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Changes in phagocytic function with glycaemic control in diabetic patients

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SUMMARY Phagocytic function was assessed by serial whole blood chemiluminescence in poorly controlled type 2 (non-insulin dependent) diabetic patients during efforts to improve glycaemic control and compared with a group of well controlled type 1 (insulin dependent) diabetic patients. Chemiluminescence (corrected to a standard polymorphonuclear count) remained below normal (0·15–0·30 photons/second/cell) for most of the type 2 patients until 12 weeks when the value was significantly increased in patients showing improved glycaemic control (mean (range) 0·25 (0·01–0·43) photons/second/cell) compared with those showing no improvement (0·12 (0·01–0·31) photons/second/cell). There was a significant inverse correlation of ΔHbA1 with Δ chemiluminescence. Although mean chemiluminescence for the type 1 diabetic patients was within the normal range, there was a wide scatter of values (0·19 (0·04–0·43) photons/second/cell) and there was no significant difference compared with the final value of type 2 patients with improved control.

Glycaemic control is therefore a major determinant of phagocytic function in diabetic patients, but other factors must contribute, particularly in type 1 (insulin dependent) patients.

It is commonly believed that diabetes mellitus increases the susceptibility of patients to infections, especially with yeasts, fungi, staphylococci and *Escherichia coli*.[1] This abnormal susceptibility seems to be related to cellular factors[2]: abnormalities of chemotaxis and adhesion of polymorphonuclear cells (PMN) to endothelial cells have been shown in diabetic patients,[3–5] but although impaired bactericidal activity has been shown in the PMNs of diabetic patients,[6–12] assessment of phagocytosis has given conflicting results. Poor glycaemic control has been associated with impaired leucocyte function,[13] and a few studies have shown an improvement with insulin treatment.[3 8 10]

Chemiluminescence is the measurement of light emission during phagocytosis, the response being due to reactive oxygen species—that is, superoxide—which is required for antimicrobial activity. This response can be enhanced by the use of zymosan particles.[14–16] Most studies of white cell function have involved purification of PMN from whole blood and this may well activate the cells thus increasing the extent of chemiluminescence. We therefore measured chemiluminescence of PMNs in whole blood in a group of poorly controlled type 2 (non-insulin dependent) diabetic patients. Assessment was made at four week intervals for up to 12 weeks during which time efforts were made to improve glycaemic control. In a parallel study we measured chemiluminescence in a group of well controlled type 1 (insulin dependent) diabetic patients for comparison with the final value in the type 2 patients.

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Patients and methods

Group A comprised 19 patients with non-insulin dependent diabetes (10 men, nine women) recruited from the outpatient clinic. The mean age was 54 years (range 34–71 years). One patient was newly diagnosed and managed on diet alone; 18 were known diabetic patients, mean duration of diabetes six years (range six months–20 years); 16 patients had secondary failure on oral hypoglycaemic agents—that is, symptomatic poor glycaemic control on maximum tablet dosage for at least six months following diagnosis of diabetes (nine started a once daily insulin regimen and seven a twice daily regimen). One patient started a sulphonylurea drug after failure to control diabetic symptoms while on a dietary regimen for 18 months and one patient with diabetes of five years duration, with recent compliance problems, resumed dietary

Table *Change in mean (SD) plasma glucose, glycosylated haemoglobin, phagocytic function and PMN cell count from initial value to final value at 12 weeks among patients with and without improvement in glycaemic control*

	Improvement in glycaemic control (n = 10)		*No improvement in glycaemic control (n = 9)*	
	Initial value	*Final value*	*Initial value*	*Final value*
Fasting blood glucose (mmol/l)	15·5 (3·9)	9·8 (3·5) p < 0·001	14·2 (3·4)	12·0 (2·2) p < 0·01†
HbA1 (%)	13·9 (2·2)	9·4 (1·2) p < 0·001*	12·8 (1·0)	12·0 (0·9) p < 0·001‡
Chemiluminescence (photons/second/cell)	0·06 (0·02–0·26)	0·25 (0·1–0·43) p < 0·001	0·03 (0·01–0·07)	0·12 (0·01–0·31) p < 0·02§
PMN count (× 10^9/l)	3·6 (1·0)	3·8 (1·3)	3·5 (0·7)	3·7 (0·9)

*†Initial *v* final values
‡§Final values, improvement *v* no improvement in glycaemic control

measures. Group B comprised 10 outpatients with insulin dependent diabetes (eight women, two men), who had been selected for good glycaemic control. Mean age was 26 years (range 20–34 years) and duration of diabetes eight years (range three–16 years). All patients had normal renal function (serum creatinine of < 120 μmol/l) and were receiving no medication other than treatment for diabetes. There was no clinical evidence of overt infection during the study.

Assessment of phagocytic cell function was performed on venous blood anticoagulated with heparin within one hour of venesection using an automated chemiluminescence detector (Picolite 6500, Canberra Packard, Caversham). Serum opsonised zymosan particles (ZAP, Canberra Packard, Caversham) were used as a stimulus and luminol as a scavenger substrate. The reaction mixture (in the dark) comprised whole blood (20 μl), ZAP (200 μl), and Hanks's balanced salt solution (200 μl). The reaction was initiated by the addition of ZAP and chemiluminescence was done for 40 minutes at 37°C.

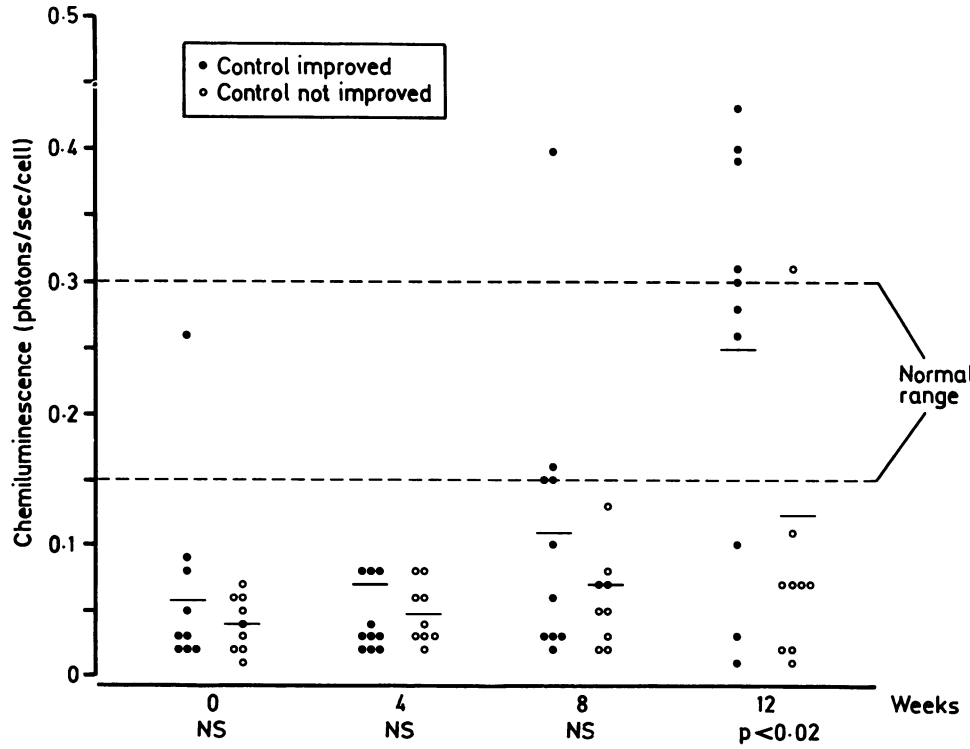

Fig 1 *Comparison of serial chemiluminescence at 0, 4, 8, and 12 weeks in diabetic patients with improved glycaemic control and those with no change in glycaemic control.*

Phagocytosis was assessed by peak output of light from PMNs (normal range 0·15–0·3 photons/second/cell). Coefficient of variation of the assay was 0·5%. An increase in chemiluminescence (△ photons/second/cell) in serial blood samples was taken as an improvement in phagocytic function. Glycosylated haemoglobin (HbA1) was measured by agar gel electrophoresis (Corning Glytrac), our laboratory normal range being 5·5–8·5%, and white cell count by Coulter S Counter (normal range for PMN count being 2·0–7·5 × 10^9/l). Chemiluminescence values were corrected to a standard PMN count of 3·5 × 10^9/l.

Statistical analysis was by the Wilcoxon rank sum test for paired and unpaired data and Spearman's rank correlation coefficient. Values were expressed as mean ± SD, and chemiluminescence, because the data were skewed, was expressed as median and range unless otherwise stated. Significance levels were taken at $p < 0·05$.

Results

Mean fasting plasma glucose for group A (n = 19) before a change in treatment was 14·9 (3·6) mmol/l, HbA1; 13·4 (1·7)% and chemiluminescence; 0·05 (0·01–0·26) photons/second/cell. Overall, glycaemic control improved in 10 patients over the study period, defined as a fall in HbA1 to within 2% of upper limit of normal (eight with insulin and two with dietary treatment, HbA1; 9·4 (1·2)%, $p < 0·001$), but showed no improvement in nine patients (eight with insulin, one with drugs), 12·0 (0·9%), NS) (table). PMN counts remained normal throughout the study (3·6 (0·9) × 10^9/l initially, to 3·7 (1·1) × 10^9/l for all patients in group A).

Only one patient in group A had normal phagocytic function initially, defined by a chemiluminescence value within the normal range (0·26 photons/second/cell). Phagocytic function, however, improved in seven out of 10 patients showing improvement in glycaemic control and failed to improve in eight out of nine patients showing no change in control—that is, chemiluminescence values remained below the normal range in the latter group in all but one patient ($p < 0·01$, Fisher's exact probability test). Serial values of chemiluminescence increased significantly between 8 and 12 weeks in the group showing improvement in phagocytosis (0·11 (0·02–0·40) *v* 0·25 (0·01–0·43), $p < 0·02$) (fig 1), and at 12 weeks in this group the value was significantly higher than the final value for the group with no improvement in phagocytosis (0·25 (0·01–0·43) *v* 0·12 (0·01–0·31) photons/second/cell, $p < 0·02$). Typical serial values of HbA1 and chemiluminescence for two sample patients are shown in fig 2.

Mean HbA1 in group B insulin dependent diabetes mellitus was within the non-diabetic range (8·3 (0·9)%) and chemiluminescence 0·19 (0·04–0·43) photons/second/cell (fig 3) was not significantly different from the final value of the type 2 patients with improved glycaemic control. PMN count was 5·2 (1·2) × 10^9/l.

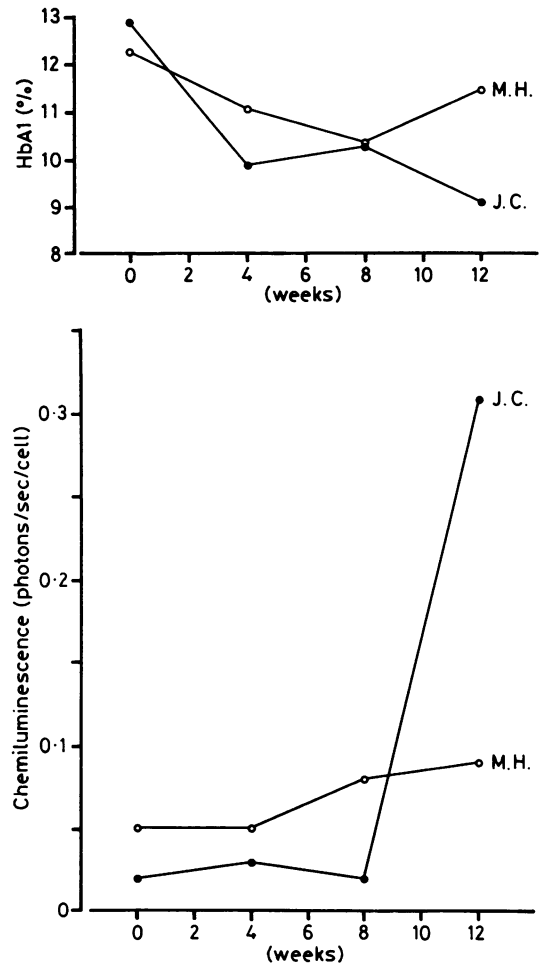

Fig 2 *Serial HbA1 and chemiluminescence for a sample patient whose glycaemic control failed to improve (M.H.) and a patient with improved glycaemic control (J.C.).*

There was a significant correlation of change in chemiluminescence with change in HbA1 ($r = -0·39$, $p < 0·05$) in the non-insulin dependent diabetes mellitus group. No significant correlations of chemiluminescence with fasting glucose, HbA1, or PMN counts were found in the study population.

Discussion

Phagocytosis depends on the ability of the white cell to

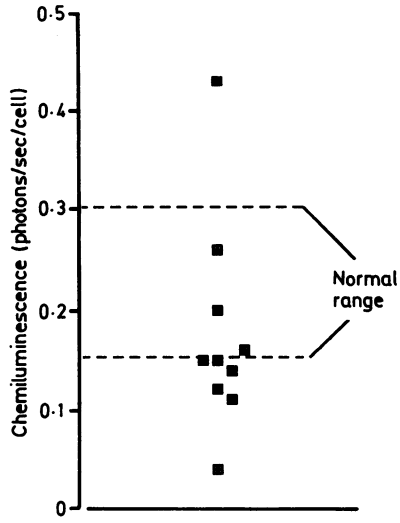

Fig 3 *Chemiluminescence in type 1 insulin dependent patients.*

deform and adapt to engulf particulate material for intracellular killing. It is possible to measure phagocytosis in PMNs by incubation techniques—that is, estimating the percentage of bacteria left extracellularly after a set period of time. Using such methods, Bybee and Rogers first showed that phagocytosis was similar in PMNs from diabetic and non-diabetic subjects unless ketoacidosis was present, this being associated with reduced phagocytic function.[17] Since then there has been a study of phagocytic cell function in diabetic children which found no difference when a comparison was made with non-diabetic control subjects,[9] and a similar study of type 1 adult diabetic patients also found no difference when compared with healthy controls.[12]

From our findings and those of others, it seems likely that glycaemic control can affect the ability of the white cell to phagocytose bacteria.[13] Exposing normal PMNs to increasing concentrations of glucose has produced conflicting results, with some studies showing reduced phagocytosis[12 18] and others showing no change.[19 20] Several incubation studies, mainly cross-sectional, in diabetic patients have related impaired phagocytic function to poor glycaemic control.[6 10] In one longitudinal study an increase in phagocytosis was found after two to three weeks of improved control,[8] and using an artificial pancreas seems to improve phagocytic function.[10] Using similar methods other studies have found reduced phagocytosis in diabetic patients but have failed to find an association with metabolic control.[7 18]

Intracellular killing of organisms is mediated by a variety of antimicrobial agents—for example, free radicals such as superoxide, singlet oxygen, and hydroxyl radicals. Superoxide is also an intermediate in the formation of hydrogen peroxide, which, along with myeloperoxidase and a halide, forms a powerful antimicrobial system. The oxidase enzyme that is responsible for superoxide generation is found in the cell membrane and oxidative metabolism is stimulated here by particle contact and phagocytosis.[21] Superoxide is a substrate for the enzyme superoxide dismutase and this enzyme will also increase as superoxide concentrations increase.

Chemiluminescence therefore reflects the respiratory burst of energy following particle contact at the cell surface. Shah *et al* found that in the resting state there was no difference in chemiluminescence between PMNs from diabetic and non-diabetic patients, but when stimulated with zymosan the response was reduced in the diabetic patients.[22] Wilson *et al,* using Candida cells as a stimulus and chemiluminescence as a measure of intracellular killing, found that killing was impaired by high glucose concentrations. They postulated that excess glucose was being metabolised via the polyol pathway to sorbitol with NADPH used as an energy source, thus depriving the membrane oxidase of energy and reducing superoxide production.[11]

Using zymosan and chemiluminescence enhanced by luminol, we have confirmed that phagocytosis in polymorphonuclear cells from whole blood is reduced in diabetic patients compared with control values. Although we found no correlation with blood glucose or HbA1 concentrations, this seems to be related to glycaemic control as values returned to normal range in those patients whose metabolic control improved during the 12 weeks studied. Interestingly, in the patients with type 1 diabetes, although they all had HBA1 concentrations within the non-diabetic range and the mean chemiluminescence value was within the normal range, most of the individual values were at the lower limit of normal with some being below normal. This would suggest that some other factor may affect phagocytic function in insulin dependent patients. In a recent study Tater *et al* found that although there was no difference in phagocytosis between type 1 diabetic patients and non-diabetic controls, phagocytosis was reduced in the non-diabetic cells when incubated with diabetic sera. They also found a reduced number of Fc receptors on the PMNs in the diabetic group.[12]

In summary, using whole blood to prevent activation of polymorphonuclear cells, we have found reduced phagocytosis in poorly controlled diabetic patients. In a longitudinal study we have shown that this can be corrected with improved glycaemic control in type 2 patients, and although phagocytic function is related to glycaemic control in type 1 diabetic patients,

other factors may be involved. The exact mechanisms affecting phagocytic function remain unclear but are probably related to changes in the white cell membrane, and further studies to examine receptors for binding organisms and microviscosity of the membrane would be of interest. Nevertheless, it remains important to maintain good glycaemic control in all diabetic patients to reduce the incidence of infection.

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