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Rapid communication

Premature cell ageing and evolution of diabetic nephropathy

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Summary The rate of development and progression of renal disease varies greatly in insulin-dependent diabetic (IDDM) patients. The cellular and molecular reasons for this difference are largely unknown but could be related to early cell differentiation, a phenomenon recently reported in IDDM patients with nephropathy. In this study we compared cell differentiation and cell volume between IDDM patients with and without nephropathy and investigated the cell ageing characteristics in relation to the rate of evolution of renal disease in the IDDM patients with diabetic nephropathy. Cell volume was larger and the percentage of post-mitotic fibrocytes was higher in skin fibroblasts derived from IDDM patients with diabetic nephropathy compared to those from IDDM patients without kidney disease (mean ± SD in arbitrary units 817.3 ± 25.7 vs 760 ± 32.8; $p = 0.005$; and mean ± SD % 33.6 ± 11.8 vs 20.8 ± 10; $p = 0.02$ respectively). Analysis of the interaction of the time to proteinuria (TTP) and the rate of change of glomerular filtration rate (GFR) with glycaemic control, arterial blood pressure and cell volume and the state of cell differentiation showed that glycated haemoglobin and the percentage of post-mitotic fibrocytes were negatively correlated to TTP ($r = -0.68$; $p = 0.008$; $r = -0.52$; $p = 0.05$ respectively) and positively associated with the rate of change of GFR ($r = 0.76$; $p = 0.03$; $r = 0.56$; $p = 0.037$ respectively). Cell volume was negatively correlated to TTP ($r = -0.53$; $p = 0.05$). Diastolic blood pressure was also related to the rate of GFR change ($r = 0.56$; $p = 0.039$). In a multiple linear regression analysis glycated haemoglobin maintained its significant independent relationship with TTP at the 1% level, while the strength of the association between the percentage of post-mitotic cells and cell volume was reduced to the 11 and 9% level, respectively. Cultured skin fibroblasts from IDDM patients with nephropathy show signs of early differentiation. Glycaemic control is a key factor in the rate of onset of proteinuria and different rates of cell ageing appear to contribute to the rate of development and progression of diabetic nephropathy. Their interaction may be responsible for the severity of renal involvement in susceptible IDDM patients. [Diabetologia (1997) 40: 244–246]

Keywords Cell ageing, nephropathy, glycaemic control, fibroblasts, glomerular filtration rate.

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; TTP, time to proteinuria; GFR, glomerular filtration rate; PMF, post-mitotic fibrocytes.

Mesangial and interstitial expansion with excess extracellular matrix deposition are characteristic renal histological features of the subset of insulin-dependent diabetic (IDDM) patients who develop diabetic nephropathy [1, 2]. We have recently described that serially cultured skin fibroblasts from IDDM patients with nephropathy exhibit hypertrophy, delayed replication and an increased percentage of terminally differentiated post-mitotic fibrocytes (PMF) [3], cells specialized for the production of extracellular matrix components.

The rate of onset and progression of kidney disease varies greatly among affected individuals [4] and has led some authors to categorise patients as "slow or fast-track progressors" [5]. The reasons for these differences in the evolution and progression of diabetic kidney disease are poorly understood, even though a number of environmental factors, including levels of blood pressure [6], blood glucose [7], serum cholesterol [8] and age of onset of diabetes have been implicated. At the cellular level early cell differentiation could be involved [3]. In this study we have compared cell volume and the percentage of PMF in IDDM patients with and without nephropathy. In the group with kidney disease we have investigated the relation between the rate of evolution of diabetic nephropathy and cell ageing, and their interaction with established environmental factors, such as arterial blood pressure and glycaemic control.

Subjects and methods

Fifteen (9 males/6 females) consecutive IDDM patients with nephropathy, attending the Diabetic Clinic at Guy's Hospital, with a median (range) age of 41 (27–67) years and duration of diabetes of 25 (19–36) years, were studied and compared to a control group of 15 patients with long-standing IDDM with normal albumin excretion rate (AER < 20 μg/min), matched for age [42 (23–69) years] and duration of diabetes [25 (21–38) years]. Nephropathy was defined by the presence of urinary albumin excretion over 300 mg/day, concomitant retinopathy, and absence of haematuria, urinary tract infections or cardiac failure or other renal disease. All IDDM patients attending Guy's Hospital Diabetic Clinic are routinely screened for clinical albuminuria at each 3–6 month visit and diagnosed as having nephropathy when albuminuria is confirmed on two successive occasions. The time to proteinuria (TTP) was calculated by subtracting the age at onset of diabetes from the age at onset of proteinuria in years. After the onset of proteinuria renal function was regularly monitored by measurements of glomerular filtration rate (GFR) with plasma clearance of ^{51}Cr-EDTA. The median (range) follow-up period in the group with nephropathy was 6 (2–14) years and the number of GFR measurements 13 (3–23). The rate of change of GFR for each patient was calculated by linear regression analysis on all GFR measurements, taking into account the number of observations and the length of follow-up. Fibroblasts were obtained from forearm skin biopsies and experiments conducted between the 6th and 8th passage. Cell volume in arbitrary units was measured on quiescent fibroblasts by flow cytometry [3]. The percentage of terminally differentiated PMF was evaluated on sparse mass cell cultures, as previously described, and scored by one of us, H. P. R., who was unaware of the patients' clinical details [3].

Results and discussion

The two groups of diabetic patients had similar blood glucose control at the time of the study (HbA_1%: 9.4 ± 2.1 vs 8.9 ± 1.8 % in IDDM patients with vs without nephropathy, respectively). Cell volume was larger in IDDM patients with nephropathy compared to that of IDDM patients without nephropathy (Mean ± SD in arbitrary units 817.3 ± 25.7 vs 760.3 ± 32.8, $p = 0.005$) and the percentage of PMF was higher (Mean ± SD 33.6 ± 11.8 vs 20.8 ± 10 %, $p = 0.02$).

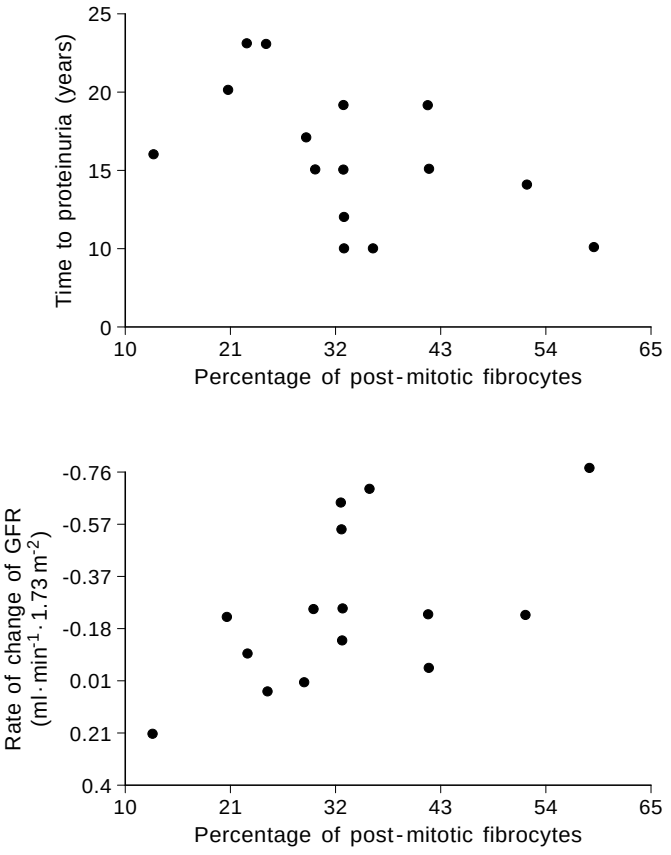

Fig. 1. Correlation between the percentage of post-mitotic fibrocytes and the time to proteinuria in years ($r = -0.52$, $p = 0.05$, upper panel) and the rate of change of glomerular filtration rate (GFR) in ml · min^{-1} · 1.73 m^{-2} ($r = 0.56$, $p = 0.037$, lower panel)

In the group with kidney disease median (range) TTP was 15 (10–23) years and rate of change of GFR was – 0.22 (– 0.76 to + 0.2) ml · min^{-1} · month $^{-1}$. At onset of proteinuria the mean ± SD HbA_1 was 10.4 ± 1.4 % and the systolic and diastolic blood pressures were 141 ± 17.7 mmHg and 86.3 ± 9.3 mmHg, respectively. In a linear regression analysis time to proteinuria was significantly and negatively associated with cell volume ($r = -0.53$; $p = 0.05$) and with the percentage of PMF ($r = -0.52$; $p = 0.05$) (Fig. 1, upper panel). The rate of change of GFR was significantly associated with the percentage of PMF ($r = 0.56$; $p = 0.037$) (Fig. 1, lower panel). Diastolic blood pressure at diagnosis of proteinuria correlated with the percentage of PMF ($r = 0.46$; $p = 0.08$) and to the rate of change of GFR ($r = 0.56$; $p = 0.039$). Glycated haemoglobin at the time of proteinuria onset was negatively correlated to the TTP ($r = -0.68$;

$p = 0.008$) and positively associated with the subsequent rate of change of GFR ($r = 0.76$; $p = 0.03$). No correlations were found between glycaemic control and cellular findings (HbA_1 vs PMF $r = 0.28$; $p = 0.876$ and vs cell volume $r = 0.12$; $p = 0.47$). Time to proteinuria correlated with the rate of change of GFR ($r = 0.633$; $p = 0.011$).

In a multiple linear regression analysis glycated haemoglobin maintained its significant independent relationship with TTP at the 1% level while the strength of the association between the percentage of post-mitotic cells and of cell volume was reduced to the 11% and 9% level, respectively. No single variable emerged as independently significantly associated at the 5% level with the rate of GFR change.

These results confirm our previous observations [3], that patients with diabetic nephropathy, as a group, show features of early cell ageing, which seem to be independent of the diabetic state. Moreover these data suggest that, in addition to the degree of hyperglycaemia, early cell differentiation is associated with a faster onset of proteinuria and a more rapid decline of renal function in IDDM patients. These abnormalities may represent the cellular background against which the development of nephropathy is more likely to occur. The degrees of glycaemic control and cell ageing, and their interaction with arterial blood pressure, are likely to account for the severity of renal involvement. In the absence of early cell differentiation, a similar exposure to diabetes, in terms of duration and intensity, is less likely to lead to the development of kidney disease. It is of interest that those patients who develop albuminuria sooner after diagnosis of diabetes also subsequently lose renal function at a faster rate. This suggests that the mechanism leading to proteinuria may be also responsible for the progression to renal failure. In the multiple linear regression analysis, the rate of change of GFR was not significantly independently associated with any of the dependent variables considered (cell volume, percentage of PMF, time to proteinuria, HbA_1 and diastolic blood pressure). However, taken together those variables explained 81.9% ($p = 0.005$) of the rate of change of GFR, suggesting that not a single factor, but the interaction of multiple factors, environmental and cellular, cooperate in determining the rate of progression of renal failure.

Early cell maturation may contribute to the development and severity of kidney involvement in diabetic patients because cells acquire specialized functions earlier in their life cycle. In the case of fibroblasts and mesangial cells this implies early production and deposition of extracellular matrix components. Excess matrix deposition leading to mesangial matrix expansion is a key event in the pathogenesis of diabetic glomerulopathy and is closely associated with albuminuria and progressive glomerular closure [9]. The assumption that abnormalities measured in the skin fibroblasts may apply to other cell types is speculative, but there are clear similarities between cell types of mesodermic origin, to which mesangial cells, renal and skin fibroblasts belong, in their function of producing extracellular matrix. Skin fibroblasts have proved useful as a model to explain cell functional abnormalities of other cell types in a variety of metabolic and genetic disorders [3, 10]. The degree of cellular abnormalities is unlikely to be secondary to the degree of renal failure in that we found no correlation between the actual level of uraemia or GFR and cellular changes [3] and have observed these cell abnormalities in patients with microalbuminuria who have normal renal function (unpublished observation).

The mechanism of the interaction between cellular abnormalities and the environmental determinants of the evolution of kidney disease in IDDM needs further investigation.

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