between BMI-SDS at diagnosis and age of diagnosis [5]. We have not shown a negative correlation in our population, despite adequate power in our cohort of white children (80% power to detect r=0.33). The diagnostic criteria used in both studies are similar. Our population is similar in age of presentation and sex ratio to that previously presented. However our lowest quartile by age was lighter (mean weight SDS 0.49 vs 0.71) and the highest quartile by age was heavier (mean weight SDS 0.37 vs 0.04) than the Middlesborough data (Table 1). Our data was collected more recently and may show that the general rise in obesity in the childhood population is most marked in adolescents. There could be socio-economic differences between the Birmingham and Middlesborough populations that account for the anthropometric differences. Our "diagnosis" weight was made at the first visit to the clinic (median 11 days after diagnosis) whereas the Middlesborough patients were measured 6 weeks after diagnosis. However both studies also looked at BMI-SDS one year after diagnosis, but we found no inverse correlation with age at diagnosis.

The fact that a relationship between age and weight at diagnosis is not found in our Asian population where it should be strengthened by greater insulin resistance is puzzling. If insulin resistance is accelerating the presentation of diabetes, one would expect Asian children to present earlier with Type 1 diabetes than white children due to greater insulin resistance [6]; this was not the case with our patients. It could be that BMI is too crude of a surrogate measure of insulin resistance. Analysing body fat composition might be a better technique, particularly when comparing ethnic groups.

In conclusion, childhood obesity is not the main influence on the age of presentation of Type 1 diabetes in our population.

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Abbreviations: BMI-SDS, body mass index standard deviation score · SDS, standard deviation score

Observations

Neutrophil antigen exposure is altered with age in relatives of patients with Type 2 diabetes

Keywords Cytoskeleton · CD11b/CD18 · Neutrophil · Type 2 diabetes · First-degree relatives

To the Editor: Neutrophil dysfunction contributes to the pathogenesis of the vascular complications of Type 2 diabetes. Adhesion of neutrophils to the vascular endothelium is mediated by surface exposure of the β_2 integrin CD11b/CD18, which is closely associated with the actin cytoskeleton [1]. We have shown that neutrophils respond to activation with phorbol ester by rapidly increasing surface expression of CD11b, followed by loss of surface CD11b in a proportion of cells [2]. Loss of surface CD11b in response to phorbol ester is associated with actin polymerisation and leads to loss of neutrophil adherence [3]. In Type 2 diabetes both the proportion of neutrophils polymerising actin and losing CD11b and the proportion of cells exocytosing primary granules (an important step in microbial killing and identified as surface exposure of the antigen CD69) is reduced [2].

Several aspects of neutrophil dysfunction in Type 2 diabetes have been attributed to the metabolic consequences of hyperglycaemia and normalisation of glycaemia has been associated with an overall improvement in neutrophil function [4]. However, it is unclear whether impaired neutrophil-cytoskeletal remodelling and abnormal trafficking of cell surface antigens in Type 2 diabetes is due to altered metabolism. Therefore, we studied the effect of phorbol ester on neutrophil actin polymerisation and antigen exposure in samples from non-diabetic first-degree relatives of patients with Type 2 diabetes to test the hypothesis that these aspects of neutrophil function are familial and unaffected by the degree of glycaemia.

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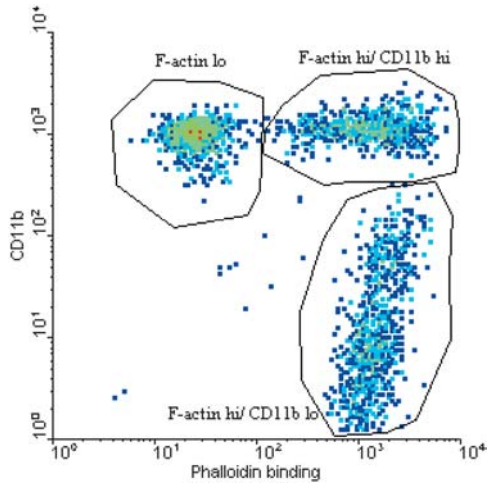

Fig. 1. Flow cytometry density plot of neutrophils incubated with 162 nmol/l PMA for 30 min and labelled with phalloidin-FITC (which binds F-actin filaments) and anti-CD11b-PE

Venous blood samples were obtained from 20 first-degree relatives (FDR) of patients with Type 2 diabetes and 17 normal control subjects (NC). All subjects gave written informed consent and ethics approval was granted by the local Joint Ethics Committee. FDR were the siblings, children or parents of patients with Type 2 diabetes, NC were recruited from the local population as well as hospital and university staff. All NC subjects had fasting venous plasma glucose concentrations of less than 6.1 mmol/l and had no personal history of diabetes, gestational diabetes, hypertension, dyslipidaemia or cardiovascular disease. Data are presented as mean ± SEM. A p value of less than 0.5 was considered statistically significant. FDR were the same age as NC (age in years: NC 36.7±3.1, FDR 33.9±2.8) but had a higher BMI and diastolic blood pressure than NC [BMI (kg/m^2) FDR 26.7±1.1, NC 22.0±0.3, p<0.01; diastolic BP (mmHg) FDR 84 (74–104), NC 73 (60–82), p<0.01] and lower HDL than NC (HDL mmol/l FDR 1.3±0.1, NC 1.6±0.1, p<0.01). There was no difference in systolic blood pressure, fasting venous plasma glucose concentrations (NC 4.9±0.1 mmol/l, FDR 5.2±0.1 mmol/l), fasting insulin, serum creatinine, total cholesterol and serum triglyceride concentrations between subject groups and all values were within normal range.

Venous blood samples from each subject were mixed with an equal volume of phosphate buffered saline (PBS) containing 5 mmol/l glucose and 5 mmol/l glutamine and incubated with 162 nmol/l phorbol 12 myristate 13 acetate (PMA) for 30 min at 37°C. Aliquots of 100 µl were then either: stained with 1 µl anti-CD69-PE and 1 µl anti-CD45-FITC on ice for 30 min in the dark, mixed with a tenfold excess of Erythrolyse (Serotec, Abingdon, UK) and washed in PBS; or stained with 1 µl anti-CD45-TRIC and 1 µl anti-CD11b-PE, fixed in 100 µl Leucoperm reagent A (Serotec, Abingdon, UK), stained with 10 µl phalloidin-FITC (which binds polymerised F-actin filaments) in 100 µl Leucoperm reagent B and washed in PBS. Cellular fluorescence was measured by flow cytometry. Neutrophils were identified from a mixed leukocyte population according to their light-scatter properties and their surface expression of CD45 as described previously [5]. Flow cytometry data were analysed using WinMDI version 2.8.

After incubation with 162 nmol/l PMA for 30 min and staining with anti-CD69-PE, two populations of neutrophil were identified with either high or low concentrations of surface CD69. When cells were stained with anti-CD11b-PE and phalloidin-FITC after PMA, three populations of neutrophil were identified (Fig. 1). The percentage of neutrophils with high surface CD69 exposure after PMA, indicating exocytosis of primary granules, was significantly reduced in samples from FDR compared to NC (% cells CD69^{hi} NC 34.5±2.0, FDR 26.1±2.0, p<0.01). Similarly, the percentage of neutrophils with high F-actin and low surface CD11b after PMA was also reduced in samples from FDR (% cells F-actin^{hi}CD11b^{lo} NC 34.8±1.8, FDR 29.3±1.6, p<0.05). These findings are consistent with our previous observations in patients with Type 2 diabetes [2].

First-degree relatives differed from normal control subjects with respect to BMI, diastolic blood pressure and HDL concentrations. To exclude metabolic differences as the cause for altered neutrophil antigen expression in FDR, neutrophil CD69 exposure was determined, after PMA activation, in samples from five control subjects with no family history of Type 2 diabetes and with an increased BMI and diastolic blood pressure. The clinical characteristics of these control subjects (NCb) were: BMI (kg/m^2) 26.9±0.7, diastolic BP (mmHg) 86 (80–90), HDL (mmol/l) 1.2±0.4. After PMA activation, the percentage of cells CD69^{hi} was significantly higher in NCb than FDR [%cells CD69^{hi} (NCb) 35.6±4.4, p<0.05]. This suggests that the abnormality observed in FDR is related to the presence of a family history of Type 2 diabetes and not to the altered metabolic state.

In FDR there was an inverse correlation between the age of subjects and the proportion of neutrophils CD69^{hi} (r=–0.64, p<0.01) and F-actin^{hi}CD11b^{lo} (r=–0.59, p<0.02). However, in NC there was no decline with age (up to 65 years) in either the percentage of cells CD69^{hi} (p=0.64) or percentage of cells F-actin^{hi}CD11b^{lo} (p=0.92) and this was significantly different to the change with age in patients (CD69^{hi} slope NC 0.12±0.16, DM –0.47±0.15, p<0.02; F-actin^{hi}CD11b^{lo} slope NC 0.18±0.11, DM –0.42±0.13, p<0.01).

These data show that altered neutrophil actin polymerisation and surface antigen exposure observed in Type 2 diabetes are also present in the non-diabetic first-degree relatives of patients. This strongly supports the hypothesis that this aspect of neutrophil dysfunction is independent of the hyperglycaemic milieu and could indicate the presence of a familial defect that pre-dates the development of Type 2 diabetes. Relatives of patients with Type 2 diabetes are at increased risk of cardiovascular disease independently of other risk factors [6] and it is possible that the basic cellular trafficking defect that leads to altered neutrophil antigen exposure contributes to this added risk.

Chronic disorders such as Type 2 diabetes have been suggested to reflect a condition of abnormal cellular ageing [7]. The present findings support this hypothesis. In the first-degree relatives of patients with Type 2 diabetes neutrophil surface antigen expression becomes more abnormal with age, whereas no association is observed in the control subjects.

In summary, neutrophil antigen exposure is altered with age in the first-degree relatives of patients with Type 2 diabetes. This aspect of neutrophil dysfunction is likely to be important in the pathogenesis of diabetic vascular complications and is familial in origin.

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Abbreviations: PMA, Phorbol 12 myristate 13 acetate · NC, normal control subjects · FDR, first-degree relatives of patients with Type 2 diabetes

SNP3 polymorphism in apo A-V gene is associated with small dense LDL particles in Type 2 diabetes

Keywords Apo A-V · SNP3 · Diabetes · LDL subfractions · Cardiovascular risk factors

To the Editor: The −1131 T>C (SNP3) polymorphism of apo A-V gene has been associated in some studies to an increase in triglycerides and in few cases to a decrease in HDL-cholesterol. Its frequency is 12% in Afro-Americans, 16% in Hispanics, 6 to 16% in Caucasians and 7% in a Spanish control cohort [1, 2, 3, 4]. The heterozygote subjects for rare SNP3 allele (C/T) are related to an increase in triglycerides in Hispanic and Caucasian men, although these data were not observed in another study where the increase in triglycerides was only statistically significant in homozygotes for the mutated allele [3]. Hypertriglyceridaemia is the main factor in determining the size of the LDL. There are many causes that influence the metabolism of triglycerides, some are primary, such as the apo E genotype, and others secondary. Type 2 diabetes mellitus is linked to different lipid disorders such as hypertriglyceridaemia, a decrease in HDL-cholesterol and an increase in small, dense LDL.

The aim of this study was to assess the frequency and the influence of SNP3 polymorphism on different lipid parameters, LPL activity and LDL subfractions in a diabetic population.

The study was approved by the Ethics Committee of our hospital. We selected 127 patients with Type 2 diabetes mellitus who had given their written consent.

Venous blood samples were taken after a 12-h fast. Cholesterol and triglyceride concentrations were measured by enzymatic techniques. LDL-cholesterol was calculated with direct measuring.

Using density gradient centrifugation according to the method described by Sanchez Quesada [5], six LDL subfractions were isolated [1, 2, 3, 4, 5, 6] from lesser to greater density: LDL1 (1.020–1.026 g/ml), LDL2 (1.026–1.030 g/ml), LDL3 (1.030–1.036 g/ml), LDL4 (1.036–1.043 g/ml), LDL5 (1.043–1.051 g/ml), LDL6 (1.051–1.056 g/ml). Plasma lipase activity was measured according to a previous study [6].

SNP 3 genotyping was done according to methodology used previously [1]. We following the Hixon's method for Apo E restriction isotyping [7].

To check the restriction technique, all samples with the mutated allele and part of those with the common allele were sequenced.

Statistical analysis was done with StatView (5.0.1 version) for Windows. Statistical significance was taken as a p value of less than 0.05. The only non-nominal variables were compared using Mann-Whitney U test. Fisher's exact test was applied to examine differences between rare and common allele groups.

We found 14 patients with the mutated allele in the SNP3 polymorphism, which gave us a mutated allele frequency of 11% in the group of 127 diabetic patients. Of the 127 patients, 16% had apo E genotype $E_3\,E_4$, 13% were $E_3\,E_2$ and 71% were $E_3\,E_3$. Among the diabetic patients with $E_3\,E_3$ genotype, there were no differences between the groups with the mutated allele and with the common one in SNP3 in the concentrations of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and apo B. The mean lipase lipoprotein activity was similar in the group of patients with the common allele and with the mutated one (230±103 pkat/ml vs 243±80 pkat/ml). The figures for the smaller, denser subfractions were higher in patients with the mutated allele in SNP3 polymorphism LDL5 (33.1±15.2 mg/dl vs 24.7±11.6 mg/dl, $p<0.05$) and LDL6 (18.9±11.0 mg/dl vs 13.2±5.8 mg/dl, $p<0.05$), whereas no differences were noted among the rest of the LDL subfractions LDL1, LDL2, LDL3 and LDL4.

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