von Willebrand Factor and Endothelial Abnormalities in Diabetic Microangiopathy

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We briefly summarize current knowledge on *1*) the abnormalities of von Willebrand factor (vWF) as an indicator of endothelial cell (EC) dysfunction in diabetes and *2*) the modifications induced in the growth of cultured ECs by high glucose in the incubation media. A MEDLINE search (1986 through Sept. 1989) was performed to update previous relevant references on vWF and ECs in healthy and diabetic subjects. Main data in the literature and personal contributions were scrutinized. Study quality, information, and relevance to the subject were assessed. vWF is synthesized and stored mainly in ECs. Its plasma levels are increased in diabetic microangiopathy but are not influenced by circulating glucose, insulin, or growth hormone, nor do they acutely affect platelet function in diabetes. Supraphysiological concentrations of glucose inhibit the replication of cultured ECs from large vessels via different possible mechanisms but appear to stimulate pathways involved in the activation of capillary ECs. vWF is a possible marker of EC damage in diabetes, and prospective studies will ascertain its role as a predictor for the development of microangiopathy. The possible dichotomy in the response of cultured ECs from large and small vessels to high glucose in the culture media may help explain some of the lesions observed in the walls of arteries and capillaries in diabetes. *Diabetes Care* 14 (Suppl. 1):167–72, 1991

The topographical situation of endothelial cells (ECs) makes them highly likely candidates for primitive involvement in the pathogenesis of diabetic vascular complications. Exposure to blood containing abnormal levels of glucose, insulin, counterregulatory hormones, and intermediate metabolites could play a role in initiating damage of the vessel wall from its intimal layer. Although studies of light microscopy and electron microscopy have failed to demonstrate specific morphological abnormalities at the cellular and ultrastructural levels, several alterations have been observed in vivo that could be interpreted as evidence for dysregulated endothelial function. Furthermore, studies in vitro have shown that the addition of supraphysiological concentrations of glucose or sera from diabetic patients to the media may alter some properties of cultured ECs. The results of the above investigations have been reviewed (1), and this study briefly focuses on some noninvasive work on the von Willebrand factor (vWF) in humans and on experiments on the replication of ECs cultured in high-glucose media.

vWF is a complex glycoprotein synthesized by vascular endothelium (2) and megakaryocytes (3) and contained in those cells and platelet α-granules, plasma, and subendothelium (4). It is probably the most important among adhesive molecules mediating hemostatic interactions between blood and the vessel wall. Other such molecules include fibrinogen, fibronectin, vitronectin, thrombospondin, collagen, laminin, and elastin.

The gene encoding vWF is located on chromosome 12, but information on its regulation and expression is still incomplete (5). It codes for an mRNA transcript of 8900 base pairs, which in turn, codes for a precursor protein of 360,000 M_r. The latter is cleaved, dimerized, and glycosylated in the endoplasmic reticulum. Subsequently, interdimer disulfide bonds are formed in the Golgi apparatus, leading to the formation of relatively small multimers. The latter can either be secreted in the

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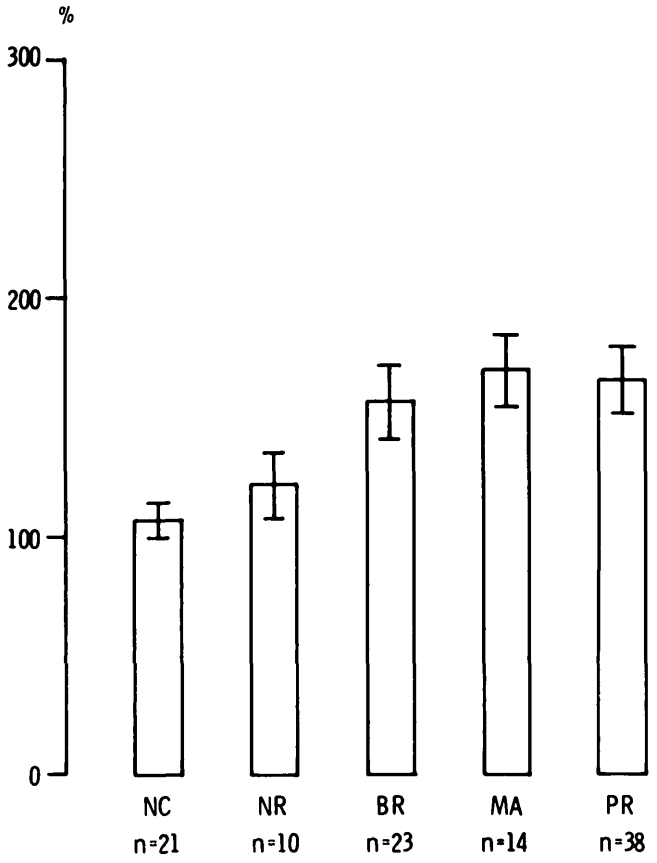

FIG. 1. Circulating levels of von Willebrand factor (expressed as percentages of values obtained testing pooled plasma from 20 healthy donors) in nondiabetic control subjects (NC) and diabetic patients with no retinopathy (NR), background retinopathy (BR; $P < 0.01$ vs. NC), maculopathy (MA; $P < 0.05$, $P < 0.001$, vs. NR and NC, respectively), and proliferative retinopathy (PR; $P < 0.01$ vs. NC). Results are means ± SE.

vessel lumen and the extracellular matrix or stored in the Weibel-Palade bodies, where they undergo further assembly with the formation of highly polymerized multimers (4). In megakaryocytes and platelets, vWF is stored in the α-granules and released with platelet activation (6). The basic subunit of circulating vWF is made up of 2050 amino acid residues and has a molecular weight of 260,000. Circulating multimers include forms from 1 to 20 × 10 M_r, the protomer being a dimer or tetramer of the above basic subunit. Multimers with the highest molecular weight are biologically more active (4,7).

Little is known about vWF turnover in health and disease. Highly polymerized multimers are released either constitutively or from the Weibel-Palade bodies in response to vasoactive or procoagulant substances or stimuli, i.e., vasopressin and some of its analogues, thrombin, and blood stasis.

vWF is necessary for the adhesion of platelets to types I and III collagen fibrils in subendothelium in conditions of high shear rate. Binding sites for collagen have been located on the vWF molecule, although the latter may interact with other constituents of the matrix as well. After contact with subendothelium, vWF is likely to undergo a steric rearrangement that permits interaction with glycoprotein (GP) Ib on the platelet membrane. Furthermore, the complex GP IIb-IIIa becomes exposed on the platelet surface and establishes a firm bond with vWF molecules in the matrix; the larger the multimers, the more effectively platelets are anchored to subendothelium (4).

Another important feature of vWF is its link with factor VIII of plasma coagulation by noncovalent bonds. This binding probably serves the purpose of stabilizing factor VIII in the circulation and providing it with a physical support at the site of platelet-subendothelial interaction, where its procoagulant activity is required.

Results obtained in our laboratory (8) and that of Coller et al. (9) have shown that the circulating levels of vWF are increased in patients with diabetic retinopathy (Fig. 1). A cross-sectional survey showed that increased plasma vWF is associated with breakdown of the blood-retinal barrier in early minimal retinopathy, suggesting that endothelial dysfunction might manifest itself in different forms once microangiopathy has become clinically detectable (10). It remains to be established whether vWF rises before the onset of retinopathy, thus being potentially involved in its pathogenesis, or merely accompanies its development. Work in vitro has shown that cultured endothelial cells grown in media supplemented with supraphysiological concentrations of glucose produce increased amounts of vWF (11). Only a longitudinal investigation could clarify the sequence of events, but none have been published so far, due to difficulties in organizing a trial that would necessarily span many years and face major technical problems. Such a study has recently been started at the Northwick Park Hospital, London, and preliminary results suggest that a rise of vWF may indeed precede the onset of retinopathy in individual patients (R. Petty, unpublished observations). If this is confirmed, then vWF might become a useful marker for identifying patients who are more likely to develop complications in the near future.

Results of oral glucose tolerance test, clamp, and 24-h studies have shown that the levels of vWF in the plasma are not influenced by changes in the plasma concentrations of factors that may be responsible for vascular damage in diabetes, e.g., blood glucose, insulin, or growth hormone (8,12). In patients with microangiopathy and high plasma vWF, more of the latter is released from vascular endothelium after appropriate stimuli, e.g., venous stasis (13) and 1-deamino-8-D-arginine vasopressin (14), suggesting that the storage pool of the glycoprotein is increased as well. There is some experimental evidence that repeated stimuli aimed at depleting the endothelial storage pool may stimulate more active synthesis of vWF in these patients than in healthy control subjects (15). vWF, both from the plasma and experimentally released from the endothelium, maintains normal electrophoretic mobility in retinopathic in-

FIG. 2. Human umbilical vein endothelial cells grown for 18 days in culture medium containing 50 mM D-glucose and stained for tubulin by indirect immunofluorescence, with rabbit primary antibody and fluorescein-conjugated goat anti-rabbit antibody. Similar images were observed when cells were grown in 5.6 mM glucose (×400).

dividuals, suggesting that multimer distribution is not altered in diabetes (13). Furthermore, the strict correlation between results of parallel assays based on immunological and biological properties of the glycoprotein suggests that nonenzymatic glycosylation, if it occurs, does not modify it functionally (8). No information is available as to whether a decrease in the clearance of vWF contributes to enhancing its levels in microangiopathy.

Finally, vWF is a cofactor of platelet function, and its increased levels might be involved in abnormal platelet adhesiveness and aggregation in diabetic patients (16). However, an acute twofold rise of plasma vWF was not found to modify platelet aggregation in vitro or in vivo, whereas platelet adhesiveness increased and then rapidly decreased to baseline values under the same experimental conditions (14,17). This suggests that antiadhesive mechanisms may be activated in the presence of high plasma vWF activity. In a cross-sectional study of insulin-dependent diabetic patients, an inverse relationship, if anything, was observed between plasma vWF and platelet sensitivity to ADP in vitro (18).

Studying the behavior of EC after the addition of extra glucose to culture media represents the natural approach to work in vitro on the pathogenesis of diabetic microangiopathy. Results obtained in our laboratory with human umbilical vein endothelial cells (HUVECs) indicate that glucose decreases the uptake of labeled thymidine through osmotic mechanisms and impairs replication via partially nonosmotic directly toxic mechanisms (19). This is in accordance with data from Lorenzi et al. (20,21) suggesting that glucose delays endothelial replication by damaging DNA and prolonging its synthesis and the G_2 phase of the cell cycle occurring between DNA synthesis and mitosis. Increased expression of mRNA for transforming growth factor-β, a known inhibitor of EC replication, has also been reported in HUVECs subjected to high glucose (22).

Observations carried out in our laboratory suggest that maintaining HUVECs in high glucose does not modify the appearance of the microtubule network but renders it colchicine resistant (23; Figs. 2–4). Because tubulin depolymerization and repolymerization provide the basis for the formation of the mitotic spindle, it is tempting to speculate that resistance to depolymerization by colchicine may reflect tubulin stabilization and that, in dividing cells, this leads to delayed mitosis. Tubulin glycosylation has been suggested to occur in peripheral

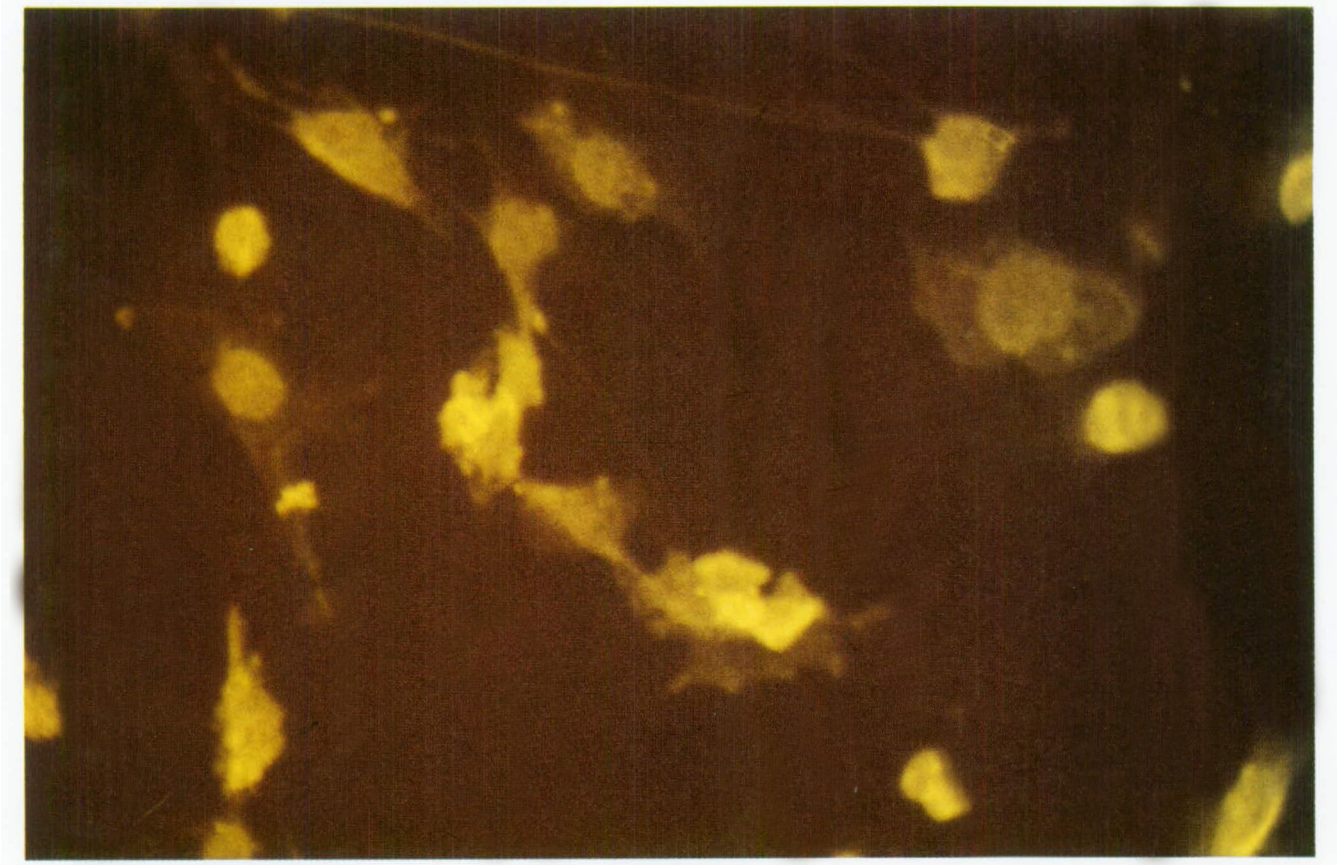

FIG. 3. Human umbilical vein endothelial cells grown for 18 days in culture medium containing 5.6 mM D-glucose, treated with 0.25 mg/ml colchicine, and stained as in Fig. 2. Cell structure and microtubular network are completely disrupted (×400).

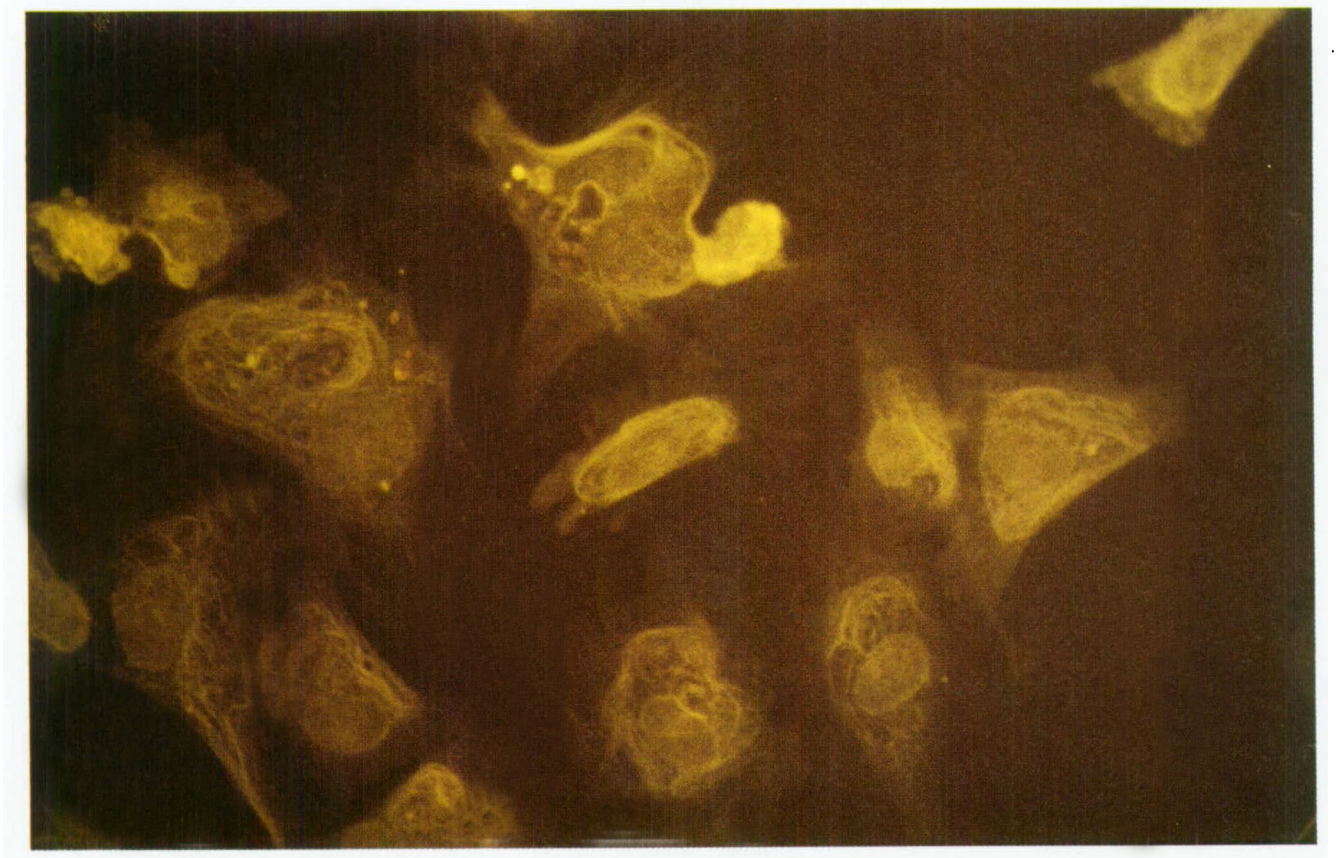

FIG. 4. Human umbilical vein endothelial cells grown for 18 days in culture medium containing 50 mM D-glucose, treated with 0.25 mg/ml colchicine, and stained as described in Fig. 2. Cell structure and microtubular network are partially preserved (×400).

nerve fibers and to play a role in the pathogenesis of diabetic neuropathy (24). Whether high glucose exerts a similar action in HUVECs and glycosylates the sites on the molecule that interact with colchicine or stabilizes tubulin through other biochemical mechanisms is being investigated in our laboratory.

Data from the Joslin Clinic group indicate that in ECs from retinal capillaries, high glucose may stimulate one of the pathways activated by some growth factors, e.g., the diacylglycerol–protein kinase C system (25). ECs from small vessels are known to differ from those of large vessels by several functional characteristics, and the above results suggest that high glucose may stimulate ECs on the capillary wall while depressing their replication on arteries and veins. Care should always be taken in comparing results from different cell culture laboratories, because the experimental conditions are far from standardized. In addition, ECs exhibit a remarkably slow rate of turnover in vivo, and this may justify some reservations on the significance of replication studies performed in vitro (27). However, the results described above may provide useful clues to our understanding of chronic processes leading to accelerated atherosclerosis of diabetic arteries on the one hand and to EC proliferation as observed in microaneurysms and new vessels in diabetic retinopathy on the other (28).

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