Published in final edited form as:
Immunotherapy. 2011 May ; 3(5): 609–628. doi:10.2217/imt.11.24.

Anti-inflammatory therapy for diabetic retinopathy

Wenbo Zhang[1,2,3,*], **Hua Liu**[1,2], **Modesto Rojas**[1,2], **Robert W. Caldwell**[3], and **Ruth B. Caldwell**[1,2,4,5,6]

[1]Vascular Biology Center, Georgia Health Sciences University, Augusta, Georgia

[2]Vision Discovery Institute, Georgia Health Sciences University, Augusta, Georgia

[3]Department of Pharmacology and Toxicology, Georgia Health Sciences University, Augusta, Georgia

[4]Department of Cellular Biology and Anatomy, Georgia Health Sciences University, Augusta, Georgia

[5]Department of Ophthalmology, Georgia Health Sciences University, Augusta, Georgia

[6]VA Medical Center, Augusta, Georgia

Abstract

Diabetic retinopathy (DR) is one of the most common complications of diabetes. This devastating disease is a leading cause of blindness in people of working age in industrialized countries and affects the daily lives of millions of people. Despite tight glycemic control, blood pressure control, and lipid-lowering therapy, the number of DR patients keeps growing and therapeutic approaches are limited. Moreover, there are significant limitations and side-effects for the current therapies. Thus, there is a great need for development of new strategies for prevention and treatment of DR. Studies have shown that DR has prominent features of chronic, subclinical inflammation. This review will focus on the role of inflammation in DR and summarize the progress of studies of anti-inflammatory strategies for DR.

Keywords

cytokine; diabetic retinopathy; inflammation; leukostasis; neovascularization; NF-κB; nitric oxide; oxidative stress; vessel degeneration; vessel leakage

According to the International Diabetes Federation (IDF-Atlas, 4th ed., 2009), diabetes currently affects nearly 285 million people worldwide and this number is expected to reach 438 million by 2030. Diabetic retinopathy (DR) is one of the most common complications of diabetes and is a leading cause of blindness in people of working age in industrialized countries [1]. Approximately 25% of type 1 diabetic patients may have signs of retinopathy after 5 years of diabetes, increasing to 60% after 10 years. Eventually, after 25 years, almost all (97%) type 1 diabetics will develop retinopathy [2, 3]. Type 2 diabetic patients may already have background retinopathy at time of diagnosis and over 60% will develop some

[*]Corresponding author: Wenbo Zhang, Vascular Biology Center, Georgia Health Sciences University, Augusta, GA, 30912-2500; wezhang@mail.georgiahealth.edu.

Disclosure: The authors have declared that no conflict of interest exists

Financial disclosure/Acknowledgements
This work was generously supported by the Juvenile Diabetes Foundation JDRF 10-2009-575 and the American Heart Association 11SDG4960005 (to W. Zhang); by NIH Grants EY11766, EY04618 and VA Merit Award (to R.B. Caldwell); and by NIH Grant HL70215 (to R.W. Caldwell).

form of retinopathy after 20 years [4]. In the USA, according to the Wisconsin Epidemiologic study of Diabetic Retinopathy (WESDR), DR causes 12,000 –24,000 new cases of blindness with costs of more than $500 million each year [5]. The number of patients with DR was 5.8 million in 2005. However this number will triple to 17.7 million in 2050 [5]. In China, it is estimated that 9.2 million Chinese people living in rural areas have DR and 1.2 million of them have vision-threatening retinopathy [6]. With changes in life style and increases in life span along with the global prevalence of diabetes, it is expected that DR will have a growing impact on large populations.

Tremendous efforts have been made to identify mechanisms and develop therapies for DR. These studies have led to the recognition of hyperglycemia, hypertension and dyslipidemia as major risk factors for DR. Consequently, tight glycemic control, blood pressure control, and lipid-lowering therapy have shown proven benefits in reducing the incidence and progression of DR [6]. Progress in developing new approaches for ocular therapy is relatively slow. Laser photocoagulation and vitrectomy remain as the two conventional approaches to treat sight-threatening conditions such as macular edema and proliferative diabetic retinopathy [7]. Anti-vascular endothelial growth factor (VEGF) therapy represents a recent breakthrough since clinical trials have shown beneficial effects of VEGF blockers (Pegaptanib, Ranibizumab, Bevacizumab) in reducing macular edema and causing neovascular regression particularly when combined with laser photocoagulation [6–8]. In spite of this progress, DR remains a major clinical challenge and the number of patients keeps growing. It is difficult to achieve tight glycemic control throughout the course of the disease. Moreover, DR can develop even after tight control is initiated due a phenomenon called “metabolic memory” in which the retinal endothelial cells manifest high glucose-induced biochemical alterations long after the initial insult [9, 10]. Laser photocoagulation and anti-VEGF therapy are not always effective and laser therapy can damage retinal neurons which may result in decreases in central vision and vision acuity and impaired night vision [6, 7]. Anti-VEGF therapy requires repeated treatment and may impair neuronal and vascular survival function [11]. Thus, there is a great need to develop new therapeutic approaches for this devastating disease.

The clinical progression of DR follows a pattern characteristic of ischemic retinopathy [2, 12]. The disease begins with biochemical and cellular alterations that are not clinically evident. Vascular alterations during this period include alterations in blood flow, death of retinal pericytes (perivascular contractile cells), basement membrane thickening and subtle increases in vascular permeability. As the disease progresses, obvious alterations in the vascular structure can be seen upon ophthalmoscopic examination. These include nonperfused vessels, microaneurysms, dot/blot hemorrhages, cotton-wool spots, venous beading, vascular loops, and significant vascular leakage. In many patients, the retinopathy progresses to proliferative DR, in which the new vessel walls are weak and allow the blood to leak out of the vessels, resulting in vitreous hemorrhage and subsequent retinal detachment. The mechanisms by which elevated blood glucose causes tissue injury and disease progression in the retina are not fully understood. However, studies have shown that DR is a multi-factorial disease involving multiple pathways, including aldose reductase pathway, oxidative stress, activation of protein kinase C, and formation of advanced glycation end products (AGEs). These pathways lead to retinal pathological changes by causing osmotic vascular damage, inducing cell dysfunction and apoptosis through activation of mitogen-activated protein kinases (MAPKs) and oxidation of intracellular components, inducing production of angiogenic cytokines, and breakdown of the vascular junction proteins. [13–18]. In addition to these pathways, recent studies indicate that inflammation is a critical player in the pathogenesis of DR and serves as a positive feedback mediator of multiple pathways.

Diabetic retinopathy has features of chronic inflammatory disease

DR has not been recognized as an inflammatory disease until recently because the retina has been considered as a tissue with immune privilege. However, recent studies in patient samples and animal models have shown that DR has features of chronic, subclinical inflammation.

Inflammation

Inflammation is the body's defense against pathogens and also a critical step in wound healing. This process involves multiple mediators such as pro-inflammatory cytokines, chemokines and adhesion molecules that initiate the interaction between leukocytes and the endothelium and guide directional leukocyte migration towards infected or injured tissue. Pro-inflammatory cytokines (such as tumor necrosis factor (TNF)-α, interleukins) and chemokines (such as CCL2, CCL5) released from infected/injured tissue activate the endothelium to increase expression of adhesion molecules (such as E-selectin, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1) and chemokines. The adhesion molecules make the endothelium "sticky", causing the leukocytes to roll along the endothelium until they meet glycosamino-glycan-immobilized chemokines. After activation by a chemokine, leukocytes transition from selectin-mediated rolling to integrin-mediated firm attachment. The attached leukocytes then transmigrate through the endothelium, moving to interstitial inflammatory sites by following chemotactic gradients [19]. Whereas acute inflammation is beneficial, chronic and dysregulated inflammation can result in undesirable effects and is critically involved in a variety of diseases such as such as atherosclerosis, rheumatoid arthritis, multiple sclerosis, and asthma [19].

DR and inflammatory molecules

Typical clinical signs of DR such as edema and neovascularization are hallmarks of inflammation and suggest a possible role of inflammation in the pathology. The potential role of inflammatory mediators in DR has been supported by extensive evidence in the past two decades. TNF-α is a pro-inflammatory cytokine which is recognized as an initiator or inflammatory reactions. High TNF-α levels have been observed in vitreous, serum, and ocular fibrovascular membranes from patients with DR and in retinas from rodent model of diabetes mellitus [20–22]. TNF-α gene polymorphism has been shown to be associated with increased susceptibility to the disease [23]. Similarly, interleukin-1β (IL-1β) and its downstream signaling molecule caspase 1 are high in vitreous and retinas from diabetic patients and rats [21, 24, 25]. Interleukin-6 (IL-6) levels in the vitreous are significantly correlated with the severity and progress of DR [26, 27]. Chemokines such as CCL2, CCL5, CXCL8, CXCL10, CXCL12 are also upregulated in vitreous samples from DR patients [28–30]. Levels of TNF-α, IL-1β, CCL5 and CXCL12 are also significantly increased in the serum of DR patients [21, 30], suggesting that systematic inflammatory reactions may contribute to this disease. In addition to increases in these inflammatory mediators, increases in adhesion molecules such as ICAM-1 and VCAM-1 have been found to be related to the progression of DR [30, 31]. ICAM-1 immunoreactivity is high in the diabetic vasculature of humans and rodents and the K469E polymorphism of the ICAM-1 confers susceptibility to proliferative DR [30, 32–34].

DR and inflammatory cells

As stated above, a typical sign of inflammation is recruitment of leukocytes via inflammatory cytokine-induced activation of the vascular endothelium. Leukocytes, the cell class responsible for innate immunity (neutrophils, monocytes, eosinophils, basophils, and mast cells) and adaptive immunity (T lymphocytes, B lymphocytes, and natural killer cells), effectively protect the host from infectious pathogens but also induce tissue injury in certain

diseases [35]. Among these cells, neutrophils and monocytes are very likely to induce microvascular injury because they have high probability to attach to the vessel wall and block blood flow due to their large population and size. Furthermore, they produce high levels of reactive oxygen species (ROS), which can injure vascular cells. The evaluation of the action of leukocytes in retinas of DR patients has been limited by the limited availability of human retinal tissue. Lutty and colleagures reported the very interesting observation that numbers of neutrophils are significantly elevated in both retinal and choroidal vessels from diabetic patients. The increase in neutrophils was found to be correlated with upregulation of ICAM-1 immunoreactivity in the vessels [33]. Monkey represents an excellent DR model that mimics the clinical aspects of human DR. In a study of retinas from diabetic monkeys, Lutty's group provided further evidence that neutrophil accumulation is associated with capillary closure, further implicating an involvement of neutrophils in this disease [36]. Diabetic rodent models share some characteristics of human DR such as increased retinal vessel permeability, vessel closure and neuronal death. Although rodents do not develop retinal neovascularization as seen in human diabetes, rodents are widely used to study mechanisms of DR owing to accessibility and possibilities for genetic manipulation. Schroder et al. conducted a detailed morphological study in diabetic rats and provided the first evidence that attachment of monocytes and neutrophils is associated with capillary occlusion, dropout and intraretinal neovascularization [37]. Subsequent studies further demonstrated a cumulative and sustained increase in leukocyte adherence to the retinal vasculature (leukostasis) along with the progression of DR [38]. The increase in leukostasis may be attributable to diabetes-induced increases in ICAM-1 and integrins in endothelial cells and leukocytes, respectively [38–40]. Blockade of ICAM-1 or deletion of CD18 (ICAM-1 receptor subunit on leukocytes) prevents diabetes-induced leukostasis. In addition to leukocytes in the circulation, microglial cells in the retina are likely to play role in DR. The microglial cell is a resident macrophage. Microglia are scattered throughout the retina and form a network with potential immunoeffector cells [41]. In DR they are rapidly activated to release inflammatory cytokines such as TNF-α [42], indicating a local immune reaction in the disease. The role of microglia in DR is further supported by a recent study which showed that A2A adenosine receptor agonist effectively blocks TNF-α production in microglia and markedly decreases hyperglycemia-induced retinal cell death (Ibrahim et. al., 2011). Whereas multiple mechanisms may be involved in diabetes-induced microglial activation, studies indicate that the formation of amadori-glycated proteins has an essential role in microglial activation and this process involves ROS-mediated activation of extracellular signal-regulated kinase and p38MAPK (Ibrahim et. al., 2011).

Overall, this clinical and pre-clinical evidence demonstrates the inflammatory nature of DR and suggests involvement of low-grade chronic inflammation in this disease.

Role of inflammation in the pathogenesis of diabetic retinopathy

DR is clinically classified into two stages: non-proliferative DR and proliferative DR [43]. Non-proliferative DR is an early stage of DR in which vision loss is mainly caused by macular edema as the consequence of increased vascular permeability due to blood-retina barrier breakdown. Proliferative DR is a more advanced stage of DR which is characterized by neovascularization on the retinal surface elicited by hypoxia after vascular function is impaired by capillary occlusion, non-perfusion and degeneration. Vision loss in proliferative DR can be also caused by macular edema as occurs in non-proliferative DR. However, a unique feature of proliferative DR is that severe vision loss or even blindness may be caused by bleeding, hemorrhage and subsequent retinal detachment because of the newly formed fragile vessels [43]. Since inflammation is found as a common feature of DR, significant efforts have been made to understand how inflammation is involved in the pathogenesis of the disease. These studies have disclosed important roles of inflammatory cytokines and

leukocytes in vascular leakage, vessel occlusion and degeneration, pathological neovascularization (Fig. 1), and neuronal death.

Inflammation and vessel leakage

The microvascular endothelium forms an effective barrier to control the movement of blood fluid and proteins across the vessel wall [44]. This barrier is formed by tight junctions and adhesion junctions that join endothelial cells to each other. The tight junction structure consists from a number of transmembrane proteins including occluding claudins, junction adhesion molecules, zonula occludens (ZO), and several regulatory proteins [45]. Adherens junctions are formed by vascular endothelial (VE)-cadherin and catenins [44]. Signaling molecules that induce disorganization/redistribution of junction proteins in the retinal endothelium may lead to breakdown of the blood-retinal barrier (BRB), resulting in an abnormal extravasation of blood components and retinal edema. Endothelial cells can respond to most pro-inflammatory cytokines and chemokines to initiate inflammatory reactions. However, signaling events induced by these cytokines/chemokines may exert multiple functions other than induction of leukocyte adhesion molecule expression. TNF-α is known to cause significant retinal endothelial permeability within a few hours by a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and protein kinase C (PKC)ζ-mediated downregulation of both claudin-5 and ZO-1 expression in the absence of any effects on apoptosis [18]. Consistent with the in vitro data, intravitreal injection of TNF-α leads to increased retinal vascular permeability which is prevented by PKCζ inhibitor [18]. Blockade of TNF-α with Etanercept, a soluble TNF-α receptor, inhibits NF-κB activation and prevents BRB breakdown in the diabetic rat model [20]. Similar increases in endothelial permeability are found when endothelial cells are activated by other cytokines such as IL-1β, IL-6, CCL2 or CXCL8 although the mechanisms may be distinct. CCL2 induces endothelial barrier breakdown via PKCα/ζ-mediated phosphorylation and translocation of occludin, claudin-5, ZO-1 and ZO-2 [46]. In contrast, CXCL8 causes endothelial permeability by inducing VE-cadherin internalization through phosphatidylinositol 3-kinases/Rac/p21 activated kinase signaling axis [47]. However, it should be noted that the in vivo effect of inflammatory molecules in inducing BRB breakdown may be more complicated than their direct effects in altering junction proteins in endothelial cells since many cytokines, such as TNF-α and IL-1β, can also cause cell death when chronically applied. Additionally, they may work in a synergistic or additive manner in DR since multiple inflammatory molecules are simultaneously increased. More importantly, leukocytes recruited in inflammation apparently have a key role in exaggerating the vascular leakage. VEGF is a potent vascular permeability factor that can directly stimulate a breakdown of endothelial permeability barrier function. However, its in vivo effect on retinal vessel permeability also involves leukocytes because blocking ICAM-1 not only reduces leukostasis but also blocks VEGF-induced vascular leakage [48]. The key role of leukostasis in breakdown of the blood-retinal barrier is also demonstrated in diabetic rat and mouse models [38, 39]. The permeability-inducing actions of leukostasis may be attributed to their effects in inducing junction protein alteration, releasing cytokines or increasing ROS, as well as inducing vessel occlusion and injury [38, 39, 49].

Inflammation and vessel closure

Retinal vessel occlusion and degeneration is a typical feature of DR and is also a cause of neovascularization. Mechanisms leading to capillary degeneration may involve inflammatory cytokine-induced endothelial cell death since inflammatory cytokines as TNF-α and IL-1β are also known to increase caspase 3 activity and potently induce endothelial cell apoptosis [18, 50]. The apoptotic effect of inflammatory cytokines may even be exaggerated in the presence of hyperglycemia [50]. However, this mechanism does not explain why the appearance of non-perfused or regressed vessels in DR or in inflammatory

cytokine-treated retinas is random instead of coordinated since vascular cells in adjacent vessels should be equally exposed to inflammatory cytokines. Thus, other mechanisms may be operative in this process. Considering the large cell volume and high rigidity of leukocytes, it is likely that leukostasis in DR may cause capillary occlusion. Supporting this possibility, analysis of diabetic retinal vessels reveals that capillary nonperfusion is closely associated with the presence of leukocytes [36, 37]. By studies using real-time analysis of leukostasis and capillary perfusion, Miyamoto et. al. provided further evidence of the temporal and spatial association between leukostasis and capillary nonperfusion [38]. Their study showed that transient leukocyte attachment to the vessel wall correlates with capillary nonperfusion and capillary reperfusion can occur when leukocytes detach and move on. However, some non-perfused vessels remain permanently closed after the leukocyte detachment [38], suggesting that leukocytes may cause vascular cell death during leukostasis. Further studies indicate that the level of Fas is increased in the retinal vascular endothelial cells during diabetes while its ligand Fas Ligand (FasL) is increased on neutrophils [51]. This change enhances the ability of neutrophils to induce endothelial cell death via Fas/FasL-mediated apoptosis [51]. Blockade of this pathway by anti-Fas neutralizing antibody reduces endothelial cell injury and apoptosis without altering leukostasis [51]. Together, these studies indicate that leukostasis has a key role in causing retinal vascular occlusion and injury in diabetes. Consistent with this possibility, blocking ICAM-1/CD18-mediated leukostasis significantly reduces retinal capillary occlusion and degeneration [39].

Inflammation and pathological neovascularization

Chronic inflammation is known to play a key role in angiogenesis in many diseases including rheumatoid arthritis and cancer where upregulated inflammatory cytokines in the tissue induce the infiltration of leukocytes. Leukocytes, in turn, actively enhance the formation of new vasculature by releasing angiogenic factors such as VEGF, angiopoietin 1, basic fibroblast growth factor, platelet-derived growth factor and transforming growth factor (TGF)-β. These agents promote recruitment of endothelial progenitor cells and enhance the survival, migration, and proliferation of pre-existing endothelial cells. They also induce matrix metallopeptidase (MMP) activity which promotes degradation of extracellular matrix [52]. A role of inflammation in proliferative DR has been suggested given that a number of inflammatory cytokines are known to increase in the disease. Nevertheless, direct investigation of inflammation in proliferative DR is hindered due to the lack of a small animal model that develops reproducible proliferative DR as occurs in human. Therefore, studies of retinal neovascularization are carried out in rodent models of oxygen-induced retinopathy by inducing retinal vessel obliteration with hyperoxia. These studies have shown that inflammatory genes are upregulated at the onset of the hypoxia as well as during the period of neovascularization [53, 54]. TNF-α levels are highly increased and blockade of TNF-α by genetic gene deletion or TNF-α receptor fusion protein (Etanercept) prominently attenuates neovascularization [55, 56]. Immunolabeling study reveals that monocyte-lineage cells are the major source of TNF-α [55], suggesting that these cells may be involved in pathological neovascularization. This possibility is supported by the finding of Ishida et al. that monocytes/macrophages are present in the neovascular tufts that grow into the vitreous and that depletion of cells of the monocyte lineage with clodronate-liposomes leads to the suppression of pathological but not physiological retinal angiogenesis [57]. However, it should be noted that some monocyte lineage cells present in oxygen-induced retinopathy are not pathogenic but instead, are necessary for removing neovascular tufts. For example, CCL2 deficiency reduces monocyte/microglia recruitment and delays neovascular tuft regression but does not prevent neovascularization [58]. Thus, although there is no doubt that inflammation is a critical player in pathological neovascularization, careful attention must be given when selecting inflammatory molecules as a therapeutic target.

Inflammation and retinal neuronal death

Inflammatory cytokines such as TNF-α and IL-1β are also proapoptotic proteins that are known to cause neuron death in many diseases [59, 60]. These cytokines, together with ROS and excessive nitric oxide (NO) released by leukocytes during inflammation, are key players in retinal neuron cell death [59, 61–64]. Blockade of leukocyte recruitment by ICAM-1 inhibition or deleting CD18 significantly suppresses leukocyte accumulation and retinal ganglion cell death in N-Methyl-D-aspartic acid-induced retinal neuropathy [65]. Presumably, similar mechanisms may be operative in DR induced neuron injury. Neuronal cell injury was first suggested as an early feature of diabetic retinopathy in the early 1960's but has only recently been widely recognized. Barber et al. reported in 1998 that retinal thickness and ganglion cell numbers are reduced while neural cell apoptosis is significantly increased in a Streptozotocin-diabetic rat model [66]. However progress in this field has been very slow. A search of PubMed for "DR" in combination with "neural cell death" or "neuron death" or "ganglion cell death" produced only 75 papers about this topic. Among these papers, one study showed that salicylate-based anti-inflammatory drugs attenuate diabetes-induced loss of ganglion cells [67]. However, there is no direct evidence to demonstrate the impact of inflammatory cytokines and leukocytes on neuron death in DR. More investigations are needed to fully address this issue.

Mechanisms underlying retinal inflammation in diabetic retinopathy

Tremendous efforts have been made to understand how hyperglycemia causes inflammatory reactions because of the fundamental role of hyperglycemia in DR. These studies have demonstrated links between hyperglycemia-induced oxidative stress, dysregulation of nitric oxide synthase (NOS), formation of AGEs and production of inflammatory molecules and leukostasis in DR. In addition, recent studies also show that two other risk factors for DR, hypertension and dyslipidemia, independently link to inflammation and/or exaggerate the inflammatory reactions induced by hyperglycemia.

Oxidative stress

Oxidative stress happens when ROS are over produced or when endogenous anti-oxidant systems are impaired. Mitochondria have long been recognized as a key source of ROS formation during diabetes [2]. Mitochondria can generate ROS by leak of electrons to molecular oxygen at electron transport chain (ETC) complexes I, II and III [68]. In diabetes, the metabolism of glucose-derived pyruvate through the ETC complexes is increased because of high glucose concentration within cells, resulting in superoxide overproduction by mitochondria [69]. This pathway not only produces more ROS by itself but also initiates other pathways leading to a breakdown in the balance between pro-oxidant and endogenous anti-oxidant systems, such as increases in glucose flux through the aldose reductase pathway, formation of AGEs, and activation of PKC [70]. All of these changes can lead to oxidative stress by decreasing activities of anti-oxidant enzymes [71, 72] or further activating the ROS generating machinery inside the cells [73–77]. This mechanism has been thought as a dominant cause of oxidative stress in diabetes. However, data showing increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in diabetic patients and animals and high glucose-treated endothelial cells suggest that NADPH oxidase is also an important and early source of ROS [78–81]. NADPH oxidase is the major source of ROS in the cardiovascular system. It consists of a NOX catalytic subunit, 4 phox subunits (p22phox, p40phox, p47phox and p67phox) and the low molecular weight G protein Rac [82]. Although NOX and p22phox form a flavocytochrome that contains all the catalytic machinery needed for superoxide production, the other subunits are required for regulation of enzyme activation. The p47phox and p67phox subunits are essential for activity. Assembly of the complex is initiated upon phosphorylation of p47phox, which is

regulated by many intracellular signaling kinases such as PKC, MAPKs and Akt [83–87]. The p67phox subunit mediates direct binding of the complex with activated Rac, which initiates the electron transfer reaction that produces superoxide [88]. These regulatory mechanisms contribute to the unique feature of NADPH oxidase-derived ROS as signaling mediator in many cellular responses. However when these mechanisms are activated because of diabetes, they can cause NADPH oxidase to overproduce ROS. It is unclear, whether mitochondrial ETC or NADPH oxidase initiate the excessive ROS formation in diabetes. However, studies in other models have suggested a positive reciprocal regulation between them [89, 90]. Overall, diabetes-induced imbalance of the pro-oxidation and anti-oxidation results in sustained oxidative stress in the body including retina.

ROS are important intracellular signaling molecules in the inflammatory cascade. ROS play a key role in inflammatory gene expression by activation of transcription factors such as NF-κB, Signal Transducers and Activators of Transcription proteins (STAT) and activator protein 1 (AP-1) [91]. Among these factors, NF-κB is the key transcription factor in most inflammatory reactions. NF-κB is a transcription factor that is ubiquitously expressed and which regulates numerous genes related to inflammation and immune responses [92]. It exists in homo- or heterodimer forms that bind to κB-binding sites in gene promoters to regulate gene transcription at least partly by interacting with transcriptional coactivators such as p300/CBP. NF-κB also cooperates with other transcription factors such as the constitutive transcription factor specificity protein 1 and the inducible transcription factors, STATs, cAMP response element-binding, and AP-1, to achieve stimulus- and promoter-specific transcriptional regulation [93]. The NF-κB dimer is sequestered in the cytosol in an inactive form when it binds to inhibitor of κB (IκB) proteins. These IκB proteins mask its nuclear translocation signal and inhibit its DNA binding activity [93, 94]. When IκB is ubiquitinated and degraded following phosphorylation by IκB kinases, it releases NF-κB resulting in NF-κB activation [94]. NF-κB and many kinases involved in its direct or indirect activation are redox sensitive molecules [95], allowing it to serve as the downstream target of ROS and mediate ROS-induced inflammatory gene expression.

In physiological conditions, the production and elimination of ROS are precisely regulated and will not produce unnecessary inflammation in the body. However, diabetes-induced sustained oxidative stress is a major cause of retinal inflammation. The activity of NF-κB is increased in high glucose treated retinal endothelial cells, pericytes or glial cells, and in diabetic retinas from patients or animal models [96–101]. This activation is significantly blocked by inhibiting redox systems by blockade of NADPH oxidase or anti-oxidant treatment [100, 101], indicating a cause-and-effect relationship. Activation of NF-κB has an essential role in diabetes-induced retinal inflammation in that inhibition of NF-κB blocks both high glucose and diabetes-induced production of inflammatory molecules in retinal cells and retinal tissue, respectively [96–98]. Correlated with decreased cytokine formation, leukostasis is also attenuated by inhibition of NF-κB [96]. Similarly, studies in animal and tissue culture models have demonstrated the involvement of NADPH oxidase in diabetes-induced inflammation and breakdown of the BRB [34, 102]. The results showed that diabetes-induced increases in retinal ROS, VEGF expression and vascular permeability are accompanied by increases in the NADPH oxidase catalytic subunit NOX2 [34, 102, 103]. The experiments further demonstrated that deleting the NOX2 gene or inhibiting NADPH oxidase prevented diabetes-induced increases in retinal permeability and ICAM-1 expression and leukostasis, indicating that the NOX2 NADPH oxidase is critically involved in the pathology of DR [34, 102, 104].

NOS dysregulation

In addition to oxidative stress, diabetes-induced vascular inflammation is very closely related to nitric oxide (NO) which is an important second messenger that regulates many

physiological and pathological events, including vascular dilation and vascular inflammation. Studies indicate that NO has a biphasic role in vascular and inflammatory diseases depending on the specific source and the amount produced [105]. The constitutively expressed NOSs, endothelial NOS (eNOS) and neuronal NOS (nNOS), are Ca2+-dependent and regulated to produce low levels of NO. NO from nNOS is involved in neural signaling and is also expressed in smooth muscle cells where it has a key role in regulating vascular responses to tissue hypoxia. NO from eNOS maintains blood flow and prevents platelet aggregation and leukostasis [106]. In fact, deleting eNOS results in elevated expression of inducible NOS (iNOS) (an NF-κB mediated inflammatory molecule) in retina which is associated with accelerated development and increased severity of DR [107]. In contrast with eNOS and nNOS, iNOS is Ca2+-independent, constitutively active and produces large amounts of NO [105]. It is not expressed in normal retinas, but is induced in retinal glial and microglial cells during inflammatory conditions. including DR. NO from iNOS plays a role in causing tissue damage and inflammation. Inhibition of iNOS by inhibitor or gene deletion prevents ICAM-1 expression, leukostasis, and vascular permeability in the diabetic retina [108]. One possible reasons for the opposite function between eNOS and iNOS is that NO produced at low levels by eNOS mainly exerts its effects directly by interactions with metal-containing proteins to maintain the normal vascular function. In contrast, high levels of NO produced by iNOS can act indirectly via the generation of reactive nitrogen oxide species (RNOS). RNOS are formed through the interaction of NO with molecular oxygen or superoxide. RNOS is strong pro-inflammatory molecule and can cause tissue damage due to lipid peroxidation, DNA damage, oxidation of thiols and nitration of tyrosine residues [109, 110].

The NOS pathway serves to link multiple pathways. On the one hand, ROS generated from NADPH oxidase or mitochondrial oxidase can rapidly react with NO to form RNOS and reduce bioavailable NO which reduces vessel dilation and increases leukocyte adhesion and platelet aggregation, leading to inflammation [105, 111]. On the other hand, NOS can be an important source of ROS due to “NOS uncoupling” in which the enzyme generates superoxide rather than NO when its substrate L-arginine is limited by increased arginase activity [105] or when the cofactor tetrahydrabiopterin is oxidized. NOS uncoupling can contribute to disease progression by multiple mechanisms. Uncoupling of eNOS can contribute to ischemia/hypoxia and tissue damage by reducing EC-dependent vasorelaxation and inducing platelet aggregation and leukostasis and also by impairing function in endothelial progenitor cells [105]. nNOS uncoupling could exacerbate the vascular damage by altering smooth muscle cell responses to hypoxia [105]. Uncoupling of iNOS could increase formation of inflammatory mediators by increasing NF-κB transcriptional activity due to decreased nitrosylation since NO is anti-inflammatory molecule which dampens inflammatory reactions by causing S-nitrosylation of the NF-κB subunits p65 and p50 [105, 112, 113]. This reduces their DNA binding which in turn decreases transcription of iNOS and other inflammatory mediators [112, 113]. Consistently, inhibition of arginase is shown to increase bioavailable NO, reduce superoxide formation, and block inflammatory reactions during retinal inflammation [104].

AGEs formation

The impact of hyperglycemia in diabetes-induced retinal inflammation is not only attributed to the direct effect of high glucose but also a consequence of the byproducts of hyperglycemia, particular AGEs. AGEs are formed by nonenzymatic glycation and oxidation of amino groups of proteins, lipids, and DNA [114]. This process is accelerated in diabetes in the presence of high glucose and oxidative stress [14, 114, 115]. In the diabetic retina, AGEs levels are prominently increased and AGE immunoreactivity is localized in vitreous, internal limiting membrane, and retinal vasculature [114]. The effect of AGEs is

mostly mediated by the receptor for advanced glycation endproducts (RAGE), a multiligand signal transduction receptor of the immunoglobulin superfamily that has been identified in numerous cells including Muller cells, endothelial cells, and neurons in the retina [114]. Mediated by RAGE, AGEs can activate many downstream signaling molecules including NF-κB and ROS to induce inflammatory reactions such as ICAM-1 expression and leukostasis in retina and retinal cells [115, 116]. Unlike other inflammatory molecules, RAGE-mediated NF-κB activation overrides the endogenous autoregulatory negative feedback loop and shows a prolonged time course [115]. This feature together with positive regulation of RAGE expression by NF-κB allows AGEs to elicit sustained inflammation reactions in DR. The role of AGEs-RAGE pathway in DR has been directly addressed by using soluble RAGE to block AGEs activity in diabetic wild type mice and transgenic mice with RAGE overexpression under the control of VEGF receptor 2 promoter [117]. Consistent with the notion that RAGE is an inflammatory mediator, normal glycemic RAGE transgenic mice display significant high levels of ICAM-1 expression, leukostasis and VEGF expression as compared with wild type mice. Interestingly, the above changes were prominently augmented in diabetic mice. However these increases were normalized to basal level by soluble RAGE treatment, indicating the AGEs-RAGE pathway is a key mediator of diabetes-induced retinal inflammation.

Hypertension

In addition to hyperglycemia, hypertension is recognized as the second key factor associated with the development of DR. Although the mechanisms by which hypertension increases the risk of DR are not fully understood, hypertension can certainly contribute to the pathogenesis of DR by inducing inflammatory reactions. The spontaneously hypertensive rats (SHR) exhibit a significant increase in monocyte/microglial number, ICAM-1 and VEGF expression, and NF-κB protein level in retina in both prehypertensive and hypertensive conditions as compared with normotensive control rats [118]. These increases are further boosted when the SHR rats were made diabetic with Streptozotocin [118], suggesting that inflammation is a common target of both hyperglycemia and hypertension. Mechanisms of hypertension-induced retinal inflammation are not completely clear. However, the over activation of renin-angiotensin system (RAS) may have a critical role.

The RAS plays a key role in the development and pathophysiology of hypertension by producing angiotensin II (Ang II), a well-known vasoconstrictive peptide [119]. However, it is now known that the function of Ang II goes far beyond its impact on systolic blood pressure. Ang II, by binding to the Ang II type 1 receptor (AT1R), potently induces NADPH oxidase activation, ROS formation, and activation of a varieties of kinases, phophatase and transcription factors [120]. Ang II-induced activation of these signaling pathways, especially ROS-modulated NF-κB activation, is critically involved in vascular inflammation by producing inflammatory cytokines and recruiting leukocytes [119, 120]. Further, the Ang II pathway interacts with other pathways to amplify the inflammatory cascades. Ang II signaling enhances AGEs formation by accelerating the process of nonenzymatic glycation and oxidation meanwhile inhibiting glyoxalase system-mediated degradation of AGE precursor [119–121]. Ang II also induces dysregulation of NOS pathways [120]. In retina, extensive studies have been performed to understand whether the RAS has a role in retinal inflammation and injury. These studies have identified that all of the components of RAS as well as Ang II receptors are expressed in retina [122, 123], suggesting an involvement of the RAS in the retinal pathophysiology. This possibility has been assessed by blockade of RAS in disease models or by intravitreal or systemic delivery of Ang II to healthy animals. In animal models of DR, levels of angiotensinogen and AT1R are significantly increased in the retina [96]. Blocking AT1R prevents NF-κB activation, ICAM-1 expression and leukostasis typically seen in DR [96]. In SHR, an AT1R antagonist (Losartan), also completely blocks

hypertension-induced retinal inflammation [124]. In the diabetic and hypertensive transgenic (Ren-2) rats that display an enhanced extra-renal RAS, an AT1R antagonist (Candesartan) attenuates diabetes-induced retinal vascular inflammatory aspects including leukostasis, NOS dysregulation, and AGEs formation [121]. In contrast, Ang II treatment induces retinal inflammatory reactions including IL-6 production, ROS formation and leukostasis in healthy animals [125, 126]. These studies indicate the RAS is necessary and sufficient for the production of retinal inflammation in diseases such as diabetes and hypertension.

In addition to the RAS, vascular stretch and activation of endothelin may also have roles in hypertension-induced retinal inflammation [127, 128]. However, more evidence is needed to address this possibility.

Dyslipidemia

Dyslipidemia is another risk factor of DR. Although it is well-known that dyslipidemia is critically involved in macrovascular inflammation in atherosclerosis, its specific role in DR is largely unclear. A recent study shows that basal VCAM-1 expression is increased in retinal vessels in hyperlipidemic mice [129], suggesting that hyperlipidemia is cause of retinal vascular inflammation. However, it is unknown whether or not this change will result in leukostasis because the basal level of other inflammatory molecules is not increased in hyperlipidemic mice. In addition, hyperlipidemia does not further increase diabetes-induced expression of inflammatory molecules in these mice. Further investigation is needed to determine the role of dyslipidemia in retinal inflammation during DR.

Anti-inflammation pathway

In addition to the above pro-inflammatory pathways, diabetes may exaggerate retinal inflammation by impairing endogenous anti-inflammatory mechanisms such as the proliferator-activated receptor (PPAR)γ pathway [101]. PPARγ is a member of a ligand-activated nuclear receptor superfamily and is involved in many biological processes such as adipogenesis, glucose metabolism, and angiogenesis [130]. Synthetic PPARγ agonists such as pioglitazone and rosiglitazone reduce biomarkers of inflammation through mechanisms involving inhibition of NF-κB activation [131]. The PPARγ pathway is operative in DR given that PPARγ agonist blocks retinal inflammation and vascular leakage in diabetic rats whereas inhibition of PPARγ exaggerates signs of DR [132]. Studies also show that the PPARγ pathway is impaired in DR in a mechanism involving NADPH oxidase [101].

In summary, risk factors of DR such as hyperglycemia, hypertension and dyslipidemia are all involved in retinal inflammation in DR through a variety of mechanisms including oxidative stress, NOS dysregulation, AGE formation, RAS activation, and inhibition of endogenous anti-inflammatory pathways (Fig. 2). However, it should be noted that inflammation is not an endpoint of the above inflammatory mechanisms. Inflammation, in turn, further activates these inflammatory mechanisms and serves as a key mediator in the positive feedback loop of inflammatory cascades (Fig. 2). For example, oxidative stress is not only a cause of inflammation but also a consequence of inflammation. During inflammation, many inflammatory cytokines induce oxidative stress and employ the NADPH oxidase pathway to induce expression of other inflammatory molecules such as CCL2 [133]. Leukocytes also release massive amounts of ROS in response to inflammatory stimuli. Consequently, inhibition of inflammatory cytokines has been shown to prevent oxidative stress and retinal pathology in experimental models [126]. As a key factor in the diabetes-induced inflammatory cascade and one of the major causes of retinal damage, inflammation serves as a promising therapeutic target for the treatment of DR.

New approaches for diabetic retinopathy: Can we stop inflammation

Since inflammation is identified as a critical mechanism for DR, significant effort has been made devoted to the development of new strategies for treatment or prevention of DR by targeting inflammation. Clinical and pre-clinical trials have tested a variety of anti-inflammatory therapeutics (Table 1).

Steroids

Corticosteroids have been used to treat ocular inflammatory conditions for more than half a century, but their application to DR was not investigated until recently [134]. Corticosteroids are known to exert anti-inflammatory actions by repressing a number of pro-inflammatory transcription factors including NF-κB and AP-1 via the direct interaction between glucocorticoid receptor and transcription factors or indirect mechanisms [135]. Oral steroids are excluded from DR therapy due to tremendous systematic side effects including exacerbation of diabetes [134]. However, intravitreal delivery of steroids has been shown to be beneficial in both pre-clinical and clinical studies. In a rat model of DR, intravitreal injection of dexamethasone suppressed up-regulation of ICAM-1, leukostasis, and prevented retinal vascular leakage [136]. Dexamethasone is not suitable for a clinical trial owing to its three-hour half-life [134]. Triamcinolone acetonide (TA), with a longer intravitreal half life (18.6 days) [137], has been tested in several clinical trials. In 2001, Jonas et al. reported the first clinical study to treat a diabetic patient with macular edema by an intravitreal injection of crystalline cortisone. Although laser coagulation failed in stopping cystoid macular edema in this patient, TA efficiently improved visual acuity [138]. A multicenter randomized clinical trial with 840 eyes to compare the effect of TA with focal/grid laser in treating diabetic macular edema was reported by the DR Clinical Research Network [139]. These studies have shown that TA (4 mg) improves visual acuity better than laser coagulation after 4 months of treatment. However, at 2 and 3 years, the mean visual acuity in TA-treated group was less than that in the laser-treated group. The potent acute effect of TA vs the longer but slower effect of laser photocoagulation suggests that combination therapy may be better. This concept has been confirmed by small randomized clinical trials which showed that TA in combination with laser coagulation improved visual acuity and decreased central macular thickness more as compared with laser photocoagulation alone [139]. These studies suggest that anti-inflammatory therapy with steroids could be a therapeutic choice for DR. However, it should also be noted that intravitreal steroid therapy increases the risk of glaucoma and cataract [139].

Nonsteroidal anti-inflammatory drug (NSAID)

NSAIDs are a group of compounds, such as aspirin and salicylate, that exert anti-inflammatory activity by inhibiting cyclooxygenase (COX) enzyme-mediated eicosanoid formation [140]. The COX enzymes are targets by NSAIDs [141]. Among the three isoforms of COX enzymes, COX-1 and COX-2 have been studied most extensively. COX-1 is expressed constitutively in many cell types and is important for homeostatic processes [141]. In contrast, COX-2 is induced mainly at sites of inflammation and produces predominately pro-inflammatory prostaglandins (PGE) such as PGE2 and PGF2α [141]. The promoter region of COX-2 contains NF-κB binding sites and COX-2 expression is under the control of NF-κB activity during inflammation [142]. Since iNOS is also an NF-κB -driven molecule, iNOS and COX-2 are often concurrently expressed in inflammation. Products from iNOS, particularly RNOS, positively regulate COX2 expression and activity [142]. COX-2 expression and its products including PGE2 are significantly increased in retinas from diabetic animals and in high glucose-treated retinal cells [143, 144]. Inhibition of COX-2 but not COX-1 reduces production of PGE2 from diabetic rat retinas [145], suggesting the dominant role of COX-2 in DR. COX-2 inhibition also blocks diabetes-

induced increases retinal inflammatory reactions such as increases in ICAM-1 expression and leukostasis [20, 143, 146]. Given that COX-2 has a role in retinal inflammation, NSAIDs may have therapeutic potential in the pathology of DR.

Pre-clinical studies have demonstrated that aspirin treatment prevents capillary cell apoptosis and vessel degeneration in the diabetic dog and rat [67, 147, 148]. Similar effects are observed in diabetic rats treated with two other salicylate-based drugs, sodium salicylate and sulfasalazine [67]. In addition to salicylates, specific COX-2 inhibitors also reduce vascular leakage, capillary cell apoptosis and vessel degeneration [20, 149, 150]. In the clinic, the beneficial effect of NSAIDs has been realized in a study showing reduced incidence of DR in patients taking salicylates for the treatment of rheumatoid arthritis [151]. This finding encouraged the launch of two clinical trials: the Early Treatment DR Study and the Dipyridamole Aspirin Microangiopathy of Diabetes Study [152, 153]. These studies showed that although treatment of patients with advanced DR with low dose of aspirin does not have any benefits, the development of retinal microaneurysms is significantly attenuated in patients with early stage of DR when treated with high dose of aspirin (900 mg/day). Clinical trial of specific COX-2 inhibitors has been discouraged given that systemic COX-2 inhibitors increase incidence of heart attacks and strokes [140]. However, in preclinical studies, topical administration of COX-2 inhibitor was shown to reduce signs of DR similar to systematic application of COX-2 inhibitor [149, 150]. It would be of importance to investigate the therapeutic benefits of topical COX-2 inhibitor in future clinical trials.

Blocking inflammatory molecules

In addition to the above blockers of general inflammation, studies have been performed to determine whether targeting specific inflammatory molecules can be beneficial for DR. As a key player in many inflammatory reactions, the pro-inflammatory cytokine TNF-α is an attractive target for many inflammatory diseases including rheumatoid arthritis [154]. By binding to its cell surface receptor (TNF-α receptor 1 and 2), TNF-α induces ROS formation, NF-κB activation and iNOS expression in inflammatory cells and rapidly upregulates the expression of adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 at the endothelial surface, resulting in leukocyte recruitment [155]. TNF-α is implicated in DR since its level is significantly increased in DR [20–22]. The effect of anti-TNF-α therapy has been studied in diabetic animal models and a few clinical cases. Subcutaneous administration of TNF-α trap (a soluble TNF-α receptor/Fc construct, Etanercept) significantly blocks retinal inflammation, retina cell injury and vessel leakage in diabetic rats [20, 156]. There is no clinic trial report so far. However in one clinical study with 4 patients, Infliximab, a TNF-α neutralizing antibody, was shown to improve visual acuity and reduce macular thickness in patients who failed to improve in response to laser photocoagulation treatment [157].

Since leukostasis is critically involved in the pathogenesis of DR, pre-clinical studies have been performed to determine whether blocking the interaction between leukocytes and endothelial cells will have beneficial effect in diabetic animal models. Leukocyte function associated antigen-1 (LFA-1, an integrin) expressed in leukocytes is important for leukocyte-endothelial cell interaction by binding to ICAM. Topical delivery of SAR 1118, a small antagonist of LFA-1, dose-dependently reduced leukostasis and retinal vascular leakage in diabetic rat model [158]. In addition to ICAM-1, interaction between very late antigen-4 (VLA-4) in leukocytes and VCAM-1 in endothelial cells is also involved in leukostasis. VLA-4 is formed by integrin alpha 4 (CD49d) and integrin beta 1. Blocking VLA-4 by anti-CD49a neutralizing antibody significantly attenuated the diabetes-induced leukostasis and vascular leakage [159]. Moreover, increases in NF-κB activity and levels of VEGF and TNF-α protein were also reduced by anti-CD49a neutralizing antibody [159],

indicating that leukocyte recruitment plays a positive feedback role in the inflammatory reactions in DR.

In addition to these specific inflammatory molecules, the AGEs/RAGE pathway is also an emerging target for DR given that it is an important mechanism leading to retinal inflammation. Blocking the RAGE axis with soluble RAGE improves neuronal dysfunction and limits the development of acellular capillaries and pericyte ghosts in diabetic mice [160]. A similar beneficial effect was also observed by inhibition of AGEs formation with LR-90, a new advanced glycation inhibitor, in a diabetic rat model [161].

Targeting oxidative stress

As a key mediator in inflammation, oxidative stress serves as an important target for anti-inflammatory therapy. A number of studies have been performed to determine whether blocking oxidative stress can be an approach to treat DR. In diabetic rats, supplement of anti-oxidants such as vitamins C and E attenuates the development of acellular capillaries and decreases the number of pericyte ghosts [162]. The effect of anti-oxidants is further boosted when a more comprehensive mixture of anti-oxidant diet is applied [162]. Clinical studies also show that high doses of vitamin E reverse some of the changes in the retinal vessels of diabetic patients [163]. However, it was also noticed that the non-selective anti-oxidants did not achieve the expected beneficial effects predicted by the pre-clinical studies and in some clinical studies the anti-oxidant therapy did not show any beneficial effect [164]. Thus, it is necessary to target specific dysregulated oxidative stress generating mechanisms and to avoid interfering with the physiological roles of reactive oxygen species. Regarding this issue, blockade of NADPH oxidase may provide better output given that NAPDH oxidase activity is dysregulated in DR and NADPH oxidase has a key role in the inflammatory cascade. This notion has been supported by several in vitro and in vivo studies. In retina and retinal cells, NADPH oxidase blockers are shown to prevent CCL2 production induced by different inflammatory stimuli [133]. In high glucose-treated retinal cells, NADPH oxidase inhibition significantly inhibits oxidative stress, NF-κB activation, RNOS formation, and inflammatory reactions [100, 102]. In diabetic animals or models of ischemic retinopathy, blockade of diabetes-induced increases in NADPH oxidase activity prevents vascular leakage and pathological neovascularization, respectively [34, 102, 103]. Moreover, these studies also reveal that statin treatment effectively blocks up-regulation of NADPH oxidase activity in DR [34], suggesting that the beneficial of statin treatment is at least partly due to its activity in blocking NADPH oxidase.

Blocking RAS

The RAS serves as a promising target for DR since this system is involved in both diabetes and hypertension-induced retinal inflammation, and is a pathway that crosstalks with multiple other pathways including oxidative stress and AGEs. Specific blockade of the RAS has been shown to prevent oxidative stress, inflammation, and vascular damage in diabetic animal models [165–167]. Several clinical trials have been performed to evaluate whether intervention of RAS can reduce the risk or slow the progression of DR. In a clinical trial involving 285 type 1 diabetic patients with normotensive and normoalbuminuria, blockade RAS with Losartan (an AT1R blocker) or Enalapril (angiotensin converting enzyme inhibitor) significantly reduced the progression of retinopathy by 70% and 65% respectively [168]. However, such dramatic benefit is not observed in the DIabetic REtinopathy Candesartan Trials which enrolled 3326 patients with type 1 diabetes and 1905 patients with type 2 diabetes to assess whether Candesartan (AT1R blocker) could reduce the incidence and progression of retinopathy in a five year period [169, 170]. All of the type 1 diabetic patients recruited in the study were normotensive and normoalbuminuric. Patients without diabetic retinopathy were assigned to treatment to study whether Candesartan can reduce the

incidence of DR. Patients with existing DR were assigned to treatment to study whether Candesartan can slow down the progression of DR. This study showed that Candesartan treatment reduced the incidence of DR but did not prevent progression of DR [169]. For a trial in type 2 diabetic patients, patients recruited were normoalbuminuric, normotensive, or treated hypertensive people with mild to moderately severe DR. This study also showed that Candesartan treatment did not prevent progression of DR but that it significantly induced the regression of DR even after adjustment for baseline characteristics and variations in systolic blood pressure [170]. Overall, blocking AT1R has a beneficial effect for DR. However further trials are needed to resolve the discrepancy between the above trials and to fully evaluate the beneficial effect of RAS blockade before its clinical usefulness is fully understood.

Conclusion

Accumulated evidence has demonstrated that retinal inflammation plays a critical role in the pathogenesis of DR. Although there is no pathogen in the diabetic retina, diabetes apparently hijacks mechanisms of inflammation such as oxidative stress formation, dysregulation of the NOS pathway, and NF-κB activation. These changes together with diabetes-induced AGEs formation and impaired endogenous anti-inflammatory pathways lead to chronic inflammatory reactions in the retina by persistently inducing expression of inflammatory cytokines, chemokines, and recruiting leukocytes. The chronic inflammation is responsible for the pathogenesis of DR by causing neurovascular damage and ischemic neovascularization. The discovery of inflammation as a key player in DR has opened a new avenue for the development of new treatments and strategies for this devastating disease.

Future perspective

The mechanism by which the inflammatory cascade is initiated and maintained in DR is currently unknown as are the individual roles of inflammatory molecules in the different stages of DR. On the one hand, the inflammatory molecules have redundant functions given that many of them cause activation of similar downstream targets such as NF-κB and several chemokines that share the same receptor. On the other hand, many inflammatory molecules are indispensible for the positive feedback loop during inflammation since retinal inflammation has been shown to be attenuated by blocking TNF-α, iNOS or integrins. These mysteries have led to challenges when considering any of the inflammatory molecules as a drug target since blockade of any one of them may be effective if the molecule is indispensible. However, the blockade can also be ineffective or even worse if compensation happens or goes in an unwanted direction. These uncertainties may be resolved by understanding the changes that occur in the inflammatory cascade after blocking an individual inflammatory molecule and selecting several candidates for combination therapy. Alternatively, drugs targeting several mechanisms, such as statin and calcium dobesilate, may be more efficient than those that target a single mechanism. Related to this notion, the use of RAS blockers also represents a possible future clinical therapeutic approach for DR given that the RAS regulates multiple key factors of inflammation including oxidative stress, AGEs formation and hypertension. A subset of Ang II receptor blockers which possess partial PPARγ agonist activity (e.g. Telmisartan and Irbesartan) may be more powerful since they will block pro-inflammatory pathways meanwhile enhancing anti-inflammatory pathways. Few studies have been performed to understand the endogenous anti-inflammation mechanisms in DR. It would be helpful to identify such mechanisms as PPARγ in DR in order to correct or enhance their anti-inflammatory activity. Finally, more studies are needed to understand the role of inflammation in neuronal damage in DR.

Abbreviations

AGEs	advanced glycation end products
Ang II	angiotensin II
AP-1	activator protein 1
AT1R	Ang II type 1 receptor
BRB	blood-retinal barrier
COX	cyclooxygenase
DR	diabetic retinopathy
eNOS	endothelial NOS
ETC	electron transport chain
ICAM-1	intercellular adhesion molecule 1
LFA-1	leukocyte function associated antigen-1
iNOS	inducible NOS
IκB	inhibitor of κB
IL-1β	interleukin-1β
IL-6	interleukin-6
MAPKs	mitogen-activated protein kinases
MMP	matrix metallopeptidase
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
nNOS	neuronal NOS
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	nonsteroidal anti-inflammatory drug
PGE	prostaglandin
PPARγ	proliferator-activated receptor gamma
RAGE	receptor for advanced glycation endproducts
RAS	renin-angiotensin system
RNOS	reactive nitrogen oxide species
ROS	reactive oxygen species
SHR	spontaneously hypertensive rats
STAT	signal transducers and activators of transcription protein
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
VCAM-1	vascular cell adhesion molecule 1
VE-cadherin	vascular endothelial-cadherin

VEGF	vascular endothelial growth factor
VLA-4	very late antigen-4
ZO	zonula occludens

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Fong DS, Aiello L, Gardner TW, et al. Retinopathy in diabetes. Diabetes Care. 2004; 27 (Suppl 1):S84–87. [PubMed: 14693935]
2. Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. Diabetes Care. 1998; 21(1):143–156. [PubMed: 9538986]
3. Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BE. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XXII the twenty-five-year progression of retinopathy in persons with type 1 diabetes. Ophthalmology. 2008; 115(11):1859–1868. [PubMed: 19068374]
4. Williams R, Airey M, Baxter H, Forrester J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular oedema: a systematic review. Eye (Lond). 2004; 18(10):963–983. [PubMed: 15232600]
5. Varma R. From a population to patients: the Wisconsin epidemiologic study of diabetic retinopathy. Ophthalmology. 2008; 115(11):1857–1858. [PubMed: 19068373]
6. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010; 376(9735):124–136. [PubMed: 20580421]
7. Yam JC, Kwok AK. Update on the treatment of diabetic retinopathy. Hong Kong Med J. 2007; 13(1):46–60. [PubMed: 17277392]
8. Arevalo JF, Sanchez JG, Lasave AF, et al. Intravitreal Bevacizumab (Avastin) for Diabetic Retinopathy at 24-months: The 2008 Juan Verdaguer-Planas Lecture((R)). Curr Diabetes Rev. 2010; 6(5):313–322. [PubMed: 20594160]
9. Kowluru RA, Zhong Q, Kanwar M. Metabolic memory and diabetic retinopathy: role of inflammatory mediators in retinal pericytes. Exp Eye Res. 2010; 90(5):617–623. [PubMed: 20170650]
10. Villeneuve LM, Natarajan R. The role of epigenetics in the pathology of diabetic complications. Am J Physiol Renal Physiol. 2010; 299(1):F14–25. [PubMed: 20462972]
11. Nishijima K, Ng YS, Zhong L, et al. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. Am J Pathol. 2007; 171(1):53–67. [PubMed: 17591953]
12. Klein R, Klein BE. Diabetic eye disease. Lancet. 1997; 350(9072):197–204. [PubMed: 9250197]
13. Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. Jama. 2002; 288(20):2579–2588. [PubMed: 12444865]
14. Stitt AW, Curtis TM. Advanced glycation and retinal pathology during diabetes. Pharmacol Rep. 2005; 57 (Suppl):156–168. [PubMed: 16415497]
15. Caldwell RB, Bartoli M, Behzadian MA, et al. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Curr Drug Targets. 2005; 6(4):511–524. [PubMed: 16026270]
16. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005; 54(6):1615–1625. [PubMed: 15919781]
17. Rahman I. Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. Biochem Pharmacol. 2002; 64(5–6):935–942. [PubMed: 12213589]

18. Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA. TNF-alpha signals through PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes. 2010; 59(11):2872–2882. [PubMed: 20693346]
19. Barreiro O, Martin P, Gonzalez-Amaro R, Sanchez-Madrid F. Molecular cues guiding inflammatory responses. Cardiovasc Res. 2010; 86(2):174–182. [PubMed: 20053659]
20. Joussen AM, Poulaki V, Mitsiades N, et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. Faseb J. 2002; 16(3):438–440. [PubMed: 11821258]
21. Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (Lond). 2006; 20(12):1366–1369. [PubMed: 16284605]
22. Limb GA, Chignell AH, Green W, LeRoy F, Dumonde DC. Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy. Br J Ophthalmol. 1996; 80(2):168–173. [PubMed: 8814750]
23. Hawrami K, Hitman GA, Rema M, et al. An association in non-insulin-dependent diabetes mellitus subjects between susceptibility to retinopathy and tumor necrosis factor polymorphism. Hum Immunol. 1996; 46(1):49–54. [PubMed: 9157089]
24. Vincent JA, Mohr S. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. Diabetes. 2007; 56(1):224–230. [PubMed: 17192486]
25. Kowluru RA, Odenbach S. Role of interleukin-1beta in the development of retinopathy in rats: effect of antioxidants. Invest Ophthalmol Vis Sci. 2004; 45(11):4161–4166. [PubMed: 15505070]
26. Mocan MC, Kadayifcilar S, Eldem B. Elevated intravitreal interleukin-6 levels in patients with proliferative diabetic retinopathy. Can J Ophthalmol. 2006; 41(6):747–752. [PubMed: 17224958]
27. Funatsu H, Yamashita H, Ikeda T, Mimura T, Eguchi S, Hori S. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. Ophthalmology. 2003; 110(9):1690–1696. [PubMed: 13129863]
28. Murugeswari P, Shukla D, Rajendran A, Kim R, Namperumalsamy P, Muthukkaruppan V. Proinflammatory cytokines and angiogenic and anti-angiogenic factors in vitreous of patients with proliferative diabetic retinopathy and eales' disease. Retina. 2008; 28(6):817–824. [PubMed: 18536597]
29. Maier R, Weger M, Haller-Schober EM, et al. Multiplex bead analysis of vitreous and serum concentrations of inflammatory and proangiogenic factors in diabetic patients. Mol Vis. 2008; 14:637–643. [PubMed: 18385799]
30. Meleth AD, Agron E, Chan CC, et al. Serum inflammatory markers in diabetic retinopathy. Invest Ophthalmol Vis Sci. 2005; 46(11):4295–4301. [PubMed: 16249511]
31. Adamiec-Mroczek J, Oficjalska-Mlynczak J. Assessment of selected adhesion molecule and proinflammatory cytokine levels in the vitreous body of patients with type 2 diabetes--role of the inflammatory-immune process in the pathogenesis of proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol. 2008; 246(12):1665–1670. [PubMed: 18682976]
32. Petrovic MG, Osredkar J, Saraga-Babic M, Petrovic D. K469E polymorphism of the intracellular adhesion molecule 1 gene is associated with proliferative diabetic retinopathy in Caucasians with type 2 diabetes. Clin Experiment Ophthalmol. 2008; 36(5):468–472. [PubMed: 18942221]
33*. McLeod DS, Lefer DJ, Merges C, Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. Am J Pathol. 1995; 147(3): 642–653. This study provides the first evidence that neutrophil numbers per square millimeter are increased in retina sections from DR patients, which is associated with the increase of ICAM-1 expression in the vessels. [PubMed: 7545873]
34. Al-Shabrawey M, Bartoli M, El-Remessy AB, et al. Role of NADPH oxidase and Stat3 in statin-mediated protection against diabetic retinopathy. Invest Ophthalmol Vis Sci. 2008; 49(7):3231–3238. [PubMed: 18378570]
35. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010; 125(2 Suppl 2):S3–23. [PubMed: 20176265]

36. Kim SY, Johnson MA, McLeod DS, Alexander T, Hansen BC, Lutty GA. Neutrophils are associated with capillary closure in spontaneously diabetic monkey retinas. Diabetes. 2005; 54(5): 1534–1542. [PubMed: 15855343]

37. Schroder S, Palinski W, Schmid-Schonbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am J Pathol. 1991; 139(1):81–100. [PubMed: 1713023]

38**. Miyamoto K, Khosrof S, Bursell SE, et al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. Proc Natl Acad Sci U S A. 1999; 96(19):10836–10841. This is a very important study which links leukostasis to retinal vessel permeability and closure. It also provides the first evidence that blockade of the interaction between leukocytes and endothelial cells can prevent vascular injury in DR. [PubMed: 10485912]

39. Joussen AM, Poulaki V, Le ML, et al. A central role for inflammation in the pathogenesis of diabetic retinopathy. Faseb J. 2004; 18(12):1450–1452. [PubMed: 15231732]

40. Barouch FC, Miyamoto K, Allport JR, et al. Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. Invest Ophthalmol Vis Sci. 2000; 41(5):1153–1158. [PubMed: 10752954]

41. Chen L, Yang P, Kijlstra A. Distribution, markers, and functions of retinal microglia. Ocul Immunol Inflamm. 2002; 10(1):27–39. [PubMed: 12461701]

42. Yang LP, Sun HL, Wu LM, et al. Baicalein reduces inflammatory process in a rodent model of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009; 50(5):2319–2327. [PubMed: 19011009]

43. Davidson JA, Ciulla TA, McGill JB, Kles KA, Anderson PW. How the diabetic eye loses vision. Endocrine. 2007; 32(1):107–116. [PubMed: 17992608]

44. Yuan SY. Protein kinase signaling in the modulation of microvascular permeability. Vascul Pharmacol. 2002; 39(4–5):213–223. [PubMed: 12747961]

45. Matter K, Balda MS. Signalling to and from tight junctions. Nat Rev Mol Cell Biol. 2003; 4(3): 225–236. [PubMed: 12612641]

46. Stamatovic SM, Dimitrijevic OB, Keep RF, Andjelkovic AV. Protein kinase Calpha-RhoA cross-talk in CCL2-induced alterations in brain endothelial permeability. J Biol Chem. 2006; 281(13): 8379–8388. [PubMed: 16439355]

47. Gavard J, Hou X, Qu Y, et al. A role for a CXCR2/phosphatidylinositol 3-kinase gamma signaling axis in acute and chronic vascular permeability. Mol Cell Biol. 2009; 29(9):2469–2480. [PubMed: 19255141]

48. Miyamoto K, Khosrof S, Bursell SE, et al. Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). Am J Pathol. 2000; 156(5):1733–1739. [PubMed: 10793084]

49. Del Maschio A, Zanetti A, Corada M, et al. Polymorphonuclear leukocyte adhesion triggers the disorganization of endothelial cell-to-cell adherens junctions. J Cell Biol. 1996; 135(2):497–510. [PubMed: 8896605]

50. Kowluru RA, Odenbach S. Role of interleukin-1beta in the pathogenesis of diabetic retinopathy. Br J Ophthalmol. 2004; 88(10):1343–1347. [PubMed: 15377563]

51*. Joussen AM, Poulaki V, Mitsiades N, et al. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. Faseb J. 2003; 17(1):76–78. Identifies that leukocytes induce retinal vascular injury via Fas-FasL-mediated endothelial cell apoptosis. [PubMed: 12475915]

52. Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? Angiogenesis. 2007; 10(3):149–166. [PubMed: 17457680]

53. Ishikawa K, Yoshida S, Kadota K, et al. Gene expression profile of hyperoxic and hypoxic retinas in a mouse model of oxygen-induced retinopathy. Invest Ophthalmol Vis Sci. 2010; 51(8):4307–4319. [PubMed: 20220049]

54. Sato T, Kusaka S, Hashida N, Saishin Y, Fujikado T, Tano Y. Comprehensive gene-expression profile in murine oxygen-induced retinopathy. Br J Ophthalmol. 2009; 93(1):96–103. [PubMed: 18838407]

55. Connor KM, SanGiovanni JP, Lofqvist C, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med. 2007; 13(7):868–873. [PubMed: 17589522]
56. Gardiner TA, Gibson DS, de Gooyer TE, de la Cruz VF, McDonald DM, Stitt AW. Inhibition of tumor necrosis factor-alpha improves physiological angiogenesis and reduces pathological neovascularization in ischemic retinopathy. Am J Pathol. 2005; 166(2):637–644. [PubMed: 15681845]
57. Ishida S, Usui T, Yamashiro K, et al. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. J Exp Med. 2003; 198(3):483–489. [PubMed: 12900522]
58. Davies MH, Stempel AJ, Powers MR. MCP-1 deficiency delays regression of pathologic retinal neovascularization in a model of ischemic retinopathy. Invest Ophthalmol Vis Sci. 2008; 49(9): 4195–4202. [PubMed: 18487365]
59. Tezel G, Wax MB. Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. J Neurosci. 2000; 20(23):8693–8700. [PubMed: 11102475]
60. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. Nat Rev Immunol. 2005; 5(8):629–640. [PubMed: 16034365]
61. Lam TT, Tso MO. Nitric oxide synthase (NOS) inhibitors ameliorate retinal damage induced by ischemia in rats. Res Commun Mol Pathol Pharmacol. 1996; 92(3):329–340. [PubMed: 8827830]
62. Fuchs C, Forster V, Balse E, Sahel JA, Picaud S, Tessier LH. Retinal-cell-conditioned medium prevents TNF-alpha-induced apoptosis of purified ganglion cells. Invest Ophthalmol Vis Sci. 2005; 46(8):2983–2991. [PubMed: 16043875]
63. Fatma N, Kubo E, Sen M, et al. Peroxiredoxin 6 delivery attenuates TNF-alpha-and glutamate-induced retinal ganglion cell death by limiting ROS levels and maintaining Ca2+ homeostasis. Brain Res. 2008; 1233:63–78. [PubMed: 18694738]
64. Neufeld AH, Kawai S, Das S, et al. Loss of retinal ganglion cells following retinal ischemia: the role of inducible nitric oxide synthase. Exp Eye Res. 2002; 75(5):521–528. [PubMed: 12457864]
65. Nakazawa T, Takahashi H, Nishijima K, et al. Pitavastatin prevents NMDA-induced retinal ganglion cell death by suppressing leukocyte recruitment. J Neurochem. 2007; 100(4):1018–1031. [PubMed: 17266736]
66. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes Early onset and effect of insulin. J Clin Invest. 1998; 102(4):783–791. [PubMed: 9710447]
67. Zheng L, Howell SJ, Hatala DA, Huang K, Kern TS. Salicylate-based anti-inflammatory drugs inhibit the early lesion of diabetic retinopathy. Diabetes. 2007; 56(2):337–345. [PubMed: 17259377]
68. Jezek P, Hlavata L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. Int J Biochem Cell Biol. 2005; 37(12):2478–2503. [PubMed: 16103002]
69. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107(9):1058–1070. [PubMed: 21030723]
70. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000; 404(6779):787–790. [PubMed: 10783895]
71. Merzouk S, Hichami A, Madani S, et al. Antioxidant status and levels of different vitamins determined by high performance liquid chromatography in diabetic subjects with multiple complications. Gen Physiol Biophys. 2003; 22(1):15–27. [PubMed: 12870698]
72. Bhatia S, Shukla R, Venkata Madhu S, Kaur Gambhir J, Madhava Prabhu K. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem. 2003; 36(7):557–562. [PubMed: 14563450]
73. Thornalley PJ. Glycation in diabetic neuropathy: characteristics, consequences, causes, and therapeutic options. Int Rev Neurobiol. 2002; 50:37–57. [PubMed: 12198817]
74. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. J Intern Med. 2002; 251(2): 87–101. [PubMed: 11905595]

75. Curtis TM, Scholfield CN. The role of lipids and protein kinase Cs in the pathogenesis of diabetic retinopathy. Diabetes Metab Res Rev. 2004; 20(1):28–43. [PubMed: 14737743]
76. Inoguchi T, Sonta T, Tsubouchi H, et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. J Am Soc Nephrol. 2003; 14(8 Suppl 3):S227–232. [PubMed: 12874436]
77. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. Circ Res. 2010; 106(8):1319–1331. [PubMed: 20431074]
78. Ellis EA, Guberski DL, Somogyi-Mann M, Grant MB. Increased H2O2, vascular endothelial growth factor and receptors in the retina of the BBZ/Wor diabetic rat. Free Radic Biol Med. 2000; 28(1):91–101. [PubMed: 10656295]
79. Inoguchi T, Tsubouchi H, Etoh T, et al. A possible target of antioxidative therapy for diabetic vascular complications-vascular NAD(P)H oxidase. Curr Med Chem. 2003; 10(17):1759–1764. [PubMed: 12871120]
80. Sonta T, Inoguchi T, Tsubouchi H, et al. Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. Free Radic Biol Med. 2004; 37(1):115–123. [PubMed: 15183199]
81. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res. 2000; 86(5):494–501. [PubMed: 10720409]
82. Cave AC, Brewer AC, Narayanapanicker A, et al. NADPH oxidases in cardiovascular health and disease. Antioxid Redox Signal. 2006; 8(5–6):691–728. [PubMed: 16771662]
83. Heyworth PG, Curnutte JT, Nauseef WM, et al. Neutrophil nicotinamide adenine dinucleotide phosphate oxidase assembly Translocation of p47-phox and p67-phox requires interaction between p47-phox and cytochrome b558. J Clin Invest. 1991; 87(1):352–356. [PubMed: 1985107]
84. Hoyal CR, Gutierrez A, Young BM, et al. Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. Proc Natl Acad Sci U S A. 2003; 100(9):5130–5135. [PubMed: 12704229]
85. Dang PM, Fontayne A, Hakim J, El Benna J, Perianin A. Protein kinase C zeta phosphorylates a subset of selective sites of the NADPH oxidase component p47phox and participates in formyl peptide-mediated neutrophil respiratory burst. J Immunol. 2001; 166(2):1206–1213. [PubMed: 11145703]
86. Korchak HM, Kilpatrick LE. Roles for beta II-protein kinase C and RACK1 in positive and negative signaling for superoxide anion generation in differentiated HL60 cells. J Biol Chem. 2001; 276(12):8910–8917. [PubMed: 11120743]
87. El Benna J, Han J, Park JW, Schmid E, Ulevitch RJ, Babior BM. Activation of p38 in stimulated human neutrophils: phosphorylation of the oxidase component p47phox by p38 and ERK but not by JNK. Arch Biochem Biophys. 1996; 334(2):395–400. [PubMed: 8900416]
88. Koga H, Terasawa H, Nunoi H, Takeshige K, Inagaki F, Sumimoto H. Tetratricopeptide repeat (TPR) motifs of p67(phox) participate in interaction with the small GTPase Rac and activation of the phagocyte NADPH oxidase. J Biol Chem. 1999; 274(35):25051–25060. [PubMed: 10455184]
89. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. Circ Res. 2008; 102(4):488–496. [PubMed: 18096818]
90. Dikalova AE, Bikineyeva AT, Budzyn K, et al. Therapeutic targeting of mitochondrial superoxide in hypertension. Circ Res. 2010; 107(1):106–116. [PubMed: 20448215]
91. Turpaev KT. Reactive oxygen species and regulation of gene expression. Biochemistry (Mosc). 2002; 67(3):281–292. [PubMed: 11970728]
92. Sanz AB, Sanchez-Nino MD, Ramos AM, et al. NF-kappaB in renal inflammation. J Am Soc Nephrol. 2010; 21(8):1254–1262. [PubMed: 20651166]
93. Hoffmann A, Baltimore D. Circuitry of nuclear factor kappaB signaling. Immunol Rev. 2006; 210:171–186. [PubMed: 16623771]
94. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008; 132(3):344–362. [PubMed: 18267068]

95. Pantano C, Reynaert NL, van der Vliet A, Janssen-Heininger YM. Redox-sensitive kinases of the nuclear factor-kappaB signaling pathway. Antioxid Redox Signal. 2006; 8(9–10):1791–1806. [PubMed: 16987032]

96*. Nagai N, Izumi-Nagai K, Oike Y, et al. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. Invest Ophthalmol Vis Sci. 2007; 48(9):4342–4350. This pre-clinical study demonstrates that RAS is upregulated in DR mouse model and plays critical role in retinal inflammation via NF-κB pathway. [PubMed: 17724226]

97. Romeo G, Liu WH, Asnaghi V, Kern TS, Lorenzi M. Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. Diabetes. 2002; 51(7):2241–2248. [PubMed: 12086956]

98. Harada C, Okumura A, Namekata K, et al. Role of monocyte chemotactic protein-1 and nuclear factor kappa B in the pathogenesis of proliferative diabetic retinopathy. Diabetes Res Clin Pract. 2006; 74(3):249–256. [PubMed: 16730843]

99. Zheng L, Szabo C, Kern TS. Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB. Diabetes. 2004; 53(11):2960–2967. [PubMed: 15504977]

100. Kowluru RA, Koppolu P, Chakrabarti S, Chen S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. Free Radic Res. 2003; 37(11):1169–1180. [PubMed: 14703729]

101. Tawfik A, Sanders T, Kahook K, Akeel S, Elmarakby A, Al-Shabrawey M. Suppression of retinal peroxisome proliferator-activated receptor gamma in experimental diabetes and oxygen-induced retinopathy: role of NADPH oxidase. Invest Ophthalmol Vis Sci. 2009; 50(2):878–884. [PubMed: 18806296]

102*. Al-Shabrawey M, Rojas M, Sanders T, et al. Role of NADPH oxidase in retinal vascular inflammation. Invest Ophthalmol Vis Sci. 2008; 49(7):3239–3244. Data show that NOX2/NADPH plays a critical role in retinal inflammatory reactions and retinal vascular leakages. [PubMed: 18378574]

103. Al-Shabrawey M, Bartoli M, El-Remessy AB, et al. Inhibition of NAD(P)H oxidase activity blocks vascular endothelial growth factor overexpression and neovascularization during ischemic retinopathy. Am J Pathol. 2005; 167(2):599–607. [PubMed: 16049343]

104. Zhang W, Baban B, Rojas M, et al. Arginase activity mediates retinal inflammation in endotoxin-induced uveitis. Am J Pathol. 2009; 175(2):891–902. [PubMed: 19590038]

105. Caldwell RB, Zhang W, Romero MJ, Caldwell RW. Vascular dysfunction in retinopathy-an emerging role for arginase. Brain Res Bull. 2010; 81(2–3):303–309. [PubMed: 19737603]

106. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A. 1991; 88(11):4651–4655. [PubMed: 1675786]

107*. Li Q, Verma A, Han PY, et al. Diabetic eNOS knockout mice develop accelerated retinopathy. Invest Ophthalmol Vis Sci. 2010; 51(10):5240–5246. eNOS upregulation has been thought to be a cause of DR. However, this paper shows that eNOS is protective for DR in that eNOS deficient diabetic mice develop earlier and more severe signs of DR than that of wildtype mice. [PubMed: 20435587]

108. Leal EC, Manivannan A, Hosoya K, et al. Inducible nitric oxide synthase isoform is a key mediator of leukostasis and blood-retinal barrier breakdown in diabetic retinopathy. Invest Ophthalmol Vis Sci. 2007; 48(11):5257–5265. [PubMed: 17962481]

109. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87(1):315–424. [PubMed: 17237348]

110. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998; 336 (Pt 1): 1–17. [PubMed: 9806879]

111. Nagaoka T, Kuo L, Ren Y, Yoshida A, Hein TW. C-reactive protein inhibits endothelium-dependent nitric oxide-mediated dilation of retinal arterioles via enhanced superoxide production. Invest Ophthalmol Vis Sci. 2008; 49(5):2053–2060. [PubMed: 18436840]

112. Kelleher ZT, Matsumoto A, Stamler JS, Marshall HE. NOS2 regulation of NF-kappaB by S-nitrosylation of p65. J Biol Chem. 2007; 282(42):30667–30672. [PubMed: 17720813]

113. Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. Biochemistry. 2001; 40(6):1688–1693. [PubMed: 11327828]

114. Barile GR, Schmidt AM. RAGE and its ligands in retinal disease. Curr Mol Med. 2007; 7(8):758–765. [PubMed: 18331234]

115. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE, the receptor for advanced glycation end products. J Mol Med. 2005; 83(11):876–886. [PubMed: 16133426]

116. Moore TC, Moore JE, Kaji Y, et al. The role of advanced glycation end products in retinal microvascular leukostasis. Invest Ophthalmol Vis Sci. 2003; 44(10):4457–4464. [PubMed: 14507893]

117. Kaji Y, Usui T, Ishida S, et al. Inhibition of diabetic leukostasis and blood-retinal barrier breakdown with a soluble form of a receptor for advanced glycation end products. Invest Ophthalmol Vis Sci. 2007; 48(2):858–865. [PubMed: 17251488]

118. Silva KC, Pinto CC, Biswas SK, de Faria JB, de Faria JM. Hypertension increases retinal inflammation in experimental diabetes: a possible mechanism for aggravation of diabetic retinopathy by hypertension. Curr Eye Res. 2007; 32(6):533–541. [PubMed: 17612969]

119. Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. Trends Pharmacol Sci. 2008; 29(7):367–374. [PubMed: 18579222]

120. Garrido AM, Griendling KK. NADPH oxidases and angiotensin II receptor signaling. Mol Cell Endocrinol. 2009; 302(2):148–158. [PubMed: 19059306]

121. Miller AG, Tan G, Binger KJ, et al. Candesartan attenuates diabetic retinal vascular pathology by restoring glyoxalase-I function. Diabetes. 2010; 59(12):3208–3215. [PubMed: 20852029]

122. Wagner J, Jan Danser AH, Derkx FH, et al. Demonstration of renin mRNA, angiotensinogen mRNA, and angiotensin converting enzyme mRNA expression in the human eye: evidence for an intraocular renin-angiotensin system. Br J Ophthalmol. 1996; 80(2):159–163. [PubMed: 8814748]

123. Wheeler-Schilling TH, Kohler K, Sautter M, Guenther E. Angiotensin II receptor subtype gene expression and cellular localization in the retina and non-neuronal ocular tissues of the rat. Eur J Neurosci. 1999; 11(10):3387–3394. [PubMed: 10564346]

124. Silva KC, Pinto CC, Biswas SK, Souza DS, de Faria JB, de Faria JM. Prevention of hypertension abrogates early inflammatory events in the retina of diabetic hypertensive rats. Exp Eye Res. 2007; 85(1):123–129. [PubMed: 17493613]

125. Chen P, Guo AM, Edwards PA, Trick G, Scicli AG. Role of NADPH oxidase and ANG II in diabetes-induced retinal leukostasis. Am J Physiol Regul Integr Comp Physiol. 2007; 293(4):R1619–1629. [PubMed: 17652361]

126. Rojas M, Zhang W, Lee DL, et al. Role of IL-6 in angiotensin II-induced retinal vascular inflammation. Invest Ophthalmol Vis Sci. 2010; 51(3):1709–1718. [PubMed: 19834028]

127. Birukov KG. Cyclic stretch, reactive oxygen species, and vascular remodeling. Antioxid Redox Signal. 2009; 11(7):1651–1667. [PubMed: 19186986]

128. Masuzawa K, Goto K, Jesmin S, et al. An endothelin type A receptor antagonist reverses upregulated VEGF and ICAM-1 levels in streptozotocin-induced diabetic rat retina. Curr Eye Res. 2006; 31(1):79–89. [PubMed: 16421022]

129. Gustavsson C, Agardh CD, Zetterqvist AV, Nilsson J, Agardh E, Gomez MF. Vascular cellular adhesion molecule-1 (VCAM-1) expression in mice retinal vessels is affected by both hyperglycemia and hyperlipidemia. PLoS One. 2010; 5(9):e12699. [PubMed: 20856927]

130. Rosen ED, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem. 2001; 276(41):37731–37734. [PubMed: 11459852]

131. Sung B, Park S, Yu BP, Chung HY. Amelioration of age-related inflammation and oxidative stress by PPARgamma activator: suppression of NF-kappaB by 2,4-thiazolidinedione. Exp Gerontol. 2006; 41(6):590–599. [PubMed: 16716549]

132. Muranaka K, Yanagi Y, Tamaki Y, et al. Effects of peroxisome proliferator-activated receptor gamma and its ligand on blood-retinal barrier in a streptozotocin-induced diabetic model. Invest Ophthalmol Vis Sci. 2006; 47(10):4547–4552. [PubMed: 17003451]

133*. Zhang W, Rojas M, Lilly B, et al. NAD(P)H oxidase-dependent regulation of CCL2 production during retinal inflammation. Invest Ophthalmol Vis Sci. 2009; 50(6):3033–3040. It shows that

NADPH oxidase/ROS pathway has a critical role in inflammation. This pathway can regulate the production of CCL2 via activation of NF-κB and Akt. [PubMed: 19234337]

134. Cunningham MA, Edelman JL, Kaushal S. Intravitreal steroids for macular edema: the past, the present, and the future. Surv Ophthalmol. 2008; 53(2):139–149. [PubMed: 18348879]
135. De Bosscher K, Haegeman G, Elewaut D. Targeting inflammation using selective glucocorticoid receptor modulators. Curr Opin Pharmacol. 2010; 10(4):497–504. [PubMed: 20493772]
136. Tamura H, Miyamoto K, Kiryu J, et al. Intravitreal injection of corticosteroid attenuates leukostasis and vascular leakage in experimental diabetic retina. Invest Ophthalmol Vis Sci. 2005; 46(4):1440–1444. [PubMed: 15790913]
137. Beer PM, Bakri SJ, Singh RJ, Liu W, Peters GB 3rd, Miller M. Intraocular concentration and pharmacokinetics of triamcinolone acetonide after a single intravitreal injection. Ophthalmology. 2003; 110(4):681–686. [PubMed: 12689886]
138. Jonas JB, Sofker A. Intraocular injection of crystalline cortisone as adjunctive treatment of diabetic macular edema. Am J Ophthalmol. 2001; 132(3):425–427. [PubMed: 11530068]
139. El-Asrar AM, Al-Mezaine HS, Ola MS. Changing paradigms in the treatment of diabetic retinopathy. Curr Opin Ophthalmol. 2009; 20(6):532–538. [PubMed: 19644368]
140. Kim SJ, Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. Surv Ophthalmol. 2010; 55(2):108–133. [PubMed: 20159228]
141. Wang D, DuBois RN. Pro-inflammatory prostaglandins and progression of colorectal cancer. Cancer Lett. 2008; 267(2):197–203. [PubMed: 18406516]
142. Surh YJ, Chun KS, Cha HH, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res. 2001:480–481. 243–268.
143. Du Y, Sarthy VP, Kern TS. Interaction between NO and COX pathways in retinal cells exposed to elevated glucose and retina of diabetic rats. Am J Physiol Regul Integr Comp Physiol. 2004; 287(4):R735–741. [PubMed: 15371279]
144. Johnson EI, Dunlop ME, Larkins RG. Increased vasodilatory prostaglandin production in the diabetic rat retinal vasculature. Curr Eye Res. 1999; 18(2):79–82. [PubMed: 10223650]
145. Ayalasomayajula SP, Amrite AC, Kompella UB. Inhibition of cyclooxygenase-2, but not cyclooxygenase-1, reduces prostaglandin E2 secretion from diabetic rat retinas. Eur J Pharmacol. 2004; 498(1–3):275–278. [PubMed: 15364005]
146. Ayalasomayajula SP, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. Eur J Pharmacol. 2003; 458(3):283–289. [PubMed: 12504784]
147. Sun W, Gerhardinger C, Dagher Z, Hoehn T, Lorenzi M. Aspirin at low-intermediate concentrations protects retinal vessels in experimental diabetic retinopathy through non-platelet-mediated effects. Diabetes. 2005; 54(12):3418–3426. [PubMed: 16306357]
148. Kern TS, Engerman RL. Pharmacological inhibition of diabetic retinopathy: aminoguanidine and aspirin. Diabetes. 2001; 50(7):1636–1642. [PubMed: 11423486]
149. Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. Invest Ophthalmol Vis Sci. 2006; 47(3):1149–1160. [PubMed: 16505053]
150. Kern TS, Miller CM, Du Y, et al. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. Diabetes. 2007; 56(2):373–379. [PubMed: 17259381]
151. Powell ED, Field RA. Diabetic Retinopathy and Rheumatoid Arthritis. Lancet. 1964; 2(7349):17–18. [PubMed: 14149196]
152. Effects of aspirin treatment on diabetic retinopathy. ETDRS report number 8 Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology. 1991; 98(5 Suppl):757–765. [PubMed: 2062511]
153. Effect of aspirin alone and aspirin plus dipyridamole in early diabetic retinopathy. A multicenter randomized controlled clinical trial The DAMAD Study Group. Diabetes. 1989; 38(4):491–498. [PubMed: 2647556]
154. Scott DL. Etanercept in arthritis. Int J Clin Pract. 2005; 59(1):114–118. [PubMed: 15707475]

155. Kleinbongard P, Schulz R, Heusch G. TNFalpha in myocardial ischemia/reperfusion, remodeling and heart failure. Heart Fail Rev. 2011; 16(1):49–69. [PubMed: 20571888]

156. Joussen AM, Doehmen S, Le ML, et al. TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. Mol Vis. 2009; 15:1418–1428. [PubMed: 19641635]

157. Sfikakis PP, Markomichelakis N, Theodossiadis GP, Grigoropoulos V, Katsilambros N, Theodossiadis PG. Regression of sight-threatening macular edema in type 2 diabetes following treatment with the anti-tumor necrosis factor monoclonal antibody infliximab. Diabetes Care. 2005; 28(2):445–447. [PubMed: 15677814]

158. Rao VR, Prescott E, Shelke NB, et al. Delivery of SAR 1118 to Retina Via Ophthalmic Drops and its Effectiveness in Reduction of Retinal Leukostasis and Vascular Leakiness in Rat Streptozotocin (STZ) Model of Diabetic Retinopathy (DR). Invest Ophthalmol Vis Sci. 2010; 51(10):5198–5204. [PubMed: 20445119]

159. Iliaki E, Poulaki V, Mitsiades N, Mitsiades CS, Miller JW, Gragoudas ES. Role of alpha 4 integrin (CD49d) in the pathogenesis of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009; 50(10):4898–4904. [PubMed: 19553613]

160. Barile GR, Pachydaki SI, Tari SR, et al. The RAGE axis in early diabetic retinopathy. Invest Ophthalmol Vis Sci. 2005; 46(8):2916–2924. [PubMed: 16043866]

161. Bhatwadekar A, Glenn JV, Figarola JL, et al. A new advanced glycation inhibitor, LR-90, prevents experimental diabetic retinopathy in rats. Br J Ophthalmol. 2008; 92(4):545–547. [PubMed: 18211931]

162. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia VII Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes. 2001; 50(8):1938–1942. [PubMed: 11473058]

163. Bursell SE, King GL. Can protein kinase C inhibition and vitamin E prevent the development of diabetic vascular complications? Diabetes Res Clin Pract. 1999; 45(2–3):169–182. [PubMed: 10588370]

164. Ali TK, El-Remessy AB. Diabetic retinopathy: current management and experimental therapeutic targets. Pharmacotherapy. 2009; 29(2):182–192. [PubMed: 19170588]

165. Zhang JZ, Xi X, Gao L, Kern TS. Captopril inhibits capillary degeneration in the early stages of diabetic retinopathy. Curr Eye Res. 2007; 32(10):883–889. [PubMed: 17963108]

166. Nagai N, Izumi-Nagai K, Oike Y, et al. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. Invest Ophthalmol Vis Sci. 2007; 48(9):4342–4350. [PubMed: 17724226]

167. Yamagishi S, Matsui T, Nakamura K, et al. Olmesartan blocks inflammatory reactions in endothelial cells evoked by advanced glycation end products by suppressing generation of reactive oxygen species. Ophthalmic Res. 2008; 40(1):10–15. [PubMed: 18025836]

168**. Mauer M, Zinman B, Gardiner R, et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med. 2009; 361(1):40–51. This clinical trial demonstrates that blockade of RAS slows the progress of DR. It also shows that there is distinct mechanism between diabetic nephropathy and DR. [PubMed: 19571282]

169. Chaturvedi N, Porta M, Klein R, et al. Effect of candesartan on prevention (DIRECT-Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes: randomised, placebo-controlled trials. Lancet. 2008; 372(9647):1394–1402. [PubMed: 18823656]

170. Sjolie AK, Klein R, Porta M, et al. Effect of candesartan on progression and regression of retinopathy in type 2 diabetes (DIRECT-Protect 2): a randomised placebo-controlled trial. Lancet. 2008; 372(9647):1385–1393. [PubMed: 18823658]

Executive summary

- DR is a vision threatening disease affects a large population globally. There is a growing need to develop new therapeutic approaches.
- DR has many features of inflammation, such as increases in inflammatory molecules, leukocyte recruitment, and activation of local immune cells.
- Inflammation plays a critical role in the development of the disease by causing vessel leakage, closure, pathological neovascularization, and neural death.
- Diabetes induces inflammatory reactions by many mechanisms including, oxidative stress NF-κB activation, NOS dysregulation, AGEs formation, hypertension, dyslipidemia and impaired anti-inflammatory pathways.
- Anti-inflammatory therapy has proven to be beneficial in both clinical and pre-clinical studies.
- Better understanding of inflammatory cascades and endogenous anti-inflammatory mechanisms may lead to better therapies in the future.

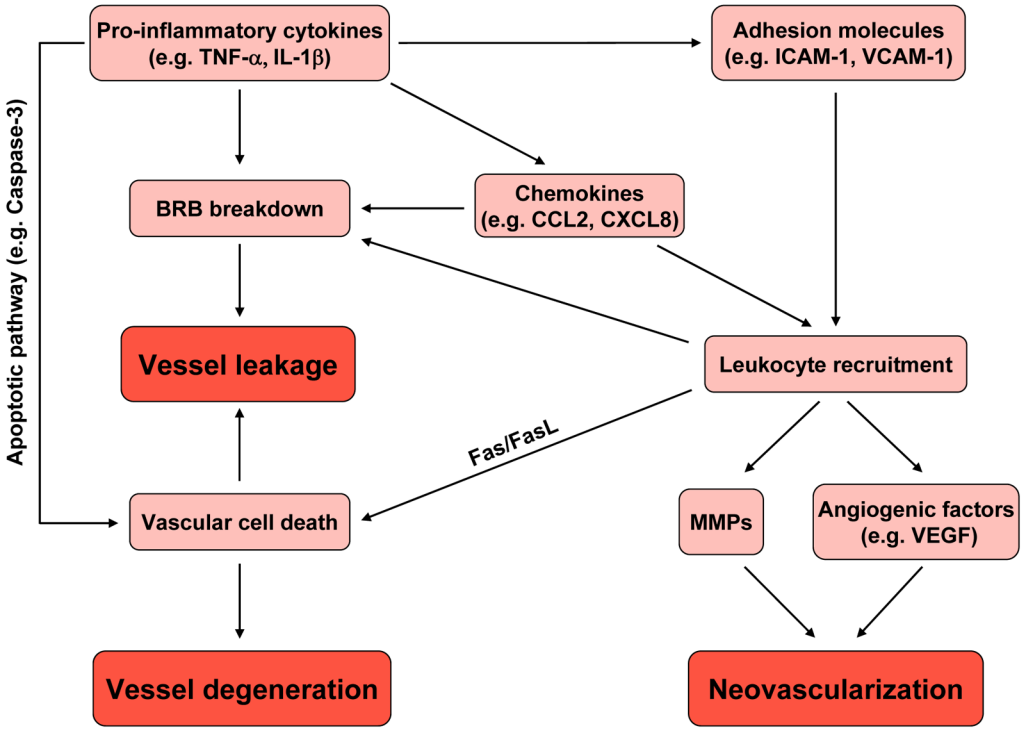

Figure 1. Role of inflammation in vascular disease in diabetic retinopathy

During inflammation, pro-inflammatory cytokines and chemokines can induce BRB breakdown and vascular cell death, resulting vessel leakage and degeneration. Alternatively, such changes can be induced by inflammatory cytokine, chemokine and adhesion molecule-mediated leukocyte recruitment. The interaction between leukocytes and vascular cells induce BRB breakdown, vascular cell death and subsequent vessel leakage and capillary degeneration. In addition, leukocytes release MMPs and angiogenic factors to induce pathological neovascularization.

Abbreviations: BRB = blood-retinal barrier, ICAM-1 = intercellular adhesion molecule 1, IL-1β = interleukin-1β, MMP = matrix metallopeptidase, TNF-α = tumor necrosis factor alpha, VEGF = vascular endothelial growth factor.

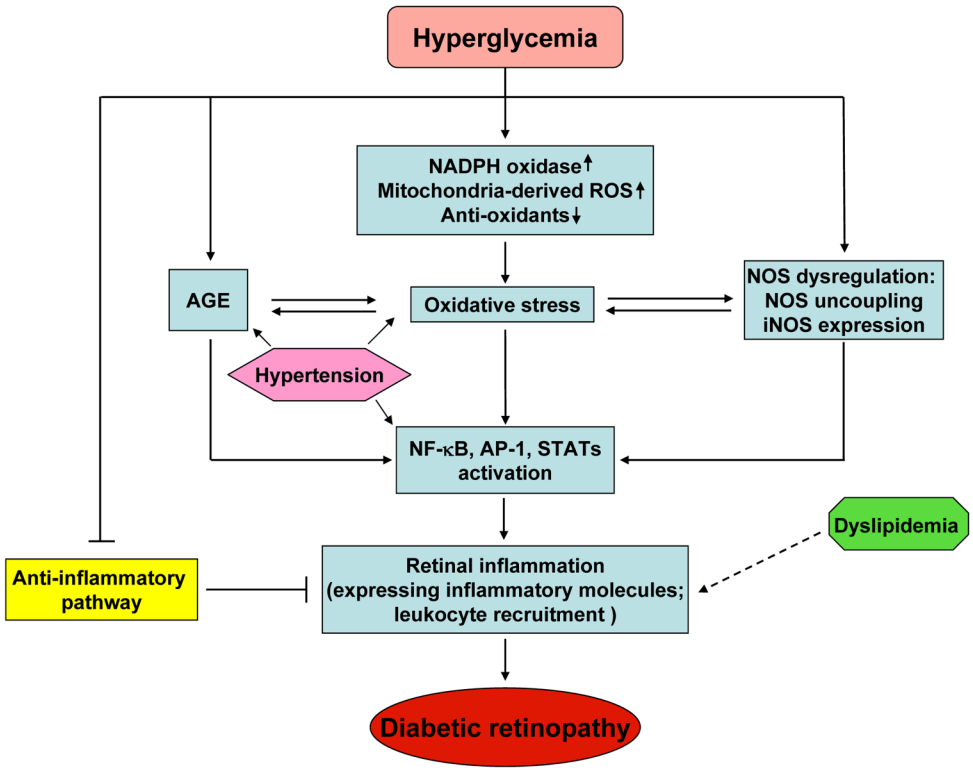

Figure 2. Mechanisms of diabetes-induced retinal inflammation

Hyperglycemia induces oxidative stress, NOS dysregulation, and AGEs formation. These changes can lead to activation of pro-inflammatory transcription factors such as NF-κB, AP-1 and STATs and inhibit anti-inflammatory pathways, resulting in retinal inflammatory reactions. Retinal inflammation can also be induced by hypertension and dyslipidemia. Retinal inflammation is enhanced by the reciprocal positive regulation among oxidative stress, NOS dysregulation, and AGEs formation.

Abbreviations: AGEs = advanced glycation end products, AP-1 = activator protein 1, NADPH = nicotinamide adenine dinucleotide phosphate, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, ROS = reactive oxygen species, STATs = signal transducers and activators of transcription proteins.

Table 1

Effect of anti-inflammatory therapy on diabetic retinopathy (DR)

Drugs	Target	Anti-inflammatory mechanisms	Effect on DR pathology
Steroids	Glucocorticoid receptor	Repress pro-inflammatory transcription factors [135]	Prevent ICAM-1 expression, leukostasis and vascular leakage [136] Improve visual acuity, decrease macular thickness [138, 139]
NSAID (e.g. Aspirin, Salicylate)	COX	Inhibit production of pro-inflammatory prostaglandins	Prevent ICAM-1 expression, leukostasis, vascular leakage, capillary cell apoptosis and vessel degeneration [20, 67, 143, 146–148, 150] Reduce incidence of DR and development of retinal microaneurysms [151–153]
Etanercept, Infliximab	TNF-α	Block TNF-α-induced inflammation	Prevent retinal inflammation, retina cell injury and vessel leakage [20, 156] Improve visual acuity and reduce macular thickness [157]
SAR 1118	LFA-1	Block leukocyte recruitment	Reduce leukostasis and vessel leakage [158]
Anti-CD49a neutralizing antibody	VLA-4	Block leukocyte recruitment	Reduce levels of VEGF and TNF-α protein, leukostasis and vessel leakage [159]
soluble RAGE, LR-90	AGEs	Block AGEs-induced inflammation	Improve neuronal dysfunction and prevent vessel degeneration [160, 161]
Vitamins C and E	Oxidative stress	Anti-oxidative stress	Reduce vessel degeneration [162] Reverse some of the changes in the retinal vessels [163]
Apocynin, Statin	NAPDH oxidase	Block NADPH oxidase activity and reduces oxidative stress	Prevent oxidative stress, retinal inflammation, vessel leakage and neovascularization [34, 102, 103]
Captopril, Telmisartan, Talsartan, Olmesartan, Candesartan, Enalapril	RAS	Block RAS-mediated inflammation	Prevent oxidative stress, inflammation, and vascular damage [96, 165, 167] Reduce the risk and the progression of retinopathy [168–170]

Abbreviations: AGEs = advanced glycation end products, COX = cyclooxygenase, DR = diabetic retinopathy, ICAM-1 = intercellular adhesion molecule 1, LFA-1 = leukocyte function associated antigen-1, NADPH = nicotinamide adenine dinucleotide phosphate, RAS = renin-angiotensin system, TNF-α = tumor necrosis factor alpha, VLA-4 = very late antigen-4.