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Short Communication

Altered rheology of lymphocytes in the diabetic mouse

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Abstract

Aims/hypothesis. Clinical complications associated with diabetes may be related to altered physical properties of leucocytes. We used micropipette techniques to examine leucocyte rheology (specifically lymphocyte rheology) in the non-obese diabetic (NOD) mouse model of diabetes mellitus. We hypothesised that diabetes affects lymphocyte rheology, and specifically that lymphocyte membranes from diabetic mammals have a higher cortical tension than those from non-diabetic mammals.
Methods. Lymphocytes were isolated from diabetic and control mice. Lymphocyte deformation and activation were assessed with a micropipette apparatus. Cellular activation was assessed visually. Projection length into the micropipette during aspiration was used to calculate the viscosity of the cell. Recovery length following expulsion from the micropipette was used to derive the recovery time constant, which is the ratio of cortical tension : viscosity (T_o/μ) for each cell. The cell cortical/surface tension was calculated from this ratio.
Results. Of 692 control lymphocytes, 29% were spontaneously activated compared with 39% of 624 diabetic cells ($p<0.06$) and 31.5% of 315 non-diabetic NOD cells ($p=0.14$). Viscosity values for diabetic lymphocytes were equivalent to those for control cells (1345.12±1420.97 Pa.s vs 996.84±585.07 Pa.s, $p=0.13$). The average T_o/μ value for diabetic lymphocytes ($35.4\pm16.5\times10^{-6}$ cm/s) was significantly higher than that for control cells ($24.8\pm11.3\times10^{-6}$ cm/s, $p<0.03$) and cells from non-diabetic NOD mice ($26.3\pm9.0\times10^{-6}$ cm/s, $p<0.005$). The mean cortical tension values for diabetic and control cells were $4.7\pm2.3\times10^{-4}$ N/m and $2.8\pm0.7\times10^{-4}$ N/m respectively ($p<0.003$).
Conclusions/interpretation. Lymphocytes from diabetic mice tend to spontaneously activate. They have an equivalent cytoplasmic viscosity but a larger recovery time constant compared with cells from control mice. The results suggest that diabetic lymphocytes are stiffer than control cells.

Keywords Cell activation · Cell deformability · Cortical tension · Diabetes · Lymphocyte · Mouse model · Rheology

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Abbreviations: NOD, non-obese diabetic

Introduction

The physical properties of leucocytes determine their ability to deform, flow through capillaries and transmigrate. On activation, changes in the mechanical and adhesive properties of leucocytes occur, which affect leucocyte interaction with the endothelium [1]. Though outnumbered by erythrocytes, leucocytes strongly influence microvascular blood flow by virtue of their large volume and high rigidity. Evidence supports the theory that the vascular complications of diabetes are associated with altered rheological properties of leucocytes [2].

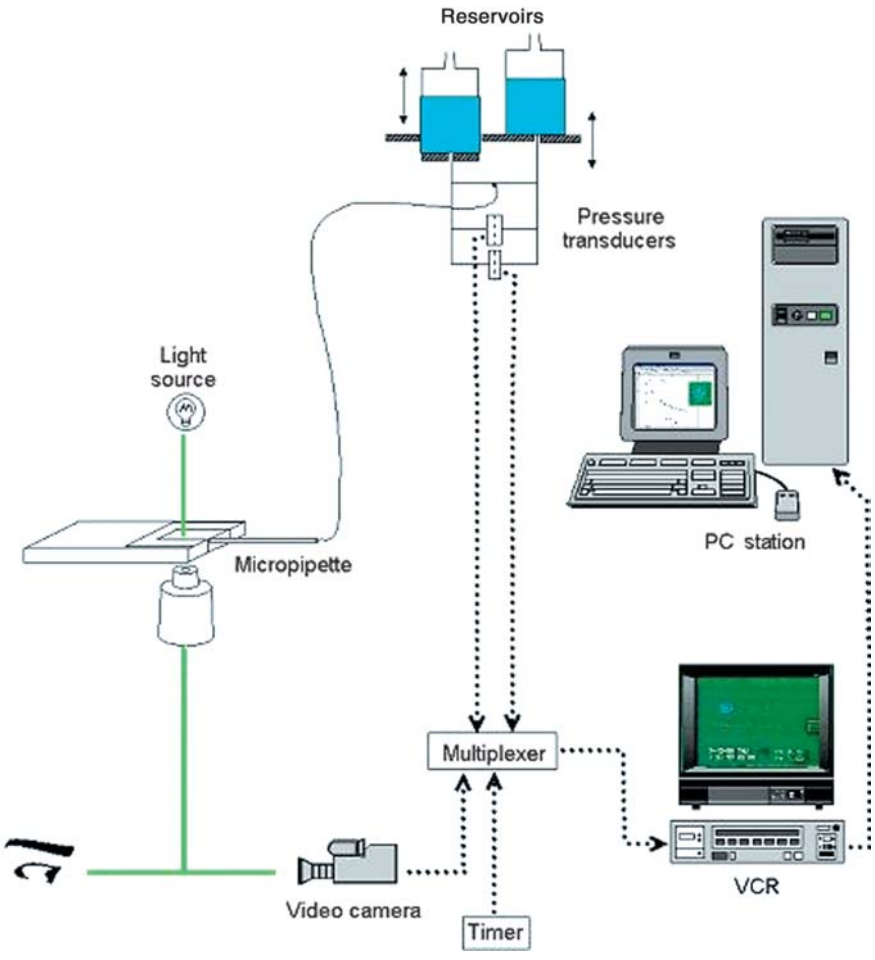

Fig. 1. Schematic representation of the micropipette apparatus

Understanding the role of altered leucocyte rheology in diabetic clinical complications requires quantitative studies of the physical characteristics of leucocytes. The majority of haemorheological studies in diabetes have been carried out using filtration techniques [3]. Because filtration techniques poorly differentiate between cells that are less deformable and those that are more adhesive to the filter pores, it is difficult to separate these effects. The micropipette technique allows the identification of activated cells and measurement of individual leucocyte deformability.

We examined diabetes-related alterations in leucocyte rheology in a specific cell population of the non-obese diabetic (NOD) mouse. We hypothesised that diabetes affects lymphocyte rheology; specifically, lymphocyte cell membranes from diabetic mammals have a higher cortical tension (i.e. they are stiffer) than those from non-diabetic mammals.

Materials and methods

Animal procedures

NIH guidelines ("Guide for the Care and Use of Laboratory Animals", revised 1996) were followed. Mice were obtained from the Jackson Laboratory (Bar Harbor, Me., USA) or from the Specific Pathogen-Free Mouse Colony, University of Florida. Diabetes was assessed by measuring glucosuria using reagent strips (Diastix; Bayer, Leverkusen, Germany). Three groups of mice were identified: (i) 18 NOD mice with clinical diabetes (positive glucosuria); (ii) seven NOD mice without evidence of diabetes (no glucosuria); and (iii) 11 B6x129 mice. Under isoflurane general anaesthesia, blood was collected from each mouse by cardiac puncture. The blood was then transferred into a 1.5-ml tube coated with 50 µl of heparin (1000 U/ml). Heparin is commonly used in rheological studies and has not been reported to increase cell activation.

Cell preparation

Lymphocytes were isolated and prepared as described previously [4, 5]. Cells were suspended in Hanks' Balanced Salt Solution. Procedures were carried out at room temperature and measurements were made within 4 h of blood collection.

Experimental system

The overall set-up consisted of a micropipette [5], a chamber on an inverted interference contrast microscope, two water reservoirs connected for micropipette hydrostatic pressure control and a video system (Fig. 1). Micropipettes were constructed from glass capillary tubes with an outer diameter of 1 mm and an inner diameter of 0.5 mm (A-M Systems, Everett, Wash., USA). The micropipettes used had an average inner diameter of 5.4 µm. Resting lymphocytes had diameters ranging from 5 to

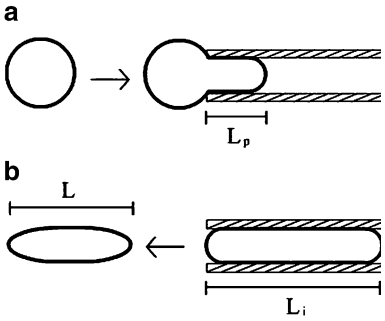

Fig. 2. Schematic representation of the rheological experiments. **a.** Aspiration experiments. For a given aspiration pressure, the length of the aspirated cell (Lp) is tracked as a function of time. **b.** Recovery experiments. Lymphocytes are drawn into a micropipette and held there for approximately 15 s before being expelled. The length of the cell (L) as it recovers its spherical shape is tracked as a function of time. L_i, initial deformed length of the cell

17 μm. Prior to use, micropipettes were flushed with 50 μl of autologous plasma to prevent adhesion to the glass surface.

Only passive lymphocytes (resting spherical lymphocytes without pseudopodia) were chosen for aspiration; these are non-adherent.

Assessment of cell activation

Cells were scanned for evidence of activation after 1 h in the chamber. Cells with pseudopodia were classified as spontaneously activated. Because activated cells are extremely rigid, only passive cells were used for rheological measurements.

Rheological measurements

Aspiration. Passive lymphocytes were aspirated at a constant pressure of 700 Pa (7 cm H_2O) into a micropipette. The length of the aspirated cell (L_p; Fig. 2a) was measured over time to generate an aspiration curve. Viscosity values (μ) were derived from the slope (dL_p/dt) of the aspiration curves using the following equation [6]:

$$\mu = \frac{(\Delta P)R_p}{(dL_p/dt)m\left(1-\frac{1}{\bar{R}}\right)} \quad (1)$$

where ΔP is the aspiration pressure, R_p is radius of the pipette, R is the radius of the cell outside the pipette, $\bar{R} = R/R_p$ and m=6.

Recovery. Lymphocytes were drawn into a micropipette and held there for about 15 s before being expelled. The length of the cell (L) as it recovered its spherical shape (Fig. 2b) was recorded as a function of time (t) and is described by the following equation [7]:

$$\frac{L}{D_o} = \frac{L_i}{D_o} + A\bar{t} + B(\bar{t})^2 + C(\bar{t})^3 \quad (2)$$

where A, B and C are known functions of (L_i/D_o). L_i and D_o are the initial deformed length and resting diameter of the cell respectively. The variable $\bar{t} = 2t/[(\mu/T_o)D_o]$ represents a dimensionless time, where μ is the cell viscosity and T_o is the cortical tension.

Statistical analysis

Results are presented as means ± SD. The chi square test was used to compare the proportion of activated cells in the three groups of mice. The two-tailed Student's *t* test was used to compare the mean values obtained in the recovery and aspiration experiments. A *p* value of less than 0.06 was considered statistically significant.

Results

Activation. Lymphocytes from diabetic NOD mice, NOD mice without evidence of diabetes and B6x129 mice were studied. Visual assessment revealed that 39±13.2% of 624 cells from diabetic mice, 31.5±5.3% of 315 cells from non-diabetic NOD mice and 29±5.4% of 692 cells from B6x129 mice were spontaneously activated. The results of the two-tailed Student's *t* test indicated that cells from B6x129 mice are essentially different to those from diabetic NOD mice ($p<0.06$) but are similar to those from non-diabetic NOD mice ($p=0.14$).

Aspiration. Aspiration experiments were performed on 30 cells from four diabetic NOD mice and 30 cells from three B6x129 control mice. Figure 3a shows a typical aspiration curve and images of a lymphocyte flowing into a micropipette. Viscosities derived from the slope of the aspiration curves were similar for the diabetic and control cells (1345.12±1420.97 Pa.s vs 996.84±585.07 Pa.s respectively, $p=0.13$).

Recovery. Recovery experiments were performed on 86 cells from 15 mice (41 cells from diabetic NOD mice, 32 cells from B6x129 control mice and 13 from non-diabetic NOD mice). Figure 3b shows data from a representative experiment and indicates that the cell recovers as a nearly Newtonian liquid drop, it also shows a lymphocyte recovering its spherical shape after being deformed in the micropipette. A recovery ratio (T_o/μ), also known as the recovery time constant, was derived from the graph generated by each cell. The average T_o/μ value for diabetic lymphocytes (35.4±16.5×10^{-6} cm/s) was significantly higher than that for control lymphocytes (24.8±11.3×10^{-6} cm/s, $p<0.003$) and non-diabetic NOD cells (26.3±9.0×10^{-6} cm/s, $p<0.005$).

Based on the results of the aspiration and recovery measurements, the cortical tension of diabetic and control cells is in the order of 4.7±2.3×10^{-4} N/m and 2.8±0.7×10^{-4} N/m ($p<0.003$) respectively.

Discussion

To control for extraneous factors that might affect leucocyte characteristics, we studied lymphocyte rheology in a murine model of human diabetes. We found that the diabetic mice possessed a greater proportion

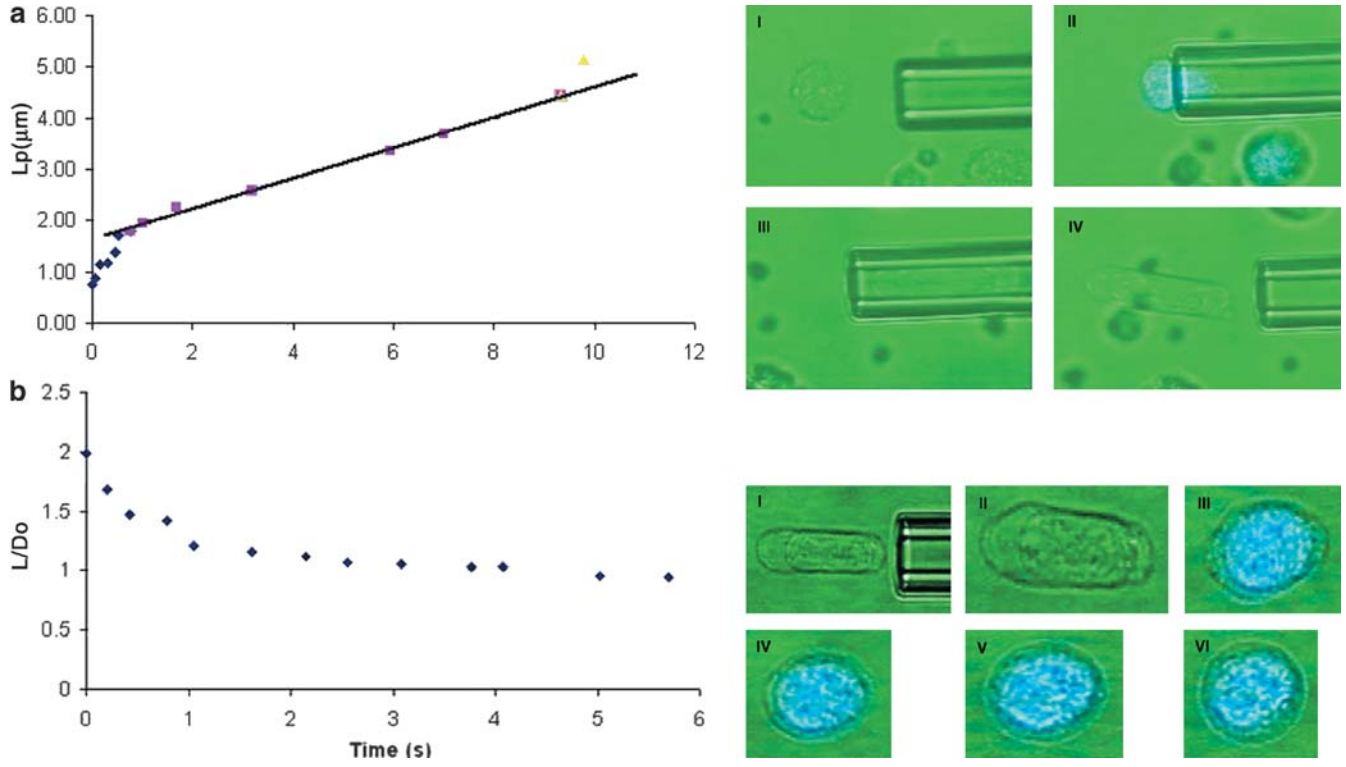

Fig. 3 a. The graph shows a typical aspiration curve. Next to it are images of a lymphocyte flowing into a micropipette. For a given aspiration pressure, the cell viscosity is determined from the slope of the line and Equation (1) (see Materials and methods section). The four images show the aspiration and expulsion of a lymphocyte (×100 oil-immersion objective lens). A 10-μm lymphocyte and its nucleus (**i**) are aspirated into a micropipette with a diameter of 5 μm (**ii, iii**) and then expelled (**iv**). L_p, length of the aspirated cell. **b.** The graph shows data from a representative recovery experiment and indicates that the cell nearly recovers as a Newtonian liquid drop. The images show a lymphocyte recovering its spherical shape after being deformed in the micropipette. Using Equation (2) (see Materials and methods section), a curve fitting to the data provides the time constant, T_o/μ. The six images (**i–vi**) show consecutive stages of the recovery experiment (×100 oil-immersion objective lens). After being aspirated inside a 5-μm diameter pipette, the lymphocyte is expelled. As the cell recovers its initial, un-deformed shape, its length is tracked as a function of time. D_o, cell resting diameter; L, cell length

of spontaneously activated cells than the control mice. These findings correlate with the report that untreated diabetic children possess a high percentage of activated neutrophils [8]. In accordance with previous reports, activated leucocytes exhibited decreased deformability and an increased tendency toward adhesion in the present study [9].

Lymphocyte viscosity was similar in the control and diabetic mice. Because the difference in viscosity was negligible whereas the difference in the T_o/μ ratio was large, cortical tension was significantly higher in diabetic cells than in control cells. This finding suggests that diabetic lymphocytes are stiffer and less deformable than control cells. Our results are consistent with previous reports that diabetic neutrophils have shorter tongue lengths (portion of cell inside the pipette during aspiration) [8]. It is important to note that only passive lymphocytes were examined in the present study. Diabetic mice were found to possess a larger proportion of activated lymphocytes, which are rigid and poorly deformable. As microcirculatory blood flow is influenced by the rheological properties of the entire population of lymphocytes (passive and active) [10], our study underestimates the overall difference in rheological properties between diabetic and control lymphocytes.

While our results provide strong evidence that hyperglycaemia alters lymphocyte rheology, additional work is required to evaluate the cortical tension of diabetic lymphocytes and to assess the effect of the presence of a large number of activated cells. Also, it should be noted that existing theoretical models of the leucocyte cell membrane cannot completely characterise cell rheological behaviour, but they can provide qualitative values for insightful comparison [5].

In summary, lymphocytes from diabetic mice tend to spontaneously activate. Diabetic lymphocytes have an equivalent cytoplasmic viscosity, but are stiffer and less deformable compared with control lymphocytes. Elucidation of the mechanisms responsible for these rheological differences may reveal novel preventative and therapeutic strategies for diabetes.

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