Inflammation and diabetic retinal microvascular complications

Wenbo Zhang1,2,3, Hua Liu1,2, Mohamed Al-Shabrawey2,4, Robert W. Caldwell3, Ruth B. Caldwell1,2,5,6,7

1Vascular Biology Center; 2Vision Discovery Institute and the 3Departments of Pharmacology and Toxicology, 4Oral Biology and Anatomy, 5Cellular Biology and Anatomy, 6Ophthalmology, Georgia Health Sciences University, Augusta, Georgia; and the 7VA Medical Center, Augusta, Georgia, USA

Address for correspondence: Dr. Wenbo Zhang, Vascular Biology Center, Georgia Health Sciences University, Augusta, Georgia 30912-2500, USA. E-mail: wezhang@georgiahealth.edu

ABSTRACT

Diabetic retinopathy (DR) is one of the most common complications of diabetes and is a leading cause of blindness in people of the working age in Western countries. A major pathology of DR is microvascular complications such as non-perfused vessels, microaneurysms, dot/blot hemorrhages, cotton-wool spots, venous beading, vascular loops, vascular leakage and neovascularization. Multiple mechanisms are involved in these alternations. This review will focus on the role of inflammation in diabetic retinal microvascular complications and discuss the potential therapies by targeting inflammation.

Key words: Diabetic retinopathy, inflammation, microvascular complications

INTRODUCTION

Diabetic retinopathy (DR) is one of the most common complications of diabetes and is a leading cause of blindness in people of the working age in industrialized countries. 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 diabetic patients will develop retinopathy. Type 2 diabetic patients may already have background retinopathy at the time of diagnosis, and over 60% will develop some form of retinopathy after 20 years. 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. 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. In clinic, laser photocoagulation and vitrectomy remain the two conventional approaches to treat sight-threatening conditions such as macular edema and proliferative DR. Vascular endothelial growth factor (VEGF) blockers (Pegaptanib, Ranibizumab and Bevacizumab) in combination with laser photocoagulation represents an emerging novel therapy to reduce macular edema and induce neovascular regression. In spite of this progress, DR remains a major clinical challenge, and the number of patients keeps growing due to difficulty in achieving tight glycemic control, metabolic memory, unresponsive to the current therapeutic approaches and significant side-effects from therapies. There is a great need to develop new therapeutic approaches for this devastating disease.

INFLAMMATORY FEATURES IN DIABETIC RETINOPATHY

DR is characterized as a microvascular complication of
diabetes.\textsuperscript{[21-1]} Vascular alterations in the early stage of the disease include alterations in blood flow, death of retinal pericytes (perivascular contractile cells), basement membrane thickening and subtle increases in vascular permeability. With the progression of disease, obvious alterations in the vascular structure can be seen upon ophthalmoscopic examination. These include non-perfused vessels, microaneurysms, dot/blot hemorrhages, cotton-wool spots, venous beading, vascular loops and significant vascular leakage. The above alterations happen in the non-proliferative stage of DR, in which vision loss is mainly caused by macular edema as the consequence of increased vascular permeability. In the proliferative stage of DR, neovascularization happens on the retinal surface after vascular function is impaired by capillary occlusion, non-perfusion and degeneration. In this stage, severe vision loss or even blindness may be caused by bleeding, hemorrhage and subsequent retinal detachment because of the newly formed fragile vessels.\textsuperscript{[13]} The mechanisms by which diabetes causes microvascular complications and disease progression in the retina are not fully understood. However, studies in patient samples and animal models have shown that DR has features of chronic, subclinical inflammation.

Inflammation is the body's defense against pathogens and is 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 toward infected or injured tissue. Pro-inflammatory cytokines (such as tumor necrosis factor [TNF]—\( \alpha \) and interleukins) and chemokines (such as CCL2 and 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. Mediated by adhesion molecules and chemokines, leukocytes attach to the vessel wall, transmigrate through the endothelium and move to the infected or injured tissue.\textsuperscript{[13]} While normal inflammation is beneficial, excessive or uncontrolled inflammation can cause tissue injury and result in diseases.\textsuperscript{[13]}

Although there are no pathogens in DR, analysis of inflammatory molecules in vitreous, serum and retina form diabetic patients or experimental animals indicate that DR is associated with significant increases in pro-inflammatory cytokines, chemokines and adhesion molecules. High TNF-\( \alpha \) levels have been observed in vitreous, serum and ocular fibrovascular membranes from patients with DR and in retinas from rodent model of diabetes mellitus.\textsuperscript{[14-16]} Interleukin-1\( \beta \) (IL-1\( \beta \)) and its downstream signaling molecule caspase 1 are significantly increased in vitreous, retinas and serum from diabetic patients and rats.\textsuperscript{[15,17,18]} Chemokines such as CCL2, CCL5, CXCL8, CXCL10 and CXCL12 are also upregulated in vitreous samples from DR patients.\textsuperscript{[19-21]} Increases in IL-6, ICAM-1 and VCAM-1 have been shown to be related to the progression of DR.\textsuperscript{[21-24]}

Correlated with increases in inflammatory molecules, DR is associated with recruitment of leukocytes. The numbers of neutrophils are significantly elevated in both retinal and choroidal vessels from diabetic patients and monkeys. The accumulation of neutrophils is correlated with upregulation of ICAM-1 immunoreactivity in the vessels and is associated with capillary closure.\textsuperscript{[25,26]} In diabetic rodent models, there is a cumulative and sustained increase in leukocyte adherence to the retinal vasculature (leukostasis) along with the progression of DR.\textsuperscript{[27]} The increase in leukostasis may be attributable to diabetes-induced increases in ICAM-1 and integrins in endothelial cells and leukocytes, respectively, in that blockade of ICAM-1 or deletion of CD18 (ICAM-1 receptor subunit on leukocytes) prevents diabetes-induced leukostasis.\textsuperscript{[27-29]} In addition to leukocytes in the circulation, microglia that are scattered throughout the retina are likely to be involved in DR.\textsuperscript{[30]} In DR, they are rapidly activated to release inflammatory cytokines such as TNF-\( \alpha \).\textsuperscript{[31]} A2A adenosine

\begin{figure}
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Cascade of events that contribute to the inflammatory response in diabetic retinopathy. Hyperglycemia induces formation of advanced glycation end products, generation of reactive oxygen species from multiple sources, mainly nicotinamide adenine dinucleotide phosphate oxidase and mitochondrial electron transport chain, and dysregulation of nitric oxide synthase (NOS) pathway. These changes activate NF-\( \kappa \)B and, in turn, upregulation of cytokines, chemokines and iNOS. This leads to upregulation of adhesion molecules and subsequent leukocyte/endothelial interaction.}
\end{figure}
receptor agonist effectively blocks TNF-α production in microglia and markedly decreases hyperglycemia-induced retinal cell death (Ibrahim et al., 2011).

In summary, this evidence demonstrates that DR has all the characters of inflammatory disease, and suggests involvement of low-grade chronic inflammation in this disease. Tremendous efforts have been made to understand how diabetes causes inflammatory reactions in DR in the absence of pathogens.

MECHANISMS OF RETINAL INFLAMMATION

Risk factors of DR include hyperglycemia, hypertension and dyslipidemia. These factors have been shown to induce inflammation by a variety of mechanisms, including oxidative stress, NF-kB activation, dysregulation of nitric oxide synthase (NOS) and formation of advanced glycation endproducts (AGEs).

Oxidative stress happens when ROS are overproduced or when endogenous anti-oxidant systems are impaired. Mitochondria can generate ROS by leakage of electrons to molecular oxygen at electron transport chain complexes I, II and III. 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. 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 protein kinase c (PKC). All these changes can lead to oxidative stress by decreasing the activities of anti-oxidant enzymes or further activating the ROS-generating machinery inside the cells. In addition to mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is another important source of ROS in diabetes, and studies in other models have suggested a positive reciprocal regulation between mitochondria and NADPH oxidase-derived ROS.

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. 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, mitogen-activated protein kinases and Akt. The p67phox subunit mediates direct binding of the complex with activated Rac, which initiates the electron transfer reaction that produces superoxide. 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. These mechanisms in fact allow NADPH oxidase to function as a key link between hyperglycemia and hypertension. Increased NAPDH oxidase activity has been found in diabetic patients and animals and high glucose-treated endothelial cells. NADPH oxidase is also potently activated by angiotensin II, a product of the renin–angiotensin system that plays a key role in hypertension. Overall, these changes lead to a net increase in ROS, and diabetes-induced sustained oxidative stress is a major cause of retinal inflammation.

ROS are important intracellular signaling molecules in the inflammatory cascade. ROS play a key role in inflammatory gene expression by activation of redox-sensitive transcription factors such as NF-kB, signal transducers and activators of transcription proteins and activator protein 1. The activity of NF-kB is increased in high glucose-treated retinal endothelial cells, pericytes or glial cells and in diabetic retinas from patients or animal models. This activation is significantly blocked by inhibiting redox systems by blockade of NADPH oxidase or antioxidant treatment, indicating a cause-and-effect relationship. Activation of NF-kB has an essential role in diabetes-induced retinal inflammation, in that inhibition of NF-kB blocks both high glucose and diabetes-induced production of inflammatory molecules in retinal cells and retinal tissue, and attenuates leukostasis. Similarly, studies in animal and tissue culture models have demonstrated the involvement of NADPH oxidase in diabetes-induced inflammation and breakdown of the blood–retinal barrier (BRB). 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. 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. Correlated with the involvement of oxidative stress in diabetes-induced retinal inflammation, studies have shown that supplement of antioxidants such as vitamins C and E attenuates the development of acellular capillaries and decreases the number of pericyte ghosts in diabetic rats. Such effects are further enhanced when
Inflammation in the development of diabetic retinopathy

Because diabetes is found to cause retinal inflammation by divergent mechanisms, significant efforts have been made to understand how inflammation is involved in the microvascular complications in the disease. These studies have demonstrated important roles of inflammatory cytokines and leukocytes in vascular leakage, vessel occlusion and degeneration and pathological neovascularization [Figure 1].

Inflammation is a cause of retinal vascular leakage. The microvascular endothelium forms an effective barrier to control the movement of blood fluid and proteins across the vessel wall. This barrier is formed by tight junctions and adhesion junctions that join endothelial cells to each other. Signaling molecules, including pro-

Although hyperglycemia has a direct impact to the pathology of DR, the byproducts of hyperglycemia, particularly AGEs, also have a significant role in retinal inflammation in DR. AGEs are formed by non-enzymatic glycation and oxidation of amino groups of proteins, lipids and DNA. This process is accelerated in diabetes in the presence of high glucose and oxidative stress. In the diabetic retina, the level of AGEs is prominently increased and AGE immunoreactivity is localized in vitreous, internal limiting membrane and retinal vasculature. The receptor for AGEs (RAGE) is expressed in numerous cells in the retina, including Muller cells, endothelial cells and neurons. The AGE–RAGE pathway can activate many downstream signaling molecules, including NF-κB and ROS, to induce inflammatory reactions such as ICAM-1 expression and leukostasis in the retina and retinal cells.

Blocking AGE with soluble RAGE or inhibiting AGE formation with LR-90 significantly reduces diabetes-induced ICAM-1 expression, leukostasis and VEGF expression.

In summary, multiple pathways are involved in diabetes-induced retinal inflammation [Figure 1]. However, it should be noted that inflammation is not an endpoint of the above inflammatory mechanisms. Inflammation, in turn, further activates these inflammatory mechanisms. For example, oxidative stress is both a cause and a consequence of inflammation. Many inflammatory cytokines induce oxidative stress and use the NADPH oxidase pathway to induce expression of other inflammatory molecules such as CCL2. Leukocytes also release ROS in response to inflammatory stimuli. Blockade of IL-6 not only prevents angiotensin II-induced leukostasis in the retina but also abolishes NADPH oxidase-mediated ROS formation. Thus, inflammation serves as a key mediator in the positive feedback loop of inflammatory cascades in DR.
Inflammatory cytokines and chemokines, which induce disorganization/redistribution of junction proteins in the retinal endothelium may lead to breakdown of the BRB, resulting in an abnormal extravasation of blood components and retinal edema. TNF-α is known to cause significant retinal endothelial permeability within a few hours by PKCζ-mediated downregulation of tight junction proteins. Intravitreal injection of TNF-α leads to increased retinal vascular permeability, which is prevented by PKCζ inhibitor. Moreover, blocking TNF-α with Etanercept, a soluble TNF-α receptor, prevents BRB breakdown in the diabetic rat model. Similar increases in endothelial permeability are also observed when endothelial cells are treated with other cytokines such as CCL2 or CXCL8. BRB breakdown can also be caused by cytokine-induced vascular cell death and by leukocyte-mediated vascular alternations. Leukocytes recruited in inflammation have a key role in causing vascular leakage by mechanisms including inducing junction protein alteration, releasing cytokines or increasing ROS as well as inducing vessel occlusion and injury. Alternatively, leukostasis in DR may cause capillary occlusion when leukocytes block blood flow because of their large cell volume and high rigidity. This notion is supported by studies showing that transient leukocyte attachment to the vessel wall correlates with capillary non-perfusion, and capillary reperfusion can occur when leukocytes detach and move on. In addition to blocking blow flow, leukocytes induce endothelial death during leukostasis via Fas ligand (FasL) and Fas-mediated apoptosis. The level of Fas is increased in retinal vascular endothelial cells during diabetes, while FasL is increased on neutrophils. Blocking Fas or inhibiting ICAM-1/CD18-mediated leukostasis significantly reduces retinal endothelial cell injury, apoptosis and retinal capillary degeneration.

Inflammation is also involved in pathological neovascularization in DR. Chronic inflammation is known as a key player in angiogenesis in many diseases such as rheumatoid arthritis and cancer. Leukocytes, recruited by inflammatory cytokines produced in the disease tissue, enhance the formation of new vasculature by releasing angiogenic factors and increasing the activity of matrix metallopeptidase. Direct investigation of inflammation in proliferative DR is not feasible because there are no small animal models that can develop reproducible proliferative DR, as occurs in humans. Therefore, studies of retinal neovascularization are carried out in rodent models of oxygen-induced retinopathy, in which retinal vessel obliteration is induced 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. Blockade of inflammatory cytokines, such as TNF-α, attenuates neovascularization. In addition to inflammatory cytokines, monocytes/macrophages are found to be present in the neovascular tufts, and depleting the monocyte lineage with clodronate-liposomes leads to the suppression of pathological but not physiological retinal angiogenesis.

**CONCLUSIONS AND PROSPECTIVE**

Although there is no pathogen in the diabetic retina, DR has all the common features of inflammation, such as increase in inflammatory molecules in retina, vitreous and plasma, leukocyte recruitment and tissue edema. These changes are caused by diabetes-induced disorders such as oxidative stress, dysregulation of the NOS pathway, AGEs formation and NF-kB activation [Figure 1]. Inflammation is now recognized as an important player in the pathogenesis of DR, and a number of studies have been designed to address whether blockade of specific inflammatory molecules can be beneficial for DR. 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. In one clinical study with four 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. 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 a diabetic rat model. In addition to ICAM-1, blockade of VCAM-1-mediated leukocyte attachment with anti-CD49a neutralizing antibody significantly attenuated the diabetes-induced leukostasis and vascular leakage. In spite of the rapid progress in this field, there are many issues that remain to be addressed before effective anti-inflammatory therapy can be designed to treat DR. For example, inflammation is involved in almost all diseases
and, therefore, it is unclear why inflammatory reactions in most diseases do not affect retinal vasculature. Is there crosstalk between diabetes-induced inflammation and other chronic disease-induced inflammation in relation to the pathogenesis of DR? Although inflammation is involved in the pathogenesis of DR, there is a higher incidence of DR in type 1 diabetic patients than in type 2 diabetic patients. Is it because of the fact that type 1 and type 2 diabetes induce different inflammatory reactions? Retinal inflammation might be more severe in type 1 diabetes as this disease is associated with aberrant autoimmunity. However, there is no study to address such difference at this moment. Given that diabetes induces the production of inflammatory molecules in both retinal local cells and blood leukocytes, what is the individual role of retinal local cells and leukocytes in inflammatory reactions and vascular injury in DR? What are the specific subtypes of leukocytes that are involved in DR? Many inflammatory molecules have redundant functions and cause activation of similar downstream targets such as NF-kB. What is their specific role and what are their reciprocal interactions in DR? These mysteries have led to challenges when considering any of the inflammatory molecules as a drug target as blockade of any of 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. Further studies may resolve these issues and bring novel therapies for DR by controlling inflammatory reactions precisely and at the right time.

ACKNOWLEDGMENTS

This work was generously supported by the American Heart Association 11SDG4960005 and the Juvenile Diabetes Foundation JDRF 10-2009-575 (to W. Zhang), by NIH Grants EY04618 and VA Merit Award (to R.B. Caldwell), by NIH Grant HL70215 (to R.W. Caldwell), by NIH Grant EY11766 (to R.B. Caldwell and R.W. Caldwell) and by the American Heart Association SDG00104 and Vision Discovery Institute (GHSU) (to M. Al-Shabrawey).

REFERENCES

1. Fong DS, Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, et al. Retinopathy in diabetes. Diabetes Care 2004;27:Suppl 1:S84-7.
2. Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL III, et al. Diabetic retinopathy. Diabetes Care 1998;21:143-56.
3. Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BE. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XXII the twenty-fifth-year progression of retinopathy in persons with type 1 diabetes. Ophthalmology 2008;115:1859-68.
4. Williams R, Airey M, Baxter H, Forrest J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular oedema: A systematic review. Eye (Lond) 2004;18:963-83.
5. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.
6. Yam JC, Kwok AK. Update on the treatment of diabetic retinopathy. Hong Kong Med J 2007;13:46-60.
7. Arevalo JF, Sanchez JG, Lasave AF, Wu L, Maia M, Bonafonte S, et al. Intravitreal Bevacizumab (Avastin) for Diabetic Retinopathy at 24-months: The 2008 Juan Verdaguer-Planas Lecture((R)). Curr Diabetes Rev 2010;6:313-22.
8. Kowluru RA, Zhong Q, Kanwar M. Metabolic memory and diabetic retinopathy: Role of inflammatory mediators in retinal pericytes. Exp Eye Res 2010;90:17-23.
9. Villeneuve LM, Natarajan R. The role of epigenetics in the pathology of diabetic complications. Am J Physiol Renal Physiol 2010;299:F14-25.
10. Nishijima K, Ng YS, Zhong L, Bradley J, Schubert W, Jo N, 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;171:53-67.
11. Klein R, Klein BE. Diabetic eye disease. Lancet 1997;350:197-204.
12. Davidson JA, Giulla TA, McGill JB, Kels KA, Anderson PW. How the diabetic eye loses vision. Endocr Rev 2007;28:107-16.
13. Barreiro O, Martin P, Gonzalez-Amaro R, Sanchez-Madrid F. Molecular cues guiding inflammatory responses. Cardiovasc Res 2010;86:174-82.
14. Joussen AM, Poultaki V, Misiadsie N, Kriebelhof B, Kozurni K, Dohmen S, et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. Faseb J 2002;16:438-40.
15. Demircan N, Safran BG, Soyla M, Ozaan AA, Szijkaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (Lond) 2006;20:1366-9.
16. 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;80:168-73.
17. Vincent JA, Mohr S. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. Diabetes 2007;56:224-30.
18. Kowluru RA, Odenbach S. Role of interleukin-1beta in the development of retinopathy in rats: Effect of antioxidants. Invest Ophthalmol Vis Sci 2004;45:4161-6.
19. Marugewzari P, Shukla D, Rajendran A, Kim R, Nampemuralsamy P, Muthukkaruppan V. Proinflammatory cytokines and angiogenic and anti-angiogenic factors in vitreous of patients with proliferative diabetic retinopathy and caxis’ disease. Retina 2008;28:817-24.
20. Maier R, Weger M, Haller-Schober EM, El-Shabrawi Y, Wedrich A, Theisl A, et al. Multiplex bead analysis of vitroovus and serum concentrations of inflammatory and proangiogenic factors in diabetic patients. Mol Vis 2008;14:637-43.
21. Meleth AD, Agron E, Chan CC, Reed GF, Arora K, Byrnes G, et al. Serum inflammatory markers in diabetic retinopathy. Invest Ophthalmol Vis Sci 2005;46:4295-301.
22. Mocan MC, Kadayifeclar S, Eldem B. Elevated intravitreal interleukin-6 levels in patients with proliferative diabetic retinopathy. Can J Ophthalmol 2006;41:747-52.
23. 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;110:1600-6.
24. 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;246:1665-70.
25. McLeod DS, Lefer DJ, Merjes C, Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. Am J Pathol 1995;147:642-53.
26. 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;54:1534-42.
27. Miyamoto K, Khosrof S, Bursell SE, Rohan R, Murata T, Clermont AC, et
al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. Proc Natl Acad Sci USA 1999;96:10836-41.

28. Jousen AM, Poulik V, Le ML, Koizumi K, Esser C, Janicki H, et al. A central role for inflammation in the pathogenesis of diabetic retinopathy. Faseb J 2004;18:1450-2.

29. Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP, et al. Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. Invest Ophthalmol Vis Sci 2000;41:1153-8.

30. Chen L, Yang P, Kijlstra A. Distribution, markers, and functions of retinal microglia. Ocul Immunol Inflamm 2002;10:27-39.

31. Yang LP, Sun HL, Wu LM, Guo XJ, Dou HL, Tso MO, et al. Baicalin reduces inflammatory process in a rodent model of diabetic retinopathy. Invest Ophthalmol Vis Sci 2009;50:2319-27.

32. Jezek P, Hlavata L. Mitochondria in homeostasis of reactive oxygen species in cells, tissues, and organism. Int J Biochem Cell Biol 2005;37:2478-503.

33. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-70.

34. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Mitochondrial respiratory chain deficiency in retinal blood vessel smooth muscle cells of hypertensive rats. J Clin Invest 2001;108:427-37.

35. Richter M, King GL. Activation of protein kinase C isoforms and its impact on diabetes and its complications. Mol Cell Endocrinol 2009;302:148-58.

36. Turpaev KT. Reactive oxygen species and regulation of gene expression. Biochemistry (Moscow) 2002;67:281-92.

37. 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;166:1206-13.

38. 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;276:8910-7.
Inducible nitric oxide synthase isoform is a key mediator of disease. Pharmacol Rep 2005;57 Suppl:156-68.

Stitt AW, Curtis TM. Advanced glycation and retinal pathology during angiogenesis. Invest Ophthalmol Vis Sci 2007;48:5257-65.

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;49:2053-60.

Morris DL, Schmidt AM. RAGE and its ligands in retinal disease. Curr Mol Med 2007;7:758-65.

Stitt AW, Curtis TM. Advanced glycation and retinal pathology during diabetes. Pharmacol Rep 2005;57 Suppl:156-68.

Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, et al. Inducible nitric oxide synthase isozyme is a key mediator of leukocyte adhesion. Proc Natl Acad Sci USA 1991;88:4651-5.

Li Q, Verma A, Han PY, Nakagawa T, Johnson RJ, Grant MB, et al. Diabetic eNOS knockout mice develop accelerated retinopathy. Invest Ophthalmol Vis Sci 2010;51:5240-6.

Leal EC, Manivannan A, Hosoya K, Terasaki C, Cunha-Vaz J, Ambrosio AF, et al. Inducible nitric oxide synthase isoform is a key mediator of leukostasis and retinal blood-barrier breakdown in diabetic retinopathy. Invest Ophthalmol Vis Sci 2007;48:2557-65.

Caldwell RB, Zhang W, Romero MJ, Caldwell RW. Vascular dysfunction in retinopathy—an emerging role for arginase. Brain Res Bull 2010;81:303-9.

Kubes P, Suzuki M, Granger DN. Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991;88:4651-5.

Journal of Cardiovascular Disease Research Vol. 2 / No 2

A new advanced glycation inhibitor, LR-90, prevents experimental diabetic retinopathy. J Biol Chem 2006;281:8379-88.

20. Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA. TNF-alpha signals through PKCzeta/p38 to activate the nuclear factor kappaB pathway in microvascular endothelial cells. J Biol Chem 2006;281:8379-88.

21. Gavard J, Hou X, Qu Y, Masedunskas A, Martin D, Weigert R, et al. Role for a CXCR2/phosphatidylinositol 3-kinase gamma signaling axis in acute and chronic vascular permeability. Mol Cell Biol 2009;29:2469-80.

22. Del Maschio A, Zanetti A, Corada M, Rival Y, Ruco L, Lampugnani MG, et al. Polymorphonuclear leukocyte adhesion triggers the disorganization of endothelial cell-to-cell adherens junctions. J Cell Biol 1996;135:497-510.

23. Kowalrzu RA, Odenbachi S. Role of interleukin-1beta in the pathogenesis of diabetic retinopathy. Br J Ophthalmol. 2004;88:1343-1347.

24. Joussen AM, Pouliki V, Mitsiades N, Cai WY, Sazuma I, Pak J , 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;17:76-8.

25. Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: Cause or consequence? Angiogenesis 2007;10:149-66.

26. Ishikawa K, Yoshida S, Kadota K, Nakamura T, Niino H, Arakawa S, et al. Gene expression profile of hyperoxic and hypoxic retinas in a mouse model of oxygen-induced retinopathy. Invest Ophthalmol Vis Sci 2010;51:4307-19.

27. Sato T, Kusaka S, Hashida N, Saishin Y, Fukushima T, Tano Y. Comprehensive gene-expression profile in murine oxygen-induced retinopathy. Br J Ophthalmol 2009;93:96-103.

28. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med 2007;13:868-73.

29. Gardner TA, Gibson DS, de Gooyer TF, de la Cruz VF, McDonald DM, Stitt AW. Inhibition of tumor necrosis factor-alpha improves physiological angiogenesis and reduces pathological neovascularization. Am J Pathol 2005;166:637-44.

30. Ishida S, Usui T, Yamashiro K, Kaji Y, Amano S, Ogura Y, et al. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. J Exp Med 2003;198:483-9.

31. Joussen AM, Doehmen S, Le ML, Koizumi K, Radecky S, Krohnke TU, 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;15:1418-28.

32. Sifakis PP, Markomichelakis N, Theodossiadis GP, Grigoropoulous 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;28:445-7.

33. Rao VR, Prescott E, Shelke NB, Trivedi R, Thomas P, Struble C, et al. Delivery of SAR 1118 to Retina Via Ophthalmic Drops and its Effectiveness in Reduction of Retinal Leukostasis and Vascular Leaks in Rat Streptozotocin (STZ) Model of Diabetic Retinopathy (DR). Invest Ophthalmol Vis Sci 2010;51:5198-204.

34. Iliali E, Pouliki 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;50:4898-904

How to cite this article: Zhang W, Liu H, Al-Shabrawey M, Caldwell RW, Caldwell RB. Inflammation and diabetic retinal microvascular complications. J Cardiovasc Dis Res 2011;2:96-103.

Source of Support: The American Heart Association 11SDG4960005 and SDG00104, the Juvenile Diabetes Foundation JDRF 10-2009-575, NIH Grants EY11766, EY04618, HL70215 and VA Merit Award and Vision Discovery Institute (GHSU), Conflict of Interest: None declared.