Diabetic retinopathy is a clinically well-defined, sight-threatening, chronic microvascular complication that eventually affects virtually all patients with diabetes. Diabetic retinopathy is characterized by gradually progressive alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vasopermeability, and in response to retinal nonperfusion, pathologic intraocular proliferation of retinal vessels (1–3).

Most diabetes researchers and clinicians are aware of the major advances made in understanding the pathobiology of proliferative diabetic retinopathy. However mechanisms underlying the progressive alterations in retinal microvessels, which precede and stimulate neovascularization, are less well-known. In this review, current information about the pathogenesis of the primary lesion of diabetic retinopathy, retinal capillary vasoregression (see Fig. 1), is presented.

Diabetic retinopathy is often considered as a complication that contrasts with other vascular sequelae of this disease because it is associated with new vessel formation, while diabetic heart disease and diabetic nephropathy are characterized by impaired angiogenesis (4). Diabetic retinopathy is generally grouped with tumor angiogenesis and is presented as a paradigm of a neovascular disease (5). As outlined in this review, the natural history of diabetic retinopathy starts with vasoregression. Recent investigations have brought new insight regarding the primary vasoregressive process that stimulates angiogenesis, provoking new directions of thinking about possible prevention and intervention (1).

Diabetic retinopathy starts with the loss of the two cellular components of retinal capillaries: the pericyte, a vessel support cell, and the endothelial cell. The exact sequence of loss in humans is not established because early human retinal samples are not available, but animal studies have provided evidence that pericytes disappear before endothelial cells start to vanish, leaving acellular capillaries with no blood flow (6). In response to progressive retinal capillary dropout, the ischemic retina mounts an angiogenic response from the surrounding capillaries leading to proliferative diabetic retinopathy. Correlative studies of fluorescein angiography and postmortem retinal digests in humans show that microaneurysms appear to cluster around areas of acellular capillaries linking structural damage in situ to clinical markers of disease progression and suggesting that microaneurysms represent abortive attempts at neovascularization (7,8).

**The mechanism of physiological (sprouting) angiogenesis.** Novel concepts have recently been proposed for physiological angiogenesis also reflecting mechanisms that might be involved in pathological angiogenesis (9,10). There are some important molecular players of interest that dominate both developmental and pathologic retinal angiogenesis. Under physiological conditions, subsets of endothelial cells in sprouting vessels qualify for different functions (Fig. 2). The tip cells guide the vessel along a gradient of heparin-bound VEGF (vascular endothelial growth factor)-A (164) sensed by VEGF receptor-2 expressed on cell extrusions (filopodia) (11). Tip cells are incapable of proliferation. In contrast, stalk cells do proliferate. These specialized endothelial cells are prevented from becoming tip cells by lateral inhibition through the Notch-Dll system, while their proliferative activity is determined by the availability of VEGF and other growth factors such as angiopoietin (Ang)-2 (12). The transition from a proliferating to a mature quiescent endothelial cell, i.e., the transition from a stalk cell to a so-called phalanx cell, is determined by the expression of Ang-1 and the firm coverage by pericytes, which render these cells resistant to growth factor depletion by mechanisms that are poorly understood. On retinal endothelial cells, Tie-2 receptor activation through Ang-1 binding combines several recruiting signals for smooth muscle cells (and probably pericytes), including hepatocyte- and heparin-binding epidermal-like growth factor, but the firm intramural positioning of pericytes has yet to be explained. As a novel finding, Notch3 is specifically involved in pericyte recruitment and survival (13).

**VEGF and the retina.** Previous work using transgenic mice with isoform-specific expression of VEGF has indicated that the VEGF-A isoform 164 is the major one required for proper three-dimensional network formation in the retina (14). One important contributing factor is the balance between VEGF’s diffusibility and its heparin-binding properties. The sole presence of the diffusible VEGF120 isoform causes severe changes in vessel outgrowth and patterning, pericyte recruitment, and vessel permeability during early vessel development in the retina. In contrast, the isolated presence of strongly matrix-associated VEGF188 impairs arterio-venous differentiation, capillary patterning, and peripheral vascular outgrowth (15). Another important issue is the relative abundance of growth factors involved in the development and maturation of the retinal vasculature. In the nondiabetic rodent retina, VEGF-A (164) is by far the most abundant factor expressed, e.g., exceeding that of tumor necrosis factor-α by 30,000-fold. The importance of VEGF-A for proper retinal vascular development is further supported by data from mice with conditional inactivation...
of VEGF-A in neuronal tissue. Mouse retinas with a depletion of VEGF in nestin-expressing cells are characterized by a sparse capillary network, a smaller capillary width, 25% fewer endothelial cells, and a sixfold increase in capillaries devoid of both endothelial cells and pericytes, indicating that VEGF is not only an important developmental growth factor, but also an important survival factor (16). When these mice are subjected to the

**FIG. 1.** Phenotype of vasoregression in the diabetic retina. In both experimental diabetic rats and diabetic humans, capillary occlusions occur. Nondiabetic (A) and 6-month diabetic rat retina with acellular capillaries (arrows) (B). Nondiabetic (C) and diabetic (D) human retinal digest preparation. Periodic acid-Schiff staining (original magnification ×250).

**FIG. 2.** Concept of physiological angiogenesis. 1) In tip cells, VEGF stimulates DLL4/NOTCH signaling via VEGF-R2, thereby inhibiting tip cell formation and inducing VEGF-R1 expression in the endothelial cells downstream. Astrocyte-derived SDF-1 acts as an additional chemoattractant, activating CXCR4 in tip cells. 2) In stalk cells, predominance of VEGF-R1 and activation of Tie-2 by Ang-2 secreted from the tip cell lead to proliferation and survival. 3) Platelet-derived growth factor receptor (PDGFR)-β—pericytes are attracted to the growing sprout by PDGF-B, released from tip cells. Interaction of recruited pericytes with endothelial cell–derived Jagged-1 induces the expression of Notch3 and activation of an autoregulatory loop that further enhances Notch3 activation, thereby promoting pericyte survival, investment, vascular branching, and induction of smooth muscle cell (SMC) genes. 4) Transforming growth factor (TGF)-β produced in endothelial cells further induces SMC differentiation and pericytes-derived Ang-1 binds to and activates the Tie-2 receptor on endothelial cells, thereby stimulating vessel maturation and stabilization.
mouse model of retinopathy of prematurity, there is a 94% reduction in new vessel formation.

In patients with active proliferative diabetic retinopathy, VEGF levels are increased, while in those eyes in which proliferative retinopathy is quiescent, VEGF levels are either normal or only modestly increased (17). Together these data suggest that VEGF is the most prominent member of a group of factors that control and facilitate physiological and pathological angiogenesis.

From a clinical standpoint, the question arises whether there are certain genetic changes that are associated with the promotion or the inhibition of retinopathy. In contrast to many other vascular diseases, diabetic retinopathy in general has not been found to be strongly and unequivocally linked to genetic defects or polymorphisms. Among the few significantly associated genes are aldose reductase, the receptor for advanced glycation end products (RAGE), the integrin α2β1, and VEGF. From the Diabetes Control and Complications Trial (DCCT), it is known that 25% of the risk for retinopathy development can be explained by genetic factors, which are determined by familial clustering (18). Al-Kateb et al. (19) analyzed the 16 single nucleotide polymorphisms of VEGF in type 1 diabetic patients of the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) cohort and tested the association of these polymorphisms with retinal outcomes after 10 years during the EDIC study. There was a strong association of VEGF polymorphisms with the development of severe retinopathy but not with retinopathy progression or diabetic macular edema. Although the familial clustering suggests a genetic contribution to the risk of developing advanced lesions, the identity and function of such contributors are completely unknown. Apart from genetic factors, epigenetic modifications induced by hyperglycemia-induced biochemical changes in the transcriptional machinery of target cells may play a role that needs to be assessed.

Context-dependent function of the Ang-Tie system.

The Ang-Tie system has received particular attention as a key regulator of adult vascular homeostasis (20–23). The receptor tyrosine kinase Tie-2 is expressed on endothelial cells, regulating vascular remodeling and maturation. The balance between the two ligands Ang-1 and -2 determines the phosphorylation status of Tie-2, thereby regulating endothelial barrier function, vessel branching, inflammatory responses, and angiogenesis (24–26). Ang-1 is considered to be an activator of Tie-2, while Ang-2 is a homologous ligand that antagonizes Ang-1 activity on Tie-2, acting as an endogenous dominant negative ligand for Ang-1. During developmental angiogenesis, Ang-1 expression appears to be the initial step of vessel maturation, causing endothelial cell differentiation and recruitment of pericyte precursors, which stabilize the new vessels (22,27). Transforming growth factor-β1 further contributes to the final maturation steps by downregulation of cell migration and promotion of cell differentiation. Adult vessel remodeling involves the interplay between VEGF and the ratio of Ang-1 and -2. When the ratio of Ang-2 to Ang-1 is high and VEGF is high in hypoxic tissues, the consequence is sprouting angiogenesis. In contrast, in the absence of VEGF, Ang-2 upregulation leads to vasoregression. Isolated downregulation or the absence of Ang-1 causes vessel destabilization through effects on pericytes (27).

**Hyperglycemia and the molecular changes in the early diabetic retina.** The most relevant morphological lesion in the diabetic retina is the acellular, nonperfused capillary segment (1). It reflects the net result of multiple damaging mechanisms involving endothelial, matrix, pericyte, and microenvironmental components. Thus retinopathy does not deviate from all the other vascular phenotypes in diabetes as is often thought. Diabetic retinopathy is part of a systemic vascular disease in which vasoregression is the primary evolutionary process. Vasoregression in the diabetic retina starts with pericyte loss. This is a long-known feature, and it has been consistently reported in diabetic animals and humans (2,6). As noted above and elaborated upon in a recent review by Betsholtz and colleagues (28), ligand-receptor systems involved in endothelial-pericyte (mural) cell signaling determine the fate of pericytes not only during vascular development, but also during incipient diabetic retinopathy (18). The concept, proposed by Hanahan, is that Ang-2 overexpression in cooperation with VEGF overexpression leads to pericyte loss and angiogenesis, while Ang-2 overexpression in the absence of VEGF leads to vasoregression (25). Figure 3 summarizes the context-dependent expression and regulation of the Ang-Tie system with the focus on diabetic vasoregression and angiogenesis. When determined in an experimental diabetic rat model in which pericyte dropout was precisely ascertained, Ang-2 upregulation (37-fold) preceded the onset of pericyte dropout (6). In young nondiabetic rats, pericyte dropout was inducible by intravitreal injection of recombinant Ang-2. Moreover in heterozygous diabetic Ang-2 knockout mice, pericyte dropout was prevented, and in mice with constitutive retinal overexpression of Ang-2, pericyte dropout was exaggerated. Together these data suggest that Ang-2 is involved in the pathogenesis of diabetic pericyte loss. Ang-2 upregulation by hypoxia is an established fact. However the early diabetic (rodent) retina is not hypoxic. Therefore hyperglycemia-induced regulation of Ang-2 was investigated. Hyperglycemia-induced mitochondrial overproduction of reactive oxygen species has been shown to induce altered gene transcription by covalent modification of coregulatory proteins. In renal endothelial cells, it was found that a complex consisting of the transcriptional co-repressor mSin3A and the transcription factor Sp3 suppresses transcriptional activity by binding to a glucose-sensitive GC box in the Ang-2 promoter. Hyperglycemia-induced formation of methylglyoxal modifies mSin3A resulting in increased recruitment of O-GlcNac transferase to an mSin3A-Sp3 complex and a subsequent increased modification of Sp3 by O-linked N-acetylglucosamine. Gluc-Nac modification of Sp3 causes decreased binding of the repressor complex to the glucose-responsive GC box in the Ang-2 promoter resulting in increased Ang-2 expression (27) (Fig. 4).

**Pericyte migration: a novel mechanism of diabetic pericyte loss.** Data from human and animal studies have suggested that diabetic pericyte loss is the result of apoptosis induced by activation of nuclear factor-κB (NF-κB) or, as has been recently pointed out, by activation of the protein tyrosine phosphatase SHP-1 in an NF-κB independent pathway. Apoptosis is determined by a transient indicator (e.g., nuclear fragmentation). Still, the number of lost pericytes from apoptotic pericytes observed in retinal digest specimens is lower than the projected number of pericytes lost in total after several months of diabetes, suggesting that additional mecha-
nisms may be involved (29,30). Moreover, the developmental origin and the morphological diversity of pericytes in retinal capillaries suggest that not all pericytes are alike. Pfister et al. (31) used quantitative retinal morphology and normal and diabetic mice with different levels of Ang-2 expression to study which pericytes were lost in the diabetic retina and whether it was due to changes in Ang-2. The investigators categorized pericytes into three classes: 1) located at vessel branches (saddle pericytes), 2) located on straight parts of capillaries, and 3) showing different degrees of detachment from adjacent endothelial cells (migrating pericytes) (Fig. 5). The investigators found that saddle pericytes remained unaffected by diabetes while only pericytes on straight parts of capillaries were reduced in diabetic retinae in parallel with an increased number of migrating pericytes. In nondiabetic Ang-2–overexpressing animals, this number was increased by 78%, while in Ang-2–deficient mice, the numbers of migrating pericytes was reduced by 36% (31). Together, these data favor pericyte migration as an important mechanism for diabetic pericyte loss.

The fate of migrating pericytes in the diabetic retina remains uncertain, but inferential evidence from work on brain pericytes suggests that they migrate away from an injured capillary for survival (32). As noted in the traumatic brain injury model, stress-induced migration of pericytes resulted in their survival. Pericytes that stayed at their vessel location and did not migrate were prone to apoptosis, suggesting that migration of pericytes away from the capillary enables the pericyte to respond to trophic signaling molecules in the perivascular compartment. The subsequent functional adaptations may include differentiation along multiple lineages reflecting the pluripotency of this enigmatic cell population (33).

After regional hypoxia occurs in the diabetic retina as a result of vasoregression and capillary dropout, retinal neovascularization occurs in an attempt to restore oxygen delivery. Both VEGF and Ang-2 are normally induced by
hypoxia and cooperate to induce angiogenesis (34, 35). Feng et al. (36), using homozygous Ang-2–deficient mice, reported spontaneous proliferative retinopathy occurring in room air that mimicked human retinopathy of prematurity (ROP) in mice. Arteriolar patterning and the formation of the primary (superficial) and secondary (deep) capillary network were impaired due to the lack of Ang-2. Over time, the relative increase of VEGF in this model declined, rendering persistent preretal proliferations less leaky. Reduced metalloproteinase (MMP) activity, which is evident in heterozygous Ang-2–deficient mice causing a reduction in proliferative retinopathy, may be overcome by VEGF-induced MMP regulation in the complete absence of Ang-2. Thus, Ang-2 appears negligible for retinal neovascularization, but it is essential for proper vessel development, in particular of the arteriolar site, and the capillaries in the depth of the retina where diabetic retinopathy starts.

**Erythropoietin in the diabetic retina.** Erythropoietin (Epo) is another ischemia-induced growth factor that was recently identified as being important in the pathogenesis of proliferative diabetic retinopathy. Work by Takagi and colleagues (37) demonstrated that Epo is increased in eyes with proliferative diabetic retinopathy, and the experimental inhibition of Epo is as effective as that of VEGF in the ROP model. More recently, a large consortium (38) reported that promoter polymorphisms of the Epo gene are associated with proliferative diabetic retinopathy in patients, with increased promoter activity giving rise to increased Epo transcription. Critically, the effect of exogenous Epo depends on the temporal relation to the induction of vasoregression (39). In the ROP model, Epo administered prior to the induction of vasoregression (i.e., before mice went into the hyperoxic chamber) reduced vasoregression and, consequently, responsive neovascularization, while Epo, administered after vasoregression had occurred, induced increased neovascularization. The effect of Epo is mediated by a complex consisting of the Epo receptor, which is expressed throughout the entire retina, and the common chain receptor, which is expressed only in the vicinity of the superficial vascular layer, i.e., in the layer in which most neovascularizations develop. Low-dose Epo, which does not induce erythropoiesis over a period of up to 6 months, inhibited oxidative stress in the retinas of STZ-diabetic rats and reduced VEGF and Ang-2 levels (40). The formation of acellular capillaries and the loss of pericytes were also reduced in these Epo-treated diabetic animals. The formation of acellular capillaries and the loss of pericytes were reduced in treated diabetic animals. Of particular note, the increased level of leukostasis (i.e., local capillary obstruction by leukocytes [see below]) remained unaffected by Epo treatment (Q. Wang et al., unpublished data), suggesting that leukostasis does not play a major role in causing vasoregression in the diabetic retina.

**Factors modulating vasoregression**

**Inflammation.** Inflammation is an important pathogenetic aspect of diabetic retinopathy based on the findings that inflammatory cytokines and mediators are upregulated in the diabetic retina, and that leukostasis occurs because of adhesion molecule upregulation (41– 45). As a novel aspect, microglial cells transdifferentiating from bone marrow–derived cells may contribute to the propagation of inflammatory vessel damage. This area has been the subject of two recent exciting reviews (46, 47) addressing the multifaceted and important link between inflammation and diabetic retinal vasoregression.

**Leukostasis.** Since the early reports by Schmidt-Schönbein, leukostasis has evolved as a mechanism by which activated bone marrow–derived cells may damage retinal capillary endothelial cells (44, 48). It was noted that leukocytes adhered to the diabetic endothelium in all vascular beds of the retina, and some leukocytes obstruct capillaries. Several studies implied that correction of leukostasis necessarily preserved retinal capillaries from occlusion (49–51). However, as outlined above, new experimental evidence suggests that the prevention of acellular capillaries succeeds without correction of leukostasis (42, 52) (Q. Wang et al., unpublished data).

**Endothelial progenitor cells.** Another bone marrow–derived cell population that is able to interact with the endothelium in damaged tissues is the endothelial progen-
However, in contrast to leukocytes, EPCs have attracted much interest because of their potential role in vascular repair. From studies using models of proliferative retinopathy, the contribution of hematopoietic stem cells to retinal neovascularization has been proposed (53). In this context, the chemokine SDF-1 was established as an important promoter of adult retinal angiogenesis in a model of laser-induced retinal vein occlusion (54). Blocking SDF-1 activity abolished the recruitment of hematopoietic stem cell–derived endothelial precursors and local endothelial cell–driven ischemic repair, preventing preretal neovascularization.

The question of whether EPCs can support the repair of damaged endothelial cells was also addressed in experimental studies. Using rodent models in which capillary endothelial cells were damaged by different mechanisms, Caballero et al. (55) used labeled CD34+/H11001 cells from both nondiabetic and diabetic origin and found that only nondiabetic cells were able to integrate into the damaged vasculature. EPCs, which have impaired function, may be restored by improvements in mobilization, e.g., by statins, or by drugs that improve function, adding to the self-repair of damaged capillaries in the diabetic retina.

Apart from being expressed on endothelial cells, Tie-2 is expressed on bone marrow–derived monocytes/macrophages (TEM), and the interaction with local tissue Ang-2 (such as in hypoxic tumor areas) promotes angiogenesis (56). High Ang-2 in the diabetic retina may cause a recruiting signal for TEM and direct them to the retina. The relevance of this pathway and the possible role in vasoregression await clarification.

**Lipids and fatty acids.** Vasoregression in the diabetic retina may also result from hyperglycemia-induced modifications of the levels or the chemical make-up of lipids and fatty acid–based lipid mediators, as suggested by clinical and experimental studies such as the DCCT and others (57–59). Two distinct entities of modifications have been proposed: 1) posttranslational modification of lipoproteins and 2) diabetes-induced alterations in the biosynthesis of eicosanoids. Examples for modified lipoproteins as possible mediators of retinal vessel toxicity have been given by Lyons and colleagues (60). They showed that oxidized LDL is toxic for pericyte and can contribute to pericyte death. Diabetes-induced changes in proinflammatory eicosanoids are derived from arachidonic acid, and anti-inflammatory resolvins and protectins are derived from ω-3 polyunsaturated fatty acids. In the diabetic retina, elongase and desaturase profiles are substantially altered leading to a decrease in these ω-3 unsaturated fatty acids (61). As they can affect retinal gene expression with subsequent changes in cell differentiation and survival, diabetes-induced reductions in ω-3 PUFAs and increases in arachidonic acid can contribute to both vasoregression and neovascularization because of the lack in antiproliferative and proinflammatory effects. In addition to their proinflammatory effects, eicosanoids can increase Ang-2 expression either directly or via the modification of VEGF expression (62).

**FIG. 5.** Schematic illustration linking hyperglycemia-induced reactive oxygen species (ROS) overproduction with Ang-2–dependent vasoregression and combined ischemia/hypoxia-induced angiogenesis. In healthy retinal capillaries, proper pericyte coverage ensures endothelial cell survival and integrity of blood-retinal barrier by Ang-1/Tie-2 signaling. Chronic hyperglycemia induces cell damage and upregulation of Ang-2 in retinal endothelial cells and Müller cells (MC), leading to retinal pericyte detachment, migration, apoptosis, and progressive vasoregression. Occluded remnants of capillaries are no longer perfused, leading to the upregulation of survival/growth factors such as VEGF. During the later stages, which is not represented in rodent models, increased expression of hypoxia-induced VEGF and increased Ang-2 levels lead to preretinal neovascularization. HXP, hexosamine pathway; EC, endothelial cell. (A high-quality color representation of this figure is available in the online issue.)
Conclusion. The sight-threatening proliferative diabetic retinopathy familiar to clinical diabetologists is not the primary pathogenic response of the retina to chronic hyperglycemia. Rather, it is an attempted compensation for retinal hypoxia caused by loss of capillary pericytes and followed by formation of acellular, nonperfused capillaries. This vasoregression is the primary pathogenic response of the retina to chronic hyperglycemia. Central mediators of pericyte loss and acellular capillary formation include hyperglycemia-induced alterations in levels of VEGF isoforms, Ang-1 and -2; recruitment of activated macrophages, microglia, and/or leukocytes from the bone marrow; increased proinflammatory eicosanoid production; and decreased anti-inflammatory resolvins and protectins derived from ω-3 polyunsaturated fatty acids. Many of these changes are mediated by consequences of hyperglycemia-induced reactive oxygen species. Further elucidation of the mechanisms by which chronic hyperglycemia causes intraretinal vasoregression (Fig. 6) will provide new targets for pharmaceutical intervention before irreversible retinal ischemia and secondary proliferative retinopathy necessitate damaging treatments such as panretinal laser photocoagulation.

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