. The administration of phosphomannopentaose sulfate (PI-88) (a sulfonated oligosaccharide which inhibits heparanase) led to the inhibition of leukostasis and preservation of the Electroretinogram (ERG) changes in diabetic rats suggesting that it may reverse retinal dysfunction by decreasing the VEGF expression.[115] It has also been reported that pro-inflammatory cytokines such as IL-1β and IL-6 could upregulate the VEGF mRNA expression.[116]

2i Monocyte chemoattractant protein-1 and 2 (MCP-1, MCP-2)

MCP-1 is a chemokine produced by multiple cell lines of the immune system as well as endothelial cells leading to the recruitment and activation of the macrophages and monocytes. It also stimulates VEGF production promoting angiogenesis and fibrosis.[117,118] It is also reported that it significantly alters retinal vascular permeability, angiogenesis, as well as contribution to the DR pathogenesis.[119,120] the higher levels of MCP-1 were noted in the vitreous of the PDR patients and retina of diabetic mice.[121-123] MCP-2 was noted to be significantly elevated in the aqueous humor of diabetics without DR as compared to non-diabetics. However, its role in DR and its therapy needs to be further evaluated.[44]

2j NF-κB

It is a transcription factor regulating genes involved in the immune and inflammatory processes as well as cellular proliferation and apoptosis. An overactivated NF-kβ leads to altered gene expression of VEGF, PDGF, and endothelin-1, and also the release of various cytokines including TNF-α, IL-1β, and IL-6. These processes ultimately lead to endothelial apoptosis and angiogenesis.[124,125] The inhibition of NF-κB activation by dehydroxymethylepoxyquinomicin reduced diabetes-induced retinal leukostasis and expression of ICAM-1 and VEGF experimentally.[126] Selective inhibition of NF-κB also resulted in reduced degeneration of the retinal capillaries and expression of inflammatory proteins.[127] Because of its potential cross-interacting broad-spectrum activity, NF-κB is a promising target whose antagonistic therapy will likely curtail DR. Further evidence is, however, required.

2k CD40 Ligand (CD40L)

The CD40L system is known to be present on the platelets. The activated platelets initiate inflammatory reaction of the endothelial cells with CD40L which is shed as soluble CD40L (sCD40L) and can serve as a marker of inflammation.[128] Yngen *et al*.[129] studied the correlation of sCD40L in type 1 diabetics with and without microangiopathy and found its levels to be significantly elevated in the blood of patients with microangiopathy suggesting it may be a part of the inflammatory cascade in diabetic microangiopathy.

2l Nitric oxide (NO)

It is produced by three nitric oxide synthase (NOS) isoforms that are expressed variably in the retina—endothelial (eNOS), neuronal (nNOS), and inducible (iNOS).[130,131] NO is a regulator of important cellular functions including vascular dilation and inflammation. eNOS and nNOS are expressed constitutively and regulate neuronal and endothelial functions.[114] iNOS is associated with increased severity and accelerated development of DR and is upregulated in DR.[132,133] It has been demonstrated in experimental mice that the deletion of iNOS led to a reduction in the DR changes of capillary degeneration and permeability.[134,135] Aminoguanidine, an inhibitor of NO, has been found to inhibit microvascular complications of diabetes in the retina in experimental models.[132] Several other studies in humans have indicated the role of NO in neurodegeneration and neovascularization.[136,137]

Inflammatory Pathways, Cellular Responses, and Clinicopathological Correlation

Clinically, DR presents with different findings including microaneurysm, edema, ischemia, new vessels, and fibrosis. These findings are used to grade the severity of the disease and assign a stage that determines the entailing management. Neuro-retinopathy has also been realized to be a part of DR now, though not included in the conventional classification systems. The pathologies discussed in Table 1 are responsible for these clinical processes. This section correlates these pathologies to the common clinical findings seen in DR.

1. **Microaneurysm** – the loss of structural support to retinal capillaries by pericyte loss and endothelial cell damage leads to microaneurysm formation which on rupture leads to retinal hemorrhage. Eventually, these outpouchings lead on to leakage resulting in edema. Inflammation has been shown to cause pericyte loss as mentioned in Table 1. As explained in Fig. 2, a large number of inflammatory molecules are involved in the pericyte loss and endothelial dysfunction which culminates in aneurysms. A significantly high level of gene expressions and protein concentrations of IL-1β, NF-κB, VEGF, TNFα, TGF-beta, and ICAM-1 were found in retinal pericytes in an experimental model where the cells were exposed to high glucose concentration suggesting changes secondary to inflammation. These changes persisted after the glucose levels became normal, thus, indicating irreversible nature.[138,139] Other inflammatory mediators causing pericytes apoptosis include TNF-alpha and reactive oxygen species.[87]
2. **BRB breakdown and edema** – BRB breakdown alters the permeability of retinal capillaries causing leakage eventually culminating in edema and exudation. The role of inflammation in BRB breakdown has been shown to occur by increased leukostasis, cytokines, and growth factors as well as pericyte loss.[88,89,140,170] TNF-alpha and VEGF have also been shown to increase the permeability of BRB. Miyamoto *et al*. showed that retinal vascular leakage and capillary non-perfusion corresponded to retina leukostasis and the diabetes-led increase in the leukostasis correlated with the elevated levels of ICAM-1. These processes were significantly inhibited by the injections of intravitreal antibody acting against ICAM-1 indicating the role of leukostasis in the BRB breakdown.[69,141,142] Therefore, ICAM-1 antibody can serve as a potential therapeutic line against DR.
3. **Ischemia** – Inflammation also leads to capillary occlusion and capillary dropout. Inflammatory cytokines including TNFα and IL-1β have been reported to increase caspase 3 activity leading to endothelial cell apoptosis.[22,84] In addition, the capillary occlusion by leukostasis due to the blockage of blood flow can also lead to ischemia.[84] As depicted in Fig. 2, leukostasis itself is a resultant of multiple pathways, especially those involving integrins and cell adhesion molecules. Endothelial cell death is also induced during leukostasis by leucocyte-mediated Fas ligand (FasL) and Fas-mediated apoptosis.[148] The role of leukostasis in DR can be adjudged from the fact that a significant reduction in diabetes-induced capillary degeneration was noted when the proteins required for the adherence of white blood cells to the endothelium (ICAM-1 and CD-18) were deleted in experimental diabetes.[39]
4. **Neovascularization** – Inflammation has been shown to cause neovascularization. Inhibition of inflammatory cytokines including TNFα attenuated neovascularization.[144-147] Macrophages and monocytes were also found in the neovascular sprout by Ishida *et al*.[148] and selective depletion of monocyte lineage with intravitreal clodronate liposomes (dichloromethylene diphosphonate), which act as anti-macrophage agents, led to the suppression of pathological neovascularization. The role of unregulated VEGF in causing the development of new vessels is very well-known.[149]

   Neurodegeneration – Neurodegeneration occurs in DR preceding clinical findings in many cases, partly a resultant of neuroinflammation. Microglial activation resulting from a persistent hyperglycemic state has been postulated to be the main factor behind neuroinflammation.[150] An increased microglial count and size have been noted in association with cotton wool spots and microaneurysms.[18] Activated microglia produce pro-inflammatory mediators such as IL-1β, IL-3, IL-6, TNF-α, VEGF, lymphotoxin, MIP-1α, MMPs, NO, ROS, COX-2, and complement factors which promote neuronal cell death.[18,151,152] Further activated Müller cells and astrocytes produce IL-1β and TNF-α, which then induce cytokine IL-8 contributing to neuroinflammation.[153,154] the presence of activated NF-κB had been noted in microglia in a hypoxic neovascular mouse model.[18,127] Topical gents like pigment epithelium-derived growth factor have been used in the mice to prevent microglial activation, and this pathway has also been exploited in phase 3 human trials using neuroprotective agents.[18] However, no therapy targeting specifically neuroinflammation in DR has been evaluated yet.
5. **Fibrosis and traction** – Though the role of inflammation in diabetic retinal detachment/tractional changes and fibrosis has not been much explored, Limb *et al*.[40] evaluated the vitreous of PDR patients. The authors reported significantly elevated levels of sICAM-1 and sE-selectin in the eyes with Tractional retinal detachment (TRD) as compared to the eyes with vitreous haemorrhage (VH) alone. IL-8 and TNF-alpha have also been reported to contribute to fibrosis after initiating ocular angiogenesis.[155] Another study showed that the upregulation of thioredoxin-interacting protein (TXNIP), a pro-inflammatory and proapoptotic protein, played a significant role in promoting retinal fibrosis and blockade of TXNIP-ameliorated retinal fibrosis in diabetic rats.[156] Additionally, toll-like receptors 2 and 4, were also shown to play a significant role in subretinal fibrosis formation.[157] All these are possible future avenues.

Linking Inflammatory Pathways to Clinical Findings and Approach to Therapy

Fig. 3 presents the link between DM, stimulus source of inflammation, the resulting inflammatory mediators, inflammatory cascade-led cellular processes, and the clinical finding manifesting as DR. It is easily seen that there are major overlaps in the proximal cascade, especially at the level of stimulus and source of inflammation. For example, AGEs contribute to the production of NF-κB and also stimulate the glial cells. The former, as discussed before, is a general transcription controlling factor that relates to the production of a host of mediators, is present in almost all the retinal layers,

and contributes to the regulation of definitive molecules like VEGF and ICAM. Similarly, as mentioned before, stimulated glial cells can lead to the production of tumor necrosis factor-alpha (TNF alpha), IL-1, IL-6, IL-8, and CRP, apart from inciting neurodegeneration. This would theoretically mean that AGEs alone are sufficient to be considered as responsible for all manifestations of DR. If we consider the source of inflammation, Müller cells are involved in the production of VEGF, IL-1β, and NF-κB, and thus, are involved in almost all the major pathways of DR [Fig. 1]. The overlap is lesser as we come closer to DR centrally in the passage of linkage, but major mediators like VEGF, IL-1B, and TNF-alpha are involved in multiple inflammatory pathways. Further, molecules like VEGF, NO, and ICAM can beget inflammation themselves leading to the production of other mediators. Moving further centrally, the overlaps are too well-known clinically where microaneurysm can lead to leakage, ischemia can lead to new vessels, and new vessels can lead to fibrosis. Thus, there is tremendous overlap and interaction at all steps in the inflammatory cascade, initiating from DM and reaching right up to the clinical manifestations of DR, though the cross-interactions are lesser centrally. Utilizing anti-inflammatory agents in the context of DR should be seen in three contrasting approaches: narrow, tailored, or broad-spectrum.

Narrow-spectrum approach: While attempting to individualize anti-inflammatory therapy with reference to Fig. 3, the inside-out approach (a clinical sign of DR to an inflammatory agent) is easier to adopt than vice versa. This has multiple reasons. First, each individual with DM and other related comorbidities can have different clinical manifestations, which will also vary depending on the stage of DR in terms of predominance. Second, at any given point in time, one pathology of DR may be more responsible for the disease or symptoms while multiple pathologies may have manifested in the retina. Third, clinical examination is an easier done evaluation in comparison to the assessment of ocular inflammatory markers while managing patients. For example, If the clinician is able to identify microaneurysms as the predominant pathology [black circle Fig. 3], it should mean that pericyte loss or endothelial dysfunction is the predominant pathogenesis [red circle Fig. 3]. Such a patient may benefit from anti-inflammatory therapy based on the VEGF, TNF, ICAM, or NF-κB pathways. Beyond this, at the levels of sources [yellow circle Fig. 3] and stimuli of inflammation [blue circle Fig. 3], there are lots of cross-interactions that do not allow an individualized approach. At these levels, any agent is likely to have a "broad-spectrum" action.

Broad-spectrum approach: the anti-inflammatory agents which target "broad-spectrum" targets like IL-1β, VEGF, TNF-α, and NO [Table 1] are likely to act in most stages and manifestations of DR, as compared to a narrow-spectrum target like IL-2 or MCP-1, though the efficiency would be variable.[171] The linkage in Fig. 3 explains why therapies that target a "broad-spectrum" mediator like VEGF work very well and have become so well-adopted into clinical practice, as also the success of the agents that have "broad-spectrum" action like the steroids.[90] This approach has easier application, but its long-term efficiency is questionable.

Tailored approach: At present, the narrow-spectrum approach for individualizing anti-inflammatory therapy to a particular manifestation of DR is challenging, and more research is needed to define the utility of a particular agent for a particular manifestation of DR. The broad-spectrum approach though may work in most cases, it may not be the most efficient in the long term. The tailored approach is another possibility but may require the assessment of ocular inflammatory markers. This approach may be feasible at least in some cases like refractory DME where, for example, the IL-8 pathway may be targeted.[91] Further, deducing the actual rise of a mediator in a particular pathology in restricted cases, as seen in the case of the correlating rise of sICAM-1 and angiographic leakage in the retina, will give vital insights into the "primary" inciting role of these mediators at that stage of the pathology.[92,142] Such approaches are likely to be more efficient in the long term.

Conclusion

Diabetes induces a chronic inflammatory state in the retina that originates due to altered systemic metabolism. Many cells of the retinal neurovascular unit contribute to its occurrence, and it is not limited to any particular retinal layer. As a consequence, a large number of heterogeneous inflammatory mediators are generated which not only participate in multiple inflammatory cascades but also influence each other's production and action. The result is a persistently active intertwined inflammation with entailing cellular responses leading to DR.

The current success of some anti-inflammatory agents in DR can be attributed in part to their "broad-spectrum" nature. Trials conducted in the last century had contrasting results. The Dipyridamole Aspirin Microangiography of Diabetes Study Group (DAMAD) showed a small reduction in the number of microaneurysms (MAs) in an aspirin = treated group whereas the Early Treatment of Diabetic Retinopathy Research Group (ETDRS) found no significant difference in the impact of aspirin on DR.[172,173] the current research has shown promising pre-clinical results with many anti-inflammatory agents in their domains, and in the future, we are likely to see a higher level of evidence as clinical trials unfold. While steroids as broad-spectrum agents have been tested extensively for DR and DME, risuteganib is a prime example of a possible targeted therapy acing at the level of integrins and is entering phase 3 trials. Which patients will benefit from such therapies is, however, yet not known.[174]

We reviewed the role of inflammation in DR in detail and attempted to develop a clinicopathological correlation between the inflammatory mediators and DR. There is a theoretical possibility of a tailored anti-inflammatory approach apart from other approaches discussed in the review. However, we found that the current literature does not present sufficient evidence to build specific linkages between the inflammatory mediators and clinic-pathological findings of DR. This will make the "future choice" of such agents difficult and arbitrary for the clinician, as well as render these therapies inefficient. Many questions need to be answered before the potential of such individualized anti-inflammatory therapies is realized, and that they may be offered in a tailored way to the patients and not just as second-line agents in refractory cases. The search should begin in realizing their actual role as well as the extent of their role in the pathogenesis of DR. Mere

extraneous presence of a mediator is not enough to suggest its "therapy-defining role," rather elucidating the correlation between the mediator activity and clinicopathologic finding should set its "therapy-defining role." This should be the prime focus of future clinical research evaluating the role of inflammation in DR.

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Conflicts of interest

There are no conflicts of interest.

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