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The cell biological level of experimental diabetic retinopathy : a role of survival factors

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VEGF is a major contributor to retinal neovascularization. The possible participation of VEGF and its high-affinity tyrosine kinase receptors, flk-1 and flt-1, in early background diabetic retinopathy was studied in the streptozotocin-induced diabetic rat model of experimental retinopathy using in situ hybridization, blotting techniques, and immunohistochemistry. Diabetic retinopathy was assessed by quantitative morphometry of retinal digest preparations. The number of acellular capillaries increased 2.7-fold in diabetic animals with diabetes' duration of six months compared with nondiabetic controls. VEGF expression was not detectable by in situ hybridization in nondiabetic rats but was highly increased in the ganglion cell layer and in the inner and outer nuclear layers of retinas from diabetic animals. VEGF protein was extractable only from diabetic retinas, and a strong immunolabeling was detected in vascular and perivascular structures. Increased flk-1 and flt-1 mRNA levels were also found in the ganglion cell and both nuclear layers of diabetic samples only. Dot blot and Western blot analyses confirmed the increase in flk-1 mRNA and protein in diabetic retinas. Also, flk-1 immunoreactivity was associated with vascular and nonvascular structures of the inner retinas from diabetic animals. These data obtained from a rodent model in which retinal neovascularization does not occur support the concept that the VEGF/VEGF receptor system is upregulated in early diabetic retinopathy.

Pericytes provide vascular stability and control endothelial proliferation. Pericyte loss, microaneurysms, and acellular capillaries are characteristic for the diabetic retina. PDGF-B is involved in pericyte recruitment, and brain capillaries of mice with a genetic ablation of PDGF-B show pericyte loss and microaneurysms. We investigated the role of capillary coverage with pericytes in early diabetic retinopathy and the contribution to proliferative retinopathy using mice with a single functional allele of PDGF-B (PDGF-$B^{+/-}$ mice). As assessed by quantitative morphometry of retinal digest preparations, pericyte numbers in nondiabetic PDGF-$B^{+/-}$ mice were reduced by 30% compared with wild-type mice, together with a small but significant increase in acellular capillaries. Pericyte numbers were reduced by 40% in diabetic wild-type mice compared with nondiabetic wild-type controls. Pericyte numbers were decreased by 50% in diabetic PDGF-$B^{+/-}$ mice compared with nondiabetic wild-type littermates, and the incidence of acellular capillaries was increased 3.5-fold when compared with nondiabetic PDGF-$B^{+/-}$ mice. To investigate the effect of pericyte loss in the context of ongoing angiogenesis, we subjected mice to hypoxia-induced proliferative retinopathy. As a result, PDGF-$B^{+/-}$ mice developed twice as many new blood vessels as their wild-type littermates. We conclude that retinal capillary coverage with pericytes is crucial for the survival of endothelial cells, particularly under stress conditions such as diabetes. At high vascular endothelial growth factor levels, such as those in the retinopathy of prematurity model, pericyte deficiency leads to reduced inhibition of endothelial proliferation in vivo.