Diabetic Macroangiopathy and Atherosclerosis

Jens L. Andresen, Lars M. Rasmussen, and Thomas Ledet

In the present study, we have compared and analyzed published data related to the pathogenesis of the large vessel disease in diabetes. The prevailing opinion appears to be that diabetes accelerates the mechanism that leads to development of classical atherosclerosis. However, as an alternative, we have amassed data that point to the presence of a diabetic macroangiopathy. This phenomenon comprises a constellation of nonatherosclerotic large vessel abnormalities. Today, we know that accumulation of periodic acid–Schiff (PAS)–positive material, as laminin, fibronectin, and type IV collagen, occurs together with hyaluronic acid and various types of connective tissue and calcium deposition. All these changes occur independent of the presence of atherosclerosis in the large vessels of diabetic patients. It seems to us that these observations emphasize that the concept of a specific diabetic macroangiopathy is a more fruitful working hypothesis than the usual theory of a link between atherosclerosis and diabetes. It provides a causal relationship (although the mechanism is unknown) between such changes and the abnormal metabolism in diabetes and a background for research strategy and tactics, aiming finally at the possibility of prevention and/or treatment of this common and dangerous disease. *Diabetes* 45 (Suppl. 3):S91–S94, 1996

The very high frequency of heart disease among diabetic patients is generally acknowledged, and it is ascribed partly to the extensive presence of atherosclerotic plaques (1). Before 1970, the problem of coronary artery disease in subjects with diabetes was mainly relocated to the atherosclerosis research field. It was suggested that the abnormalities occur in the lipoproteins either before or after penetration of the arterial wall, where the smooth muscle cells develop the atherosclerotic plaque. Since 1970, however, we have worked on the hypothesis of a diabetic macroangiopathy that proposes a series of particular alterations to be present in the arterial wall in diabetes (2). It is important to emphasize that the concept of diabetic macroangiopathy is the development of nonatherosclerotic large vessel abnormalities. These changes may be the background that increases the susceptibility to atherogenic factors in those with diabetes.

The phrase "diabetic macroangiopathy" was introduced 25 years ago by Lundbæk (3) as a term for nonatherosclerotic changes in the larger blood vessels. It is confusing, however, that the same term (diabetic macroangiopathy) is also being used to mean a large vessel disease occurring in diabetic patients in the form of an early and severe atherosclerosis. Three important points are relevant for the understanding of a particular diabetic macroangiopathy. At first, a causal relationship may exist between the classical metabolic disorder of diabetes and the development of vessel wall damage. Second, the changes in the large vessel may be a part of the diabetic angiopathy that affects the whole vascular system. However, the abnormalities may be present as a variety of pictures in various organs. Finally, the alterations may be specific diabetic phenomena indicating that the changes in a strict sense of the word only can be seen among patients with diabetes. However, some of the abnormalities may be seen in other diseases; nevertheless, the constellation of changes in diabetes has a unique character. This is the situation with diabetic retinopathy, in which most of the individual abnormalities can be seen in other diseases. However, the constellation is so typical for diabetes that the disease can be diagnosed by ophthalmoscopy. It is noteworthy that when diabetic macroangiopathy is considered from that particular point of view, it is not a complication of diabetes. It is now an essential part of the disease and may then be regarded as a long-term manifestation of diabetes.

Most reports have dealt with the prevalence and severity of arterial disease in diabetic patients as it appears from the studies performed in Tecumseh, Framingham, and Whitehall and from the World Health Organization Multinational Study (4–7). All these studies reported an increased incidence of heart disease among diabetic patients with an almost equal affliction of men and women. Recently, two studies have demonstrated a link between diabetic nephropathy and the high incidence of cardiac disease (8,9). Most studies on diabetic patients have been designed to describe aspects of the lipid-atherosclerosis hypothesis; very few analyses have focused on the presence of a diabetic macroangiopathy. Diabetic macroangiopathy has been discussed mainly on the basis of studies of large arteries of the lower extremities and of the coronary arteries. It is clear that an elucidation of this proposed entity is much more easily arrived at by studies of either young patients in whom atherosclerosis will not blur the picture or on selected areas of the vessel wall in which atherosclerosis is not present.

In the late 1940s, Root (10) demonstrated in a study of 83 young diabetic patients (26–34 years of age) a clear-cut relationship between the duration of diabetes and the percentage of calcification. From low roentgenograms of the large vessels, a more pronounced medial calcification and lumen reduction of the metatarsal arteries was found among diabetic patients (11,12). It was disclosed that the linear type of calcification was observed to be a characteristic phenomenon in diabetes, different from the spotty lesions usually

From the Research Laboratory for Biochemical Pathology, Kommunehospitalet, Denmark.

Address correspondence and reprint requests to Dr. Thomas Ledet, Research Laboratory for Biochemical Pathology, Kommunehospitalet, 8000 Aarhus-C, Denmark.

Accepted for publication 28 September 1995.

ELISA, enzyme-linked immunosorbent assay; PAS, periodic acid–Schiff; TGF, transforming growth factor.

seen in atherosclerosis. In a later study, the calcium deposition in tunica media was statistically correlated to a decrease in the glucose tolerance in a group of NIDDM patients (13). When the angiographic technique was applied on 47 IDDM patients, a significant correlation was demonstrated between uniform narrowing of the femoral artery and the duration of diabetes (14).

Information about the structural and biochemical changes in the large vessel wall are of utmost importance for the understanding of the development of abnormal changes in the arteries. Thus, we have examined the large coronary arteries from 10 IDDM patients and 10 nondiabetic subjects who were matched with regard to sex and age, using a quantitative morphological procedure (15). Fields of tunica media with and without atherosclerosis were analyzed. The area covered by periodic acid–Schiff (PAS)–positive material was significantly larger in the vessel wall from the diabetic patients. However, the field occupied by glycosaminoglycans stained with Alcian blue was significantly reduced in tunica media compared with vessels from diabetic patients. It appeared that the histochemical changes were independent of both the presence of atherosclerosis and the location of the arterial specimens.

To characterize the PAS-positive glycoprotein in detail, our studies were expanded using a combination of morphometry and immunohistochemistry (16). The area fraction of fibronectin was obtained from tunica media of aorta from a group of NIDDM and IDDM subjects. A larger area of fibronectin was demonstrated in tunica media without atherosclerotic plaque from subjects with diabetes compared with those without diabetes.

The diabetic microangiopathy that is considered to be a specific diabetic phenomenon has, as the histopathological denominator, the presence of a PAS-positive but glycosaminoglycan-poor deposition in the vessel wall. The same phenomenon, a wide reaction for PAS-positive glycoprotein and a reduced area of the glycosaminoglycans, seems to occur in the tunica media from the larger blood vessels from subjects with diabetes. This is in agreement with our proposed concept of the presence of a diabetic macroangiopathy in the larger vessels.

Various biochemical approaches, including extraction procedures and enzyme-linked immunosorbent assay (ELISA) techniques, have been applied on tissue from the aorta. It seemed that fibronectin occurred in larger amounts in tunica media without atherosclerotic plaque from diabetic subjects compared with the nondiabetic individuals (16). Collagen type IV, a specific marker for the basement membrane, was isolated from a group of IDDM and NIDDM subjects. The results demonstrated that the quantity of type IV collagen was increased in tunica media without atherosclerosis from the diabetic individuals compared with nondiabetic subjects. In contrast, the amount of type IV collagen was the same in tunica intima. The concentration of type V collagen was unchanged comparing diabetic and nondiabetic subjects (17).

The amount of glycosaminoglycans was evaluated in tunica media with and without atherosclerotic plaque using the classical cellulose-acetate technique. The aortas were obtained from 15 diabetic patients (7 with IDDM and 8 with NIDDM). There was a relative and absolute increase in the quantity of hyaluronic acid in normal tunica media from individuals with IDDM. A significant positive correlation was found between the content of hyaluronic acid and duration of diabetes but not to age. In tunica media with atherosclerotic plaque, a significantly increased proportion of dermatan sulfate and reduced hyaluronic acid was seen in diabetic than in nondiabetic patients (18).

It has been demonstrated in clinical studies that the large vessels are more stiff in patients with than without diabetes (19) and can be correlated to the duration of diabetes (20). Increased stiffness was observed in areas without atherosclerotic plaque from subjects with diabetes, using biomechanical techniques (21).

Our data raise two important questions. First, what is the functional consequence of the changes in the extracellular matrix around the smooth muscle cells? Second, are the alterations a result of the diabetic metabolism disorder?

We have tried to elucidate part of the second question using tissue cultures of aortic smooth muscle cells. Collagen type I and type III are the main collagen types in the vessel wall. We have therefore investigated the effect of normolipemic serum from diabetic and nondiabetic individuals on the synthesis of these collagen types. The data demonstrated that serum from patients with diabetes contains one or more factors that increase the production of type I collagen from rabbit aortic smooth muscle cell culture. The synthesis of fibronectin was significantly enhanced, but the collagen type III secretion unchanged. A series of factors were consequently tested for the effect on the synthesis rate of collagen from the rabbit aortic smooth muscle cell cultures. Insulin and growth hormone both acted, but in opposite directions. Growth hormone increased the synthesis of type I collagen and fibronectin, whereas a reduction was seen after insulin addition. Glucose and ketones had no influence on the synthesis of either components (22).

The individual smooth muscle cells in the arterial wall are surrounded by a basement membrane. The basement membrane in the glomerulus is a PAS-positive structure that contains components as fibronectin, laminin, type IV collagen, entactin, and heparan sulfate proteoglycans (Perlecan) (23). The basement membrane from the cultures of arterial smooth muscle cells was isolated by a modification of the sonication differential centrifugation technique (24). This procedure has previously been used with great success for the isolation of the glomerular basement membrane from kidneys (25). We have characterized the basement membrane around the arterial smooth muscle cells with respect to protein, amino acids, and monosaccharides and glycosaminoglycans (24,26,27).

The synthesis of this arterial basement membrane was studied using normolipimic serum from a group of IDDM and NIDDM patients, as well as nondiabetic individuals (28). It turned out that serum from both types of patients with diabetes enhanced the synthesis of the basement membrane. However, growth hormone at 1 ng/ml increased the synthesis of basement membrane material, whereas the production was unaffected by glucose, insulin, glucagon, and ketones (29,30). It was shown that the increased incorporation of radioactive amino acid into the basement membrane structure could be partly ascribed to a reduced degradation. To describe the degradation in more detail, the endocytosis rate was measured after growth hormone addition. However, no influence could be demonstrated, and the same result was obtained when the activity of the lysosomal enzymes was estimated. Presently, it is not known how growth hormone

mediates its effect on the degradation of basement membrane. In a series of investigations, radioactive glucosamine and sulfate were used as precursors. When growth hormone was added at a concentration of 1 ng/ml, the incorporation rate was decreased for both substances, which indicates that growth hormone reduced the glucosamine- and sulfate-containing components in the basement membrane.

In recent years, the effect of growth hormone has been suggested to be mediated through IGF-I. However, in diabetes, the IGF-I level in serum is either normal or reduced, but the concentration around the cells is unknown. The last point may be of particular interest since many cells, including the arterial smooth muscle cells, have been shown to produce IGF-I.

We have tested the influence of IGF-I at various concentrations on the synthesis of the basement membrane from arterial smooth muscles cells. The results demonstrate that IGF-I stimulates the synthesis of basement membrane, simultaneously with an increased cell growth. However, at present, it is unclear whether the growth hormone results are due to increased synthesis of IGF-I from the smooth muscle cells or whether it is a direct effect of growth hormone.

In recent years, a number of growth factors have been discovered and among those is transforming growth factor (TGF) β. This component is synthesized by arterial smooth muscle cells, and the production increased after cell damage (31). It is also known that the collagen production is enhanced, whereas the proliferation of smooth muscle cells is reduced. The synthesis rate of basement membrane from the human arterial smooth cells was investigated after supplementation of TGF-β to the cultures. The results obtained show that basement membrane production is increased although the growth of cells is reduced (T.L., J.L.A., L.M.R., unpublished data). These data are compatible with the hypothesis that TGF-β can be involved in the development of diabetic macroangiopathy.

It has been demonstrated that normolipimic serum from either animals with experimental diabetes or diabetic subjects stimulated growth (32–34). Increased growth was not obtained with glucose concentrations corresponding to that seen in patients with diabetes (32,35). The growth effect of insulin, glucagon, and ketone bodies was investigated in the same in vitro system. The results obtained show the same effect of 10% serum and 10% serum added with either 10, 100, 200, or 2,000 μU/ml insulin. Moreover, when serum was used from patients with recent-onset diabetes with a insulin concentration of 0 or 0.6 μU/ml, higher growth was still obtained compared with serum from nondiabetic subjects (36). It seems that the growth factor in serum from diabetic patients cannot be insulin, although previous studies have arrived at the opposite result (31,37,38).

In 1970, the increased serum growth hormone level was put forward as one causal factor for the development of diabetic angiopathy (18). Consequently, we have studied the effect of various concentrations of growth hormone added to 10% serum. It seems clear that increased growth of rabbit aortic smooth muscle cells occurs with higher concentrations of human growth hormone (39). When the effect of a neutralizing growth hormone antibody was tested, it became clear that the increased growth effect of serum from diabetic patients could be almost abolished (36).

If we put all of these data together, do they then support the presence of a diabetic macroangiopathy? The results obtained by morphological and biochemical procedures show the presence of a series of changes in tunica media. These alterations seem to be independent of and different from the alterations seen in presence of atherosclerotic plaque. Moreover, some of the morphological abnormalities are similar to those seen in diabetic microangiopathy. The pathophysiological data are provided from the arterial smooth muscle cell culture studies. It is not possible, of course, to go from the in vitro situation to the prevailing condition in vivo. Nevertheless, the fact is that cell growth and a large number of the extracellular matrix components in the vessel wall can be changed. This occurs after addition of either serum from diabetic patients, hormones, or various metabolites to the culture system.

The results are compatible with the proposal that the diabetic macroangiopathy is related to metabolism in diabetes. We find the concept of a particular diabetic macroangiopathy more fruitful for generating new experiments than the idea of diabetes as one of miscellaneous risk factors in atherosclerosis.

ACKNOWLEDGMENTS

These studies were supported by The Danish Heart Association, The Danish Diabetes Association, Carl P. Petersen's Foundation, Thomas Frølunds Foundation, and the University of Aarhus.

REFERENCES

1. Robertson WB, Strong JP: Atherosclerosis in persons with hypertension and diabetes mellitus. *Lab Invest* 18:78–91, 1968
2. Ledet T, Heickendorff L, Rasmussen LM: Cellular mechanisms of diabetic large vessel disease. In *International Textbook of Diabetes Mellitus*. Alberti KGMM, DeFronzo RA, Keen H, Zimmet P, Eds. London, Wiley, 1992, p. 1436–1446
3. Lundbæk K: Blood vessel disease. *Acta Diabetol* 8 (Suppl. 1):3–4, 1971
4. Epstein FH, Ostrander LD, Johnson BC, Payne MV, Hayner NS, Keller J: Epidemiological studies of cardiovascular disease in a total community: Tecumseh, Michigan. *Ann Intern Med* 2:1170–1187, 1965
5. Kannel WB, McGee DL: Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* 2:920–926, 1979
6. Reid DD, Brett GZ, Hamilton PJS, Jarret RJ, Keen H, Rose R: Cardiorespiratory disease and diabetes among middle aged male civil servants. *Lancet* I:469–473, 1974
7. WHO Multinational Study of Vascular Disease in Diabetics: prevalence of small vessel and large vessel disease in diabetic patients from 14 centres. *Diabetologia* 28:615–640, 1985
8. Królewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR: Magnitude and determinants of coronary artery disease in juvenile-onset insulin-dependent diabetes mellitus. *Am J Cardiol* 14:55–60, 1987
9. Borch-Johnsen K, Kreiner S: Value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J* 294:1651–1654, 1987
10. Root HF: Diabetes and vascular disease in youth. *Am J Med Sci* 217:545–553, 1949
11. Ferrier TM: Radiologically demonstrable arterial calcifications in diabetes mellitus. *Aust Ann Med* 13:222–228, 1964
12. Christensen NJ: Muscle blood flow, measured by xenon^{113} and vascular calcifications in diabetics. *Acta Med Scand* 183:449–454, 1968
13. Neubauer B: A quantitative study of peripheral arterial calcification and glucose tolerance in elderly diabetics and non-diabetics. *Diabetologia* 7:409–413, 1971
14. Neubauer B, Gundersen HJG: Calcifications, narrowing, and rugosities of the leg arteries in diabetic patients. *Acta Radiol* 24:401–413, 1983
15. Dybdahl H, Ledet T: Diabetic macroangiopathy: quantitative histopathological studies of the extramural coronary arteries from type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 30:882–886, 1987
16. Rasmussen LM, Heickendorff L: Accumulation of fibronectin in aortas from diabetic patients: a quantitative immunohistochemical and biochemical study. *Lab Invest* 61:440–446, 1989
17. Rasmussen LM, Ledet T: Aortic collagen alterations in human diabetes mellitus: changes in basement membrane collagen content and in the susceptibility of total collagen to cyanogen bromide solubilisation. *Diabetologia* 36:445–453, 1993

18. Heickendorff L, Ledet T, Rasmussen LM: Glycosaminoglycans in the human aorta in diabetes mellitus: a study of tunica media from areas with and without atherosclerotic plaque. *Diabetologia* 37:286–292, 1993
19. Thordarson H, Thorgeirsson G, Helgason T: Aortic stiffness in insulin-dependent diabetics: an echocardiographic study. *Diabetic Med* 3:449–454, 1986
20. Christensen T, Neubauer B: Increased arterial wall stiffness and thickness in medium-sized arteries in patients with insulin-dependent diabetes mellitus. *Acta Radiol* 29:299–302, 1988
21. Oxlund H, Rasmussen LM, Andreassen TT, Heickendorff L: Increased aortic stiffness in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 10:748–752, 1989
22. Ledet T, Vuust J: Arterial procollagen type I, type III, and fibronectin effects of diabetic serum, glucose, insulin, ketone, and growth hormone studied on rabbit aortic myomedial cell cultures. *Diabetes* 29:964–970, 1980
23. Mohan PS, Spiro RG: Macromolecular organization of basement membranes characterization and comparison of glomerular basement membrane and lens capsule components by immunochemical and lectin affinity procedures. *J Biol Chem* 261:4325–4336, 1986
24. Heickendorff L, Ledet T: Arterial basement-membrane-like material isolated and characterized from rabbit aortic myomedial cells in culture. *Biochem J* 211:397–404, 1983
25. Spiro RG: Studies on the renal glomerular basement membrane preparation and chemical composition. *J Biol Chem* 242:1915–1922, 1967
26. Heickendorff L, Ledet T: The carbohydrate components of arterial basement-membrane-like material: studies on rabbit aortic myomedial cells in culture. *Biochem J* 211:735–741, 1983
27. Heickendorff L, Ledet T: Glycosaminoglycans of arterial basement membrane-like material from cultured rabbit aortic myomedial cells. *Biochim Biophys Acta* 798:276–282, 1984
28. Rasmussen LM, Ledet T: Serum from diabetic patients enhances synthesis of arterial basement membrane-like material in cultured smooth muscle cells. *APMIS* 96:77–83, 1988
29. Ledet T, Heickendorff L: Growth hormone effect om accumulation of arterial basement membrane-like material studied on rabbit aortic myomedial cell cultures. *Diabetologia* 28:922–927, 1985
30. Ledet T, Heickendorff L: Insulin, ketones, glucose and glucagon: effects on the arterial basement membrane in vitro. *Acta Endocrinol* 115:139–143, 1987
31. Wolf Y, Rasmussen ML, Ruoslahti E: Antibodies against TGF-β suppresses intimal hyperplasia in a rat model. *J Clin Invest* 93:1172–1178, 1994
32. Ledet T, Fischer Dzoga K, Wissler RW: Growth of rabbit aortic smooth-muscle cells cultured in media containing diabetic and hyperlipemic serum. *Diabetes* 25:207–215, 1976
33. Ledet T: Growth of rabbit aortic smooth muscle cells in serum from patients with juvenile diabetes. *Acta Pathol Microbiol Scand Sect A* 84:508–516, 1976
34. Koschinsky T, Bünting CE, Rütter R, Gries FA: Sera from type 2 (non-insulin-dependent) diabetic and healthy subjects contain different amounts of a very low molecular weight growth peptide for vascular cells. *Diabetologia* 28:223–228, 1985
35. Turner IL, Bierman EL: Effects of glucose and sorbitol on proliferation of cultured human skin fibroblasts and arterial smooth muscle cells. *Diabetes* 27:583–588, 1978
36. Ledet T: Growth hormone antiserum suppresses the growth effect of diabetic serum studies on rabbit aortic medial cell cultures. *Diabetes* 26:798–803, 1977
37. Pfeifle B, Hamann H, Fussganger R, Ditschuneit H: Insulin as a growth regulator of arterial smooth muscle cells: effect of insulin of I.G.F.I. *Diabete Metab* 13:326–330, 1987
38. Stout RW, Bierman EL, Ross R: Effect of insulin on the proliferation of cultured primate arterial smooth muscle cell. *Circ Res* 36:319–327, 1975
39. Ledet T: Growth hormone stimulating the growth of arterial medial cells in vitro absence of effect of insulin. *Diabetes* 25:1011–1017, 1976