REVIEW ARTICLE

MECHANISMS OF DISEASE

Diabetic Retinopathy

David A. Antonetti, Ph.D., Ronald Klein, M.D., M.P.H., and Thomas W. Gardner, M.D.

Until recently, the treatment for diabetic retinopathy relied almost exclusively on managing the metabolic dysregulation of diabetes mellitus until the severity of vascular lesions warranted laser surgery. Intensive metabolic control remains a highly effective means of controlling retinopathy and other diabetes-related complications in many patients. Recent research has identified the central role of vascular endothelial growth factor (VEGF) in the vascular lesions observed in diabetic retinopathy, and new agents that block VEGF action provide an effective treatment for this debilitating disease in patients for whom metabolic control alone is insufficient. The fact that treatment of vascular complications in the retina preserves visual acuity in patients with diabetic retinopathy highlights the interconnectedness of the neural retina with the retinal vasculature and the functional neurovascular unit in the retina.

In this article, we highlight the principles underlying metabolic control and anti-VEGF therapies in the treatment of diabetic retinopathy. We also explore the molecular interactions of neuronal, glial, and vascular cells in the retina as the basis of the neurovascular unit and examine the effect of diabetes on the function of the neurovascular unit in order to highlight new therapeutic approaches that are needed to address the large increase in the worldwide prevalence of diabetes.

From the Departments of Ophthalmology and Visual Sciences and Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor (D.A.A., T.W.G.); and the Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison (R.K.). Address reprint requests to Dr. Gardner at Kellogg Eye Center, 1000 Wall St., Ann Arbor, MI 48105, or at tomwgard@umich.edu.

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DIABETIC RETINOPATHY IN THE PAST AND PRESENT

The features of diabetic retinopathy, as detected by ophthalmoscopy, were described in the 19th century. They begin with microaneurysms and progress into exudative changes (leakage of lipoproteins [hard exudates] and blood [blot hemorrhages]) that lead to macular edema (Fig. 1), ischemic changes (infarcts of the nerve-fiber layer [cotton-wool spots]), collateralization (intraretinal microvascular abnormalities) and dilatation of venules (venous beading), and proliferative changes (abnormal vessels on the optic disk and retina, proliferation of fibroblasts, and vitreous hemorrhage). Persons with mild-to-moderate nonproliferative retinopathy have impaired contrast sensitivity and visual fields that cause difficulty with driving, reading, and managing diabetes and other activities of daily living. Visual acuity, as determined with the use of Snellen charts, declines when the central macula is affected by edema, ischemia, epiretinal membranes, or retinal detachment.

Fifty years ago, proliferative diabetic retinopathy was treated by means of pituitary ablation, but the high frequency of complications related to hypopituitarism, including death, prompted the development of panretinal photocoagulation. In 1968, the Airlie House Symposium led to a standard classification system for diabetic retinopathy and laid the groundwork for the Diabetic Retinopathy Study (ClinicalTrials.gov number, NCT00000160) and the Early Treatment Diabetic Retinopathy Study (NCT00000151) in the 1970s and 1980s, respectively.[1,2] These clinical trials showed

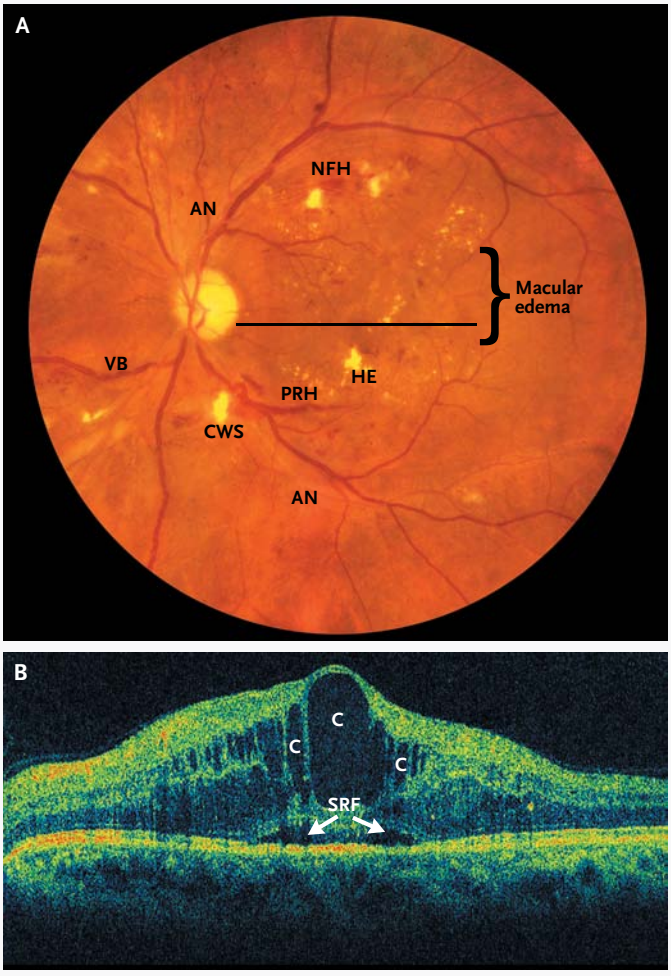

Figure 1. Clinical Features of Diabetic Retinopathy.

A fundus photograph (Panel A) shows the left eye of a 57-year-old man with 20/200 visual acuity, signs of hypertension, and proliferative diabetic retinopathy with macular edema (the region of macular edema is indicated by the bracket). Notable features include arteriolar narrowing (AN), nerve-fiber hemorrhage (NFH), hard exudates (HE), cotton-wool spots (CWS), venous beading (VB), and preretinal hemorrhage (PRH). Optical coherence tomography (Panel B) with a horizontal scan through the central fovea (corresponding to the horizontal line in Panel A) reveals marked thickening and edema of the macula with cysts (C) and subretinal fluid (SRF). (Images courtesy of Richard Hackel, M.A., C.R.A.)

the dramatic effects of retinal photocoagulation, which significantly reduced the severe visual loss due to proliferative diabetic retinopathy and macular edema, and led to guidelines and screening programs for the timely detection and treatment of diabetic retinopathy.

The incidence and the risk of progression of diabetic retinopathy have both declined over the past 30 years, from up to 90% to less than 50%. The population-based Wisconsin Epidemiologic Study of Diabetic Retinopathy showed that, from 1980 to 2007, the estimated annual incidence of proliferative diabetic retinopathy decreased by 77% and vision impairment decreased by 57% among persons with type 1 diabetes.[3] Persons with recently diagnosed type 1 or type 2 diabetes have a much lower risk of proliferative diabetic retinopathy, macular edema, and visual impairment (Fig. 2A), as compared with patients from earlier periods.[4-8]

The marked reduction in the prevalence and incidence of retinopathy and vision impairment over the past few decades reflects improved management of glycemia, blood pressure, and lipid levels.[8] These improvements have resulted from the introduction of new devices for self-monitoring of blood-glucose levels and the administration of insulin, new medications (e.g., statins and hypoglycemic agents), surgical interventions (including vitrectomy), an increased awareness of the need for intensive control of glycemia and blood pressure, and the implementation of educational and screening programs (Fig. 2B).[9-12] The benefits of intensive control are offset, however, by a 33% increase in the frequency of hypoglycemia and a 100% increase in the prevalence of overweight or obesity among adults with diabetes. The percentage of persons with type 2 diabetes who meet the target levels for glycated hemoglobin, blood pressure, or serum total cholesterol, as recommended by the American Diabetes Association, increased by 30 to 50% from 2000 to 2006.[13] However, only 7% of patients meet all three targets,[14] and non-Hispanic blacks and Mexican Americans meet them less commonly than whites.[15]

THE DIABETES EPIDEMIC

The number of persons with diabetes worldwide is predicted to grow to 429 million by 2030, owing to the rising frequency of obesity, increasing life span, and improved detection of the disease.[16,17] In India, an estimated 32 million persons had diabetes in 2000, and roughly 79 million will be affected by 2030.[18] If the prevalence of complications remains unchanged, approximately 0.7 million Indians will have proliferative diabetic retinopathy and 1.8 million will have clinically significant macular edema.[18] Improved delivery of health care re-

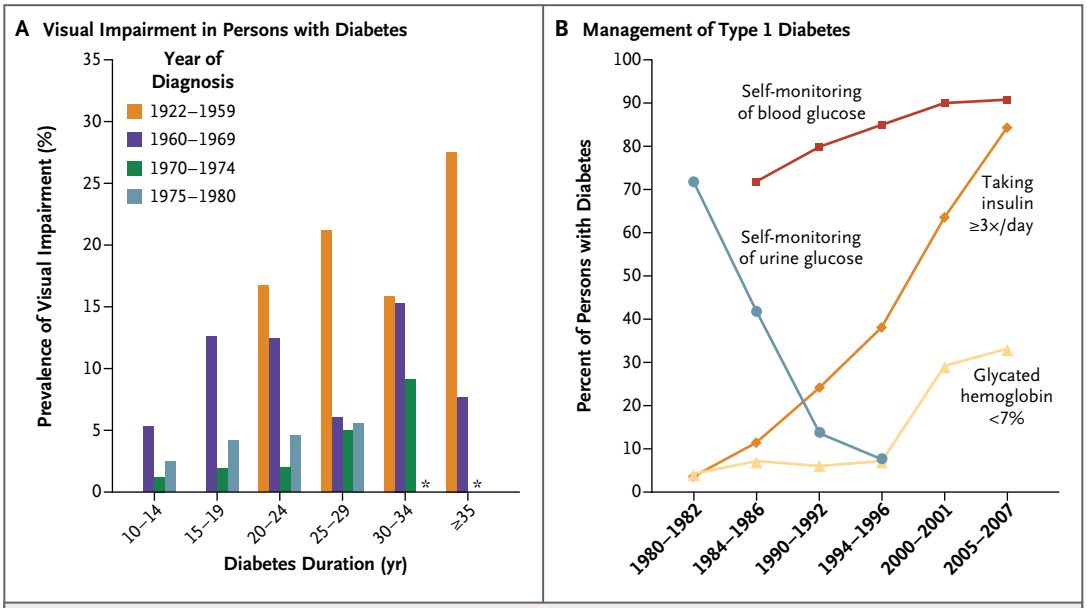

Figure 2. Improvement in Visual Prognosis and Diabetes Care.

For a specific duration of diabetes, persons with recently diagnosed type 1 or type 2 diabetes have a lower prevalence of visual impairment, defined as a best corrected visual acuity of 20/40 or worse in the better eye, as compared with patients who received a diagnosis in earlier periods (Panel A). The asterisks indicate that the numbers were too few to establish a reliable estimate. Since 1980, there have been changes in the management of diabetes, as shown by the proportion of persons with type 1 diabetes who use blood versus urine glucose testing, who have glycated hemoglobin values of less than 7%, and who are administering three or more injections of insulin per day (Panel B). (Graphs in both panels adapted from Klein and Klein.[8])

duces the incidence of vision impairment in whites in developed countries (e.g., Denmark, Sweden, and the United States), but it remains uncertain whether the lifestyle changes that are associated with urbanization in India and other developing countries will result in uncontrolled glycemia, blood pressure, and lipid levels and a higher frequency of severe diabetic retinopathy in persons with type 2 diabetes. These data portend a huge population of persons at high risk for diabetes-induced visual impairment for whom current approaches to treatment are inadequate. Little information exists on the risk of retinopathy and other diabetes-related complications in developing countries, so continued epidemiologic surveillance is needed to determine trends, properly allocate resources, and develop cost-effective preventive interventions.

Epidemiologic studies have shown the effects of hyperglycemia, hypertension, and dyslipidemia — and, to a lesser extent, a high body-mass index, a low level of physical activity, and insulin resistance — on the incidence and progression of diabetic retinopathy and clinically significant macular edema. The Diabetes Control and Complications Trial (DCCT; NCT00360815) showed that intensive metabolic control reduces the incidence and progression of diabetic retinopathy. Although the glycated-hemoglobin level is the strongest risk factor for predicting the development and progression of diabetic retinopathy, glycated hemoglobin accounted for only 11% of the risk of retinopathy in the DCCT.[19] Similarly, the values for glycated hemoglobin, blood pressure, and total serum cholesterol together accounted for only 9 to 10% of the risk of retinopathy in the Wisconsin Epidemiologic Study of Diabetic Retinopathy.[20] Therefore, the prevention and treatment of diabetic complications should include other modifiable factors. Data from several studies suggest roles for other factors, including sleep apnea,[21] nonalcoholic fatty liver disease,[22] and serum prolactin, adiponectin, and homocysteine levels,[23-25] as well as genetic factors, including mutations in the erythropoietin gene promoter.[26] However, the relative contributions of these factors to the risk of retinopathy in populations remain uncertain.

Despite advances in diabetes care, complications persist for various reasons. Proliferative diabetic retinopathy and other complications develop after 30 years in up to 20% of persons with diabetes who have been treated with intensive metabolic control,[27] and ideal metabolic control is difficult to achieve because of the increased risk of hypoglycemia and the nonphysiologic route of insulin administration. Only 17% of persons in the DCCT who were followed in the Epidemiology of Diabetes Interventions and Complications study (NCT00360893) had glycated-hemoglobin levels less than 7% at their last visit.[27] In developing countries, the resources needed to implement good diabetes control are generally unavailable. Therefore, greater emphasis must be placed on preventing complications, which will require both a better understanding of the mechanisms by which diabetes affects the retina and an improved means of detecting retinopathy.

CLINICAL TRIALS OF RETINOPATHY THERAPIES

Large, randomized trials have shown the benefits of systemic and ocular therapies for the prevention or treatment of diabetic retinopathy (Table 1) and have revealed that metabolic control, the renin–angiotensin system, peroxisome proliferator–activated receptor α (PPAR-α), and VEGF contribute to human pathophysiology. Notably, renin–angiotensin system inhibitors reduce the incidence and risk of progression of diabetic retinopathy in persons with type 1 diabetes and are now standard therapy.[12,29,32,33] The PPAR-α agonist, fenofibrate, reduces the risk of progression by up to 40% among patients with nonproliferative retinopathy, as shown in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD; Current Controlled Trials number, ISRCTN64783481)[37] and the Action to Control Cardiovascular Risk in Diabetes (ACCORD; NCT00000620) studies.[36,38] Whether the mechanism of action underlying this preventive effect of fenofibrate is related to its lipid-lowering action remains unclear. The ACCORD study did not show an effect of intensive blood-pressure control on retinopathy progression but did show the benefit of intensive glycemic control in preventing the progression of retinopathy.

Eye-specific treatments are beneficial in patients whose vision is threatened by macular edema. Use of the VEGF-neutralizing antibodies bevacizumab and ranibizumab improves visual acuity by an average of one to two lines on a Snellen chart, with an improvement of three or more lines in 25 to 30% of patients, and loss of visual acuity decreased by one third.[35,39,40] These improvements, which are seen over a period of 2 years after approximately 10 intraocular injections, are significantly better than the results of laser treatment alone. The VEGF aptamer, pegaptanib, improves visual acuity by approximately one line.[41] Sustained intravitreal delivery of fluocinolone yields a similar likelihood of gaining three or more lines of acuity but with a 60% increase in the risk of glaucoma and a 33% increase in the need for cataract surgery.[42] The same implant technology delivering a lower dose of fluocinolone did not increase the risk of cataract or glaucoma.[43] Glucocorticoids such as fluocinolone reduce retinal inflammation and may restore the integrity of the blood–retina barrier by increasing tight-junction protein expression.[44,45] These initial treatments for diabetic retinopathy reflect the gains in our understanding of how diabetes impairs vision and set the stage for further advances in the management of this disorder.

THE NEUROVASCULAR UNIT

New insights into retinal physiology suggest that the retinal dysfunction associated with diabetes may be viewed as a change in the retinal neurovascular unit. The neurovascular unit refers to the physical and biochemical relationship among neurons, glia, and specialized vasculature and the close interdependency of these tissues in the central nervous system (Fig. 3). This intimate association of glia with neurons allows for energy homeostasis and neurotransmitter regulation. Furthermore, glial-cell, pericyte, and neural interactions promote formation of the blood–brain and blood–retina barriers, which control the flux of fluids and blood-borne metabolites into the neural parenchyma.[46,47] Neurodegenerative conditions such as stroke, Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease alter the neurovascular unit, with changes in neural function and neurotransmitter metabolism and loss of the blood–brain barrier.[48-50] If the neurovascular unit is similarly involved in diabetes, then new therapeutic approaches addressing both vascular dysfunction and neural degeneration may be required. Table 2

Table 1. Major Pharmacologic Clinical Trials in Diabetic Retinopathy.*

Drug Trial	Primary End Point	Stage of Diabetic Retinopathy at Baseline	Size of Effect	Comments
Intensive insulin in type 1 diabetes (DCCT; NCT00360815)[9,28]	Development or progression of DR	None or mild-to-moderate NPDR	Reduced incidence by 76% and risk of progression by 54%	Conclusively showed benefits and risks of intensive metabolic control in type 1 diabetes
Lisinopril in type 2 diabetes (EUCLID)[29]	Progression of DR	None or mild-to-moderate NPDR	Reduced risk of PDR by 50%	First study to show effect of RAS inhibition on DR
Metabolic (sulfonylurea or insulin) and blood-pressure control (ACE inhibitors or beta-blockers) in type 2 diabetes (UKPDS)[30,31]	Development or progression of DR	None or mild-to-moderate NPDR	Metabolic and blood-pressure control reduced risk of incident DR, reduced need for laser surgery by one third	Conclusively showed benefits and limitations of metabolic and blood-pressure control in type 2 diabetes
Enalapril and losartan in type 1 diabetes (RASS; NCT00143949)[12]	Progression or development of DR	None or mild-to-moderate NPDR	Reduced risk of progression by 65%	Showed benefits of RAS inhibition in type 1 diabetes
Candesartan in prevention and progression of DR in type 1 diabetes (DIRECT-1; NCT00252720)[32,33]	Development and progression of DR	Prevent: no DR Protect: mild to moderately severe NPDR	Reduced incidence by 18% but no effect on risk of progression	Showed that candesartan reduces incidence of DR but has no effect on risk of progression in type 1 diabetes
Candesartan in progression of DR in type 2 diabetes (DIRECT-2; NCT00252694)[34]	Progression of mild-to-moderate NPDR	Mild-to-moderate NPDR	No reduction of progression but more regression	Showed that candesartan may ameliorate existing NPDR
Ranibizumab in type 1 and type 2 diabetes (DRCR; NCT00445003)[35]	Diabetic macular edema	Mild-to-moderate NPDR	Increased visual acuity by 9 letters, increase of ≥3 lines in 30% of patients	Showed that VEGF inhibition improves visual acuity
Fenofibrate plus simvastatin in type 2 diabetes (ACCORD-Eye; NCT00542178)[36]	Progression of DR or development of PDR	Mild-to-moderate NPDR	Reduced risk of progression by 40%	Showed additive effects of fenofibrate plus simvastatin
Fenofibrate in type 2 diabetes (FIELD; ISRCTN647833481)[37]	Progression of DR	Mild-to-moderate NPDR	Reduced risk of progression and macular edema by one third	Showed that fenofibrate reduces need for laser treatment

* ACCORD denotes Action to Control Cardiovascular Risk in Diabetes, ACE angiotensin-converting enzyme, DCCT Diabetes Control and Complications Trial, DIRECT Diabetic Retinopathy Candesartan Trials, DR diabetic retinopathy, DRCR Diabetic Retinopathy Clinical Research, EUCLID Eurodiab Controlled Trial of Lisinopril in Insulin-Dependent Diabetes, FIELD Fenofibrate Intervention and Event Lowering in Diabetes, NPDR nonproliferative diabetic retinopathy, PDR proliferative diabetic retinopathy, RAS renin–angiotensin system, RASS Renin–Angiotensin System Study, UKPDS United Kingdom Prospective Diabetes Study, and VEGF vascular endothelial growth factor.

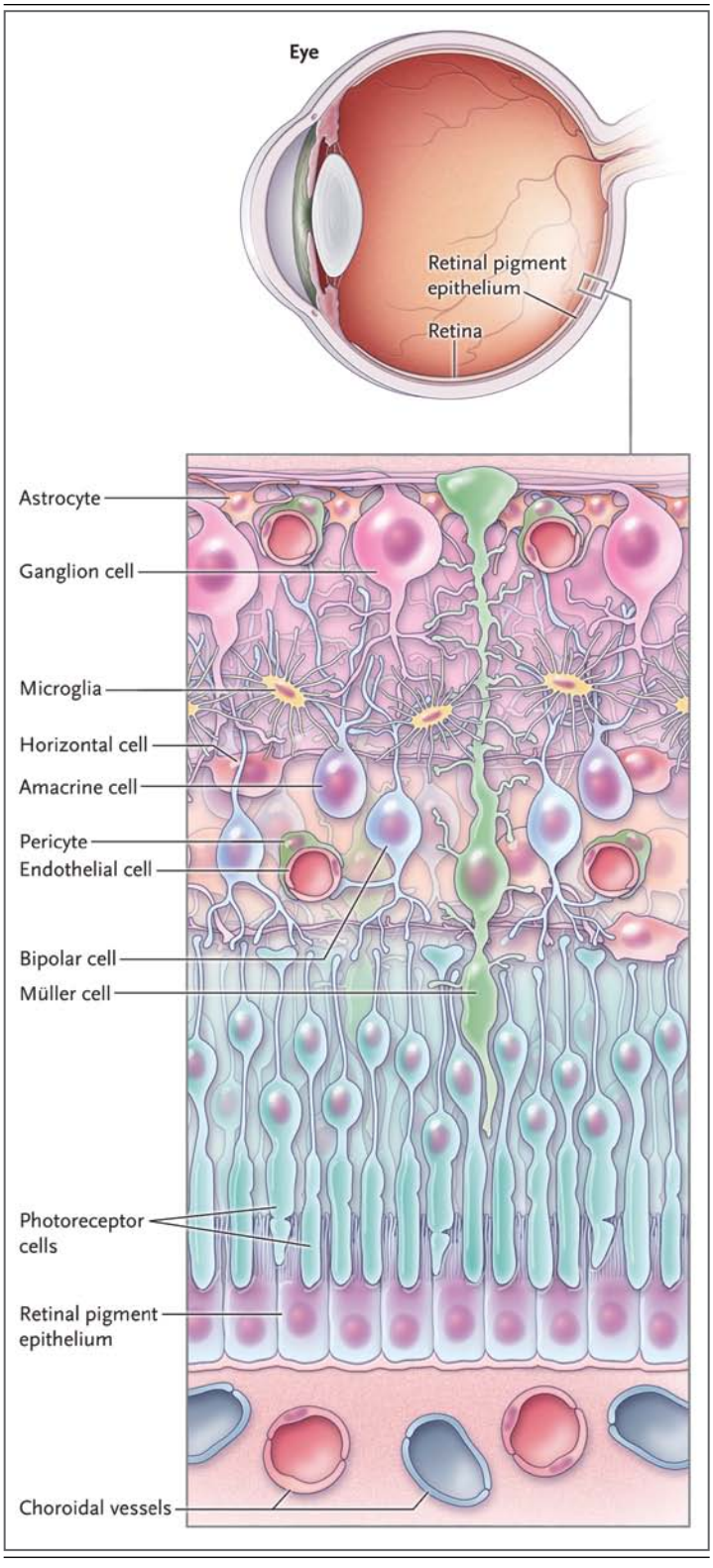

Figure 3. The Neurovascular Unit of the Retina.
Under normal conditions, blood-vessel endothelial cells and pericytes, astrocytes, Müller cells, and neurons are intimately connected to establish the blood–retina barrier to control nutrient flow to the neural retina affording energy balance, to maintain the proper ionic environment for neural signaling, to regulate synaptic transmission, and to provide adaptable responses to the environment to allow vision.

lists alterations in the neurovascular unit in diabetic retinopathy.

The retinal architecture confers unique characteristics to the neurovascular unit. The inner retina has capillary beds in the ganglion-cell and inner nuclear layers. The neurovascular unit includes astrocytes and Müller cells, and amacrine and ganglion neurons reside in close proximity to microvascular segments that deliver oxygen and nutrients. The close coupling of neurovascular units is shown by the autoregulation of retinal vascular blood flow by local metabolite levels (the lactate level and the partial pressure of oxygen and of carbon dioxide) and glial cells.[51] The outer retina consists of photoreceptor neurons and Müller cells, which are metabolically coupled to support the generation of electrochemical impulses in response to stimulation with light, with nutrients and oxygen diffusing from choroidal vessels through the pigmented epithelial-cell layer.

VASCULAR LEAKAGE AND ANGIOGENESIS

Diabetic retinopathy involves occlusion and leakage of retinal vessels, leading to macular edema in the nonproliferative phase and angiogenesis and to tufts of highly permeable vessels in the proliferative phase. Macular edema (present in 25% of persons with diabetes) remains the clinical feature most closely associated with vision loss, with thickening of the central fovea evident on optical coherence tomography and fluorescein leakage visible on angiographic testing. The duration of central foveal thickening and the degree of fluorescein leakage are major factors in accounting for reduced visual acuity.[52,53] The efficacy of treatment with the anti-VEGF agents ranibizumab and bevacizumab indicates that VEGF contributes to the pathogenesis of diabetic macular edema and reflects successful translational research.

Conditional deletion of the *VEGF* gene from Müller cells reveals the importance of glial cells for VEGF production in oxygen-induced retinopathy models of angiogenesis, and this finding underscores the consequences of altered glial–vascular communication.[54,55] The mechanism of

VEGF-induced vascular permeability involves activation of classical protein kinase C isoforms, particularly protein kinase C beta.[56,57] Recently, the tight-junction protein occludin was identified as a target of protein kinase C beta, leading to ubiquitin-mediated endocytosis of tight-junction components and increased vascular permeability[58,59] and providing a molecular mechanism for the regulation of the properties of the blood–retina barrier in response to VEGF. Studies in animals and initial clinical reports suggest that inhibiting protein kinase C beta with ruboxistaurin reduces diabetic macular edema. In a combined analysis of data from two clinical trials of oral ruboxistaurin, the proportion of patients with sustained moderate visual loss was smaller in the group of patients who received ruboxistaurin than in the placebo group (6.1% vs. 10.2%)[60,61]; however, the Food and Drug Administration asked to see additional confirmatory phase 3 clinical-trial results before approving the drug for clinical use.

Other potential targets of VEGF-receptor signaling include inhibition of the soluble tyrosine kinase Src to regulate vascular permeability. Using small-molecule inhibitors of Src in animals with *src*-gene deletion, Scheppke and colleagues[62] found that the requirement for Src activation in VEGF induced retinal vascular permeability. Although no data from clinical trials are available yet, topical application to the cornea of a dual Src and VEGF receptor inhibitor prevented VEGF-induced vascular permeability in animals.[62]

Signaling pathways also contribute to vascular permeability in diabetic retinopathy. Mass spectrometry analysis of vitreous fluid in patients with proliferative retinopathy allowed Gao and colleagues[63] to identify the plasma kallikrein system that leads to bradykinin-receptor activation. Kallikrein inhibitors prevent retinal vascular permeability in diabetic rodents, and kallikrein injection acts synergistically with diabetes to increase retinal vascular leakage.[64] Other extracellular proteases, such as urokinase plasminogen activator[65] and matrix metalloproteases 2 and 9, may also contribute to the degradation of tight-junction protein and to retinal vascular permeability.[66]

The blood–retina barrier requires proper pericyte function, and loss of pericytes may contribute to vascular permeability (Fig. 4). Pericyte dropout is a feature of diabetic retinopathy, and genetic ablation of platelet-derived growth factor (PDGF) β causes pericyte loss and a phenotype that resembles diabetic retinopathy, with increased vascular damage and angiogenesis.[67] Geraldes et al.[68] recently found that hyperglycemia induces expression of protein kinase C delta, which upregulates Src-homology 2 domain–containing tyrosine phosphatase 1. This tyrosine phosphatase inhibits PDGF signaling through the Akt survival pathway, contributing to pericyte-cell death and vascular derangement. These findings underscore the cell-to-cell communication necessary for proper retinal function and maintenance of the blood–retina barrier.

Retinal angiogenesis (neovascularization) usually arises on the optic disk and at the junction of nonperfused retinal vessels and perfused vessels that are leaking, with growth into the posterior surface of the vitreous. Untreated neovascularization leads to vitreous contraction, vitreous hemorrhage, and tractional retinal detachment. An increased ratio of proangiogenic factors (VEGF and erythropoietin) to antiangiogenic factors (pigment-epithelium–derived factor) promotes neovascularization,[69-71] and the ratio is decreased after laser treatment. However, VEGF inhibition and regression of active neovascularization are associated with increased expression of connective-tissue growth factor in the vitreous, which contributes to vitreoretinal fibrosis.[72]

NEURONAL DYSFUNCTION

In addition to vascular abnormalities, the neurosensory retina is altered in diabetes. The neurosensory retina generates vision but is transparent and largely undetectable by standard clinical examination, so its role in diabetic retinopathy has been difficult to determine in humans. However, most retinal neurons and glial cells are altered concomitantly with the development of microvascular lesions and are progressively impaired with worsening retinopathy. These alterations include biochemical defects, such as impaired control of glutamate metabolism (the major neurotransmitter),[73] as well as loss of synaptic activity and dendrites,[74,75] apoptosis of neurons primarily in the ganglion-cell and inner nuclear layers,[76] and activation of microglial cells that may protect the inner retina from injury and contribute to the inflammatory response.[77]

In experimental models of diabetes, insulin-receptor signaling is impaired in the retina as it is in peripheral tissues,[74-81] and the actions of brain-derived neurotrophic factor are also reduced.[68,82] Thus, just as the loss of PDGF signaling contributes to pericyte loss,[68] the loss of neu-

rotrophic signals that support cell survival and cell–cell interactions at synapses probably contributes to the pathological features of retinopathy. Furthermore, changes in retinal blood flow and vasoreactivity in response to oxygen may indicate impaired autoregulation and impaired control of vascular integrity by the neural retina.[51] Diabetic retinopathy includes reduced electrical activity[83] and alterations of nerve fibers.[74,75] Together with reduced corneal-nerve sensation and impaired autonomic innervation of the pupil, altered function of the retinal sensory nerve indicates that diabetes causes denervation of multiple sensory inputs to the eye. Thus, although the retinal neuronal structure differs from the peripheral sensory system, diabetic retinopathy resembles diabetic peripheral sensory neuropathy.

INFLAMMATION IN DIABETIC RETINOPATHY

The concept of the neurovascular unit extends to the presence of activated microglia in diabetic retinopathy. Systemic inflammation is an intrinsic response to overfeeding, obesity, and diabetes,[84] and diabetes increases the release of retinal inflammatory mediators (interleukin-1β, tumor necrosis factor α [TNF-α], intercellular adhesion molecule [ICAM] 1, and angiotensin II)[85] and activation of microglial cells[77] in early retinopathy. Leukostasis occurs in diabetic mice and rats, and deletion of the genes for the adhesion protein ICAM or its leukocyte binding partner, CD18, ameliorates leukostasis and permeability.[34] Vascular permeability, leukostasis, CD18 and ICAM expression, and nuclear factor κB activation are normalized by treatment with high-dose aspirin, a cyclooxygenase-2 inhibitor, meloxicam, or a soluble TNF-α receptor–Fc hybrid, such as etanercept.[86] These findings suggest that TNF-α and cyclooxygenase-2 contribute to diabetic retinopathy, perhaps by preventing endothelial-cell damage from adhering leukocytes.[34] Furthermore, a newly discovered inhibitor of atypical protein kinase C prevents

Table 2. Changes in Vascular, Glial, Neuronal, and Microglial Cells in the Retina That Are Associated with Diabetic Retinopathy.*

Tissue injury
Nonproliferative diabetic retinopathy
Microaneurysms (vessel outpouching and leaks)
Lipid exudates
Microhemorrhages
Cotton-wool spots associated with nerve-fiber damage
Basement-membrane thickening
Venous tortuosities and beading
Fibrotic proliferative diabetic retinopathy
Angiogenesis (growth of vessels into retina and vitreous)
Hemorrhages
Tractional retinal detachment caused by proliferative vitreoretinopathy
Macular edema
Altered b-wave oscillatory potential on electroretinogram
Decrease in visual acuity
Vascular events
Microvascular permeability: altered tight-junction and adherens-junction expression and post-translational modifications
Focal hypoxic events
Production of GF and related cytokines, including vascular endothelial GF, platelet-derived GF, basic fibroblast GF, connective-tissue GF, erythropoietin, and angiotensin II
Loss of pigment-epithelium–derived factor
Protease changes: matrix metalloproteinases, serine proteases (urokinase), kallikrein, and bradykinin
Pericyte and endothelial-cell apoptosis
Receptor-signaling defects

Table 2. (Continued.)

Metabolic events and glial dysregulation
Generation of free radicals
Mitochondrial dysfunction and NADPH-oxidase activity
Generation of nitric oxide
Peroxynitration
Protein oxidation
Lipid peroxidation
Altered metabolism of glutamine synthetase and branched-chain aminotransferases
Altered lipid profiles with decrease in polyunsaturated n–3 fatty acids
Proteolysis
Protein synthesis
Neuronal dysfunction
Neuronal swelling
Altered synaptic protein expression
Neuronal apoptosis of ganglion cells and amacrine cells
Inflammation
Microglial morphologic changes and activation
Leukostasis: expression of intercellular adhesion molecule
Cytokine production by glia (microglia or adherent leukocytes)
Tumor necrosis factor α
Interleukin-1β, 6, and 8
Chemokine ligand 2

* GF denotes growth factors.

TNF-α–induced retinal vascular permeability[87] and VEGF-induced permeability (according to an unpublished study), providing a broad target for potential control of edema. Interleukin-1β and TNF-α levels increase in the vitreous of patients with proliferative diabetic retinopathy.[88,89] Progressive retinal injury may impair the blood–retina barrier and lead to macrophage migration into the neurosensory retina or increased adherence to the vasculature, as well as accumulation of inflammatory and angiogenic mediators in the vitreous cavity.

Collectively, the data suggest that inflammation contributes to the development and progression of retinopathy. Antiinflammatory treatment with intravitreal glucocorticoids and anti-VEGF therapy reduce the overall severity of retinopathy and macular edema and restore the blood–retina barrier.[35,46] Further investigation is needed to develop therapies that control inflammation in diabetic retinopathy.

FUTURE CHALLENGES AND OPPORTUNITIES

The large worldwide increase in diabetes provides an imperative to prevent retinopathy and other complications before the advanced stages of disease. Improved outcomes of treatments for cancer have resulted from advances in clinical-trial end points that reflect the pathophysiology of the disease, such as molecular biomarkers of tumor activity and positron-emission–tomographic scanning. Likewise, new end points reflecting the pathophysiological features and full phenotype of diabetic retinopathy are needed for sensitive, quantitative, and predictive assessment of the severity of retinopathy. Vascular lesions change slowly, and photographic staging alone cannot facilitate short-term (<1 year) proof-of-concept trials to evaluate pathophysiological mechanisms and therapies.

Standard measures are now being supplement-

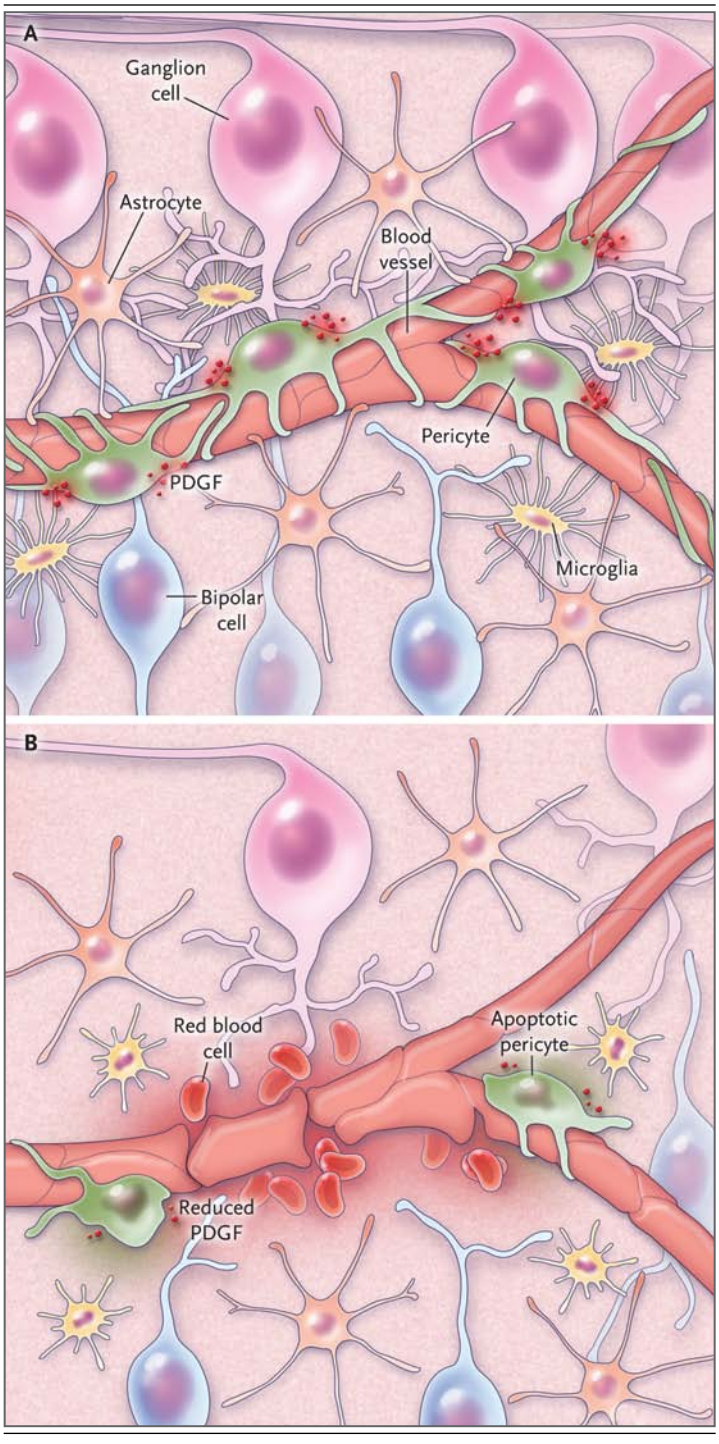

Figure 4. Disruption of the Neurovascular Unit of the Retina by Diabetes.

Panel A shows the neurovascular unit in the retina. Pericytes and glial cells, including astrocytes, promote formation of the blood–retina barrier in the vasculature, helping to create the environment for proper neural function. Microglial processes monitor the retinal environment. Panel B shows how normal cellular communication is altered in diabetes, with elevated VEGF from glial cells, combined with increased inflammatory cytokines, in part from activated microglia and adherent leukocytes (not shown), and the loss of platelet-derived growth factor (PDGF) signaling in pericytes, contributing to the breakdown of the blood–retina barrier and, in some cases, to angiogenesis. Blocking VEGF signaling has provided new therapeutic options to improve the treatment of patients with diabetic retinopathy and restore the neurovascular unit. In addition to microvascular complications, the loss of insulin receptor signaling and damage from inflammatory cytokines may contribute to synaptic degeneration and neuronal apoptosis and impairment of visual function in patients with diabetes.

ed with sensitive indexes of retinal function and structure to determine the nature of early retinopathy. Flavoprotein spectrophotometry reveals defects in mitochondrial metabolism,[90] reduced electroretinographic responses suggest reduced cellular signal transmission and predict subsequent microvascular lesions[91] and responses to improved metabolic control,[92] and subtle defects in visual function are detected by contrast sensitivity and visual-field defects.[93,94] Optical coherence tomography detects thinning of the neuronal and synaptic layers of mild retinopathy.[95,96]

Metabolic and blood-pressure control have reduced the incidence of diabetic retinopathy and vision impairment and remain the foundation for controlling retinopathy and other complication of diabetes. However, these approaches do not ameliorate visual impairment and may have adverse effects. Furthermore, economic barriers often prevent the implementation of these approaches among patients who are poor and underinsured. Research into the molecular causes of diabetic retinopathy reveals changes affecting all cells within the retina, including those in the microvasculature, glia, neurons, and microglia. These changes in the retina, which can be viewed as a disruption of the neurovascular unit, contribute to the pathophysiology of diabetic retinopathy. Intraocular administration of VEGF inhibitors and glucocorticoids has launched an era of biologically based pharmacologic treatment that complements surgical approaches for advanced stages of retinopathy. Further advances require an understanding of how the metabolic changes in diabetes disrupt the neurovascular unit, as well as focused efforts to develop clinical-trial end points and biomarkers. The expected increase in diabetic retinopathy due to the increasing incidence of type 2 diabetes requires the elimination of socio-

economic barriers so that research advances can be translated into effective, accessible care for all persons with diabetes.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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