Altered Red and White Blood Cell Rheology in Type II Diabetes

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SUMMARY

Twenty-three patients suffering from type II, non-insulin-dependent diabetes were compared with matched controls. Suspensions with standardized white and red cell counts were filtered in a novel device capable of discriminating filter occlusion and cell transit time. Results confirm previous studies indicating that red cell deformability is impaired in diabetes. According to our findings, this may be caused by a slight overall loss of red cell fluidity together with the existence of a subpopulation of more markedly rigid erythrocytes. Furthermore, we demonstrate that white cell filterability is reduced in type II diabetes. This could be due to decreased white cell deformability, increased white cell adhesion, or both. Analysis of diabetic subgroups indicates that the white cell rheology is impaired to a greater extent in patients taking oral antidiabetic drugs than in patients controlled by diet alone. Altered white cell rheology could help to explain the pathological blood cell filterability frequently reported in diabetes. More important, impaired white cell rheology might significantly contribute to microcirculatory flow abnormalities jeopardizing O_2 exchange in the terminal vascular bed. DIABETES 1986; 35:1412–15.

The microcirculatory involvement in long-standing diabetes is well recognized.[1] Blindness due to retinopathy is 25 times more frequent and nephropathy is 17 times more common in diabetics than in nondiabetics. Largely because of the vascular sequelae, the average life span of a diabetic patient is reduced by ~35%.[2] Despite our growing knowledge, there is still uncertainty about several aspects of the pathogenesis of diabetic microangiopathy. Many authors have emphasized that in addition to a vascular component, hemorheological disturbances can play a role in the impairment of diabetic microvascular flow.[3,4] Recently, hemorheological research has focused on the diabetic red cell, and results have been conflicting. Leukocytes can also be demonstrated to affect blood flow in the supply vessels. There are no published data on the rheological properties of diabetic leukocytes. Therefore, the aim of this study was to investigate possible deterioration of white cell rheology in diabetes. This was achieved by employing an innovative filtration method capable of discriminating between the two essential components in blood filtration, i.e., cell transit time and filter-pore occlusion.

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SUBJECTS AND METHODS

Subjects. Twenty-three ambulatory, type II, non-insulin-dependent diabetics were randomly selected (12 women, 11 men). The mean age was 54 yr (range, 42–67), the average history of diabetes was 14 yr (range, 3–31), the mean fasting blood glucose was 231 mg/dl (range, 132–274), the mean body weight was 124% of ideal, and there were concomitant cardiovascular risk factors in 15 cases (9 with hypertension, 8 cigarette smokers, and 2 with hyperlipoproteinemia), 2 cases of retinopathy, 1 case of nephropathy, 2 cases with clinical signs of neuropathy, and 1 case with a history of heart infarction. Exclusion criteria were clinically overt cerebral or peripheral vascular disease, brain or heart infarction within the past 12 mo, insulin dependency, ketoacidosis, grossly insufficient metabolic control [fasting blood glucose >300 mg/dl (16.6 mM)] and medication with drugs with known hemorheological effects. Two subgroups were formed: one only under dietary control, the other on oral biguanides, sulfonylureas, or both. The control group consisted of 23 nondiabetics (11 women, 12 men). The mean age was 51 yr (range, 34–69), the mean body weight was 121% of ideal, and there were concomitant cardiovascular risk factors in 16 cases (9 with hypertension, 8 cigarette smokers, and 3 with hyperlipoproteinemia). Three controls had histories of stroke and 4 had histories of myocardial infarction (both >12 mo ago). Exclusion criteria were identical with those of diabetics.

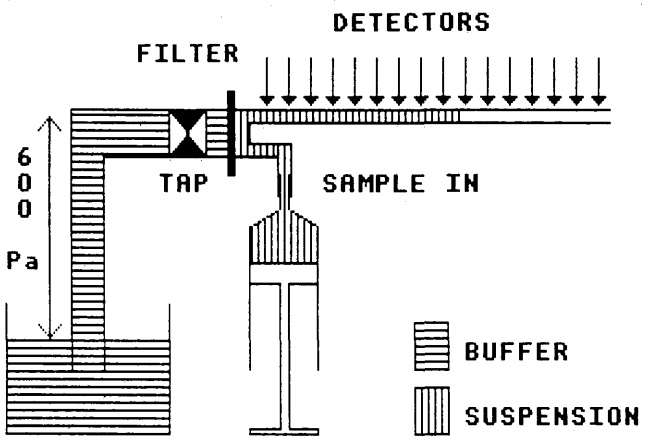

FIG. 1. Schematic drawing of filtration apparatus. Tubing to *left* of filter is filled with buffer. Pressure head can be adjusted by controlling liquid level in dish. Tubing to *right* of filter contains suspension, added from syringe, to be filtered. Filtration is started by opening tap. Filtration rate is measured by meniscus passing 39 photodiodes along horizontal tube (*arrows*).

Methods. Venous blood was drawn after an overnight fast with minimal occlusion or suction under standardized conditions[5] and anticoagulated with 1.5 mg EDTA/ml. All measurements were concluded within 1 h of venipuncture.

Preparation of cell suspensions. Two milliliters of whole blood were used for platelet, erythrocyte, and leukocyte counts (TOA cell counter). Eight milliliters were centrifuged (15 min, 3500 × *g*), and the buffy coat was carefully removed. Two milliliters of concentrated red cells were aspirated from the middle of the red cell column, removing at least 98% of the leukocytes and 97% of the platelets.[6] Blood cell count in this red cell mass was determined as above—the leukocyte count was invariably <20/μl (Buerker chamber).

Red cell suspensions (RCS) containing 10^6 red cells/μl and few leukocytes (<30/μl) or platelets (<4 × 10^3/μl) were prepared by adding 4500 × 10^6 red cells from the buffy coat–depleted red cell mass to 4 ml phosphate-buffered Dulbecco saline solution. Red and white cell suspensions (RWCS) containing 10^6 red cells/μl, 400 leukocytes/μl, and few platelets (<20 × 10^3/μl) were composed by adding whole blood and packed red cells into the buffer. The required volumes were computed individually for each sample according to the cell counts in whole blood and concentrated red cell mass. The amount of trapped plasma in the red cell mass was assumed to be 0.1 vol fraction in each case. Three RCSs and three RWCSs were prepared from each patient's blood sample. Coefficients of variation based on three measurements of identically reconstituted RCSs or RWCSs averaged 2% for red cell, 3% for leukocyte, and 3% for platelet counts.

To verify the effect of platelets in the filtration tests, platelet-rich plasma was added to the RCS. Red cell and platelet suspensions corresponding to concentration in RWCS but without leukocytes were filtered in five controls and five diabetics and compared with results obtained with platelet-poor suspensions.

Filtration. The filtration apparatus has been described elsewhere,[7] and its accuracy has been verified recently.[8] Cell suspensions were filtered through a vertical polycarbonate membrane filter (Nuclepore, Pleasanton, CA; batch no. 54P4B10; nominal pore diam, 5 μm; filter diam, 13 mm; effective filtration area, 0.78 cm^2). The filtration rate was measured by optical detection of the retreating meniscus at 39 sites in a horizontal glass tube (Fig. 1). The filtered volume between each detector was 23 μl. The pressure head, chosen to yield sufficient flow in this system, was 600 Pa. Filter-to-filter variation of hydrodynamic conductivity was ±7%.

The initial filtration rate (F_0) of the cell suspensions was quantified by extrapolating the progressively decreasing filtration rate to time zero (Fig. 2). Red cell deformability was expressed as incremental red cell volume (IRCV): IRCV = $[F_m/(F_0 - 1)]$/RCC, where F_m is the initial filtration rate of the suspending medium and RCC is the red cell count in the suspension. IRCV depends on red cell transit time only. It reflects the volume of suspending medium hindered in flow because of the passage of an average red cell through an average pore, i.e., the drop of integral flow during the passage time of a red cell.[9] The effect of variations in red cell volume and the error inherent in measuring low hematocrits are eliminated by this method. Filter clogging rate was determined from the decrease of filter conductance during the filtration of the first 0.25 ml (Fig. 2). The concentration of particles initially plugging the pores of the filter (CP, 1/ml) was calculated as CP = $N_p \times 4(F_0 - F_{0.25})/F_0$, where N_p is the estimated initial number of free pores (3 × 10^5) and $F_{0.25}$ is the filtration rate of the suspension after filtering 0.25 ml (Fig. 2). Therefore, $(F_0 - F_{0.25})/F_0$ is the relative decrease of filtration rate due to clogging when 0.25 ml of suspension has been filtered. The CP calculation describes a rate process.

Three RCSs and three RWCSs of each sample were filtered in duplicate within 10 min after resuspending the cells in

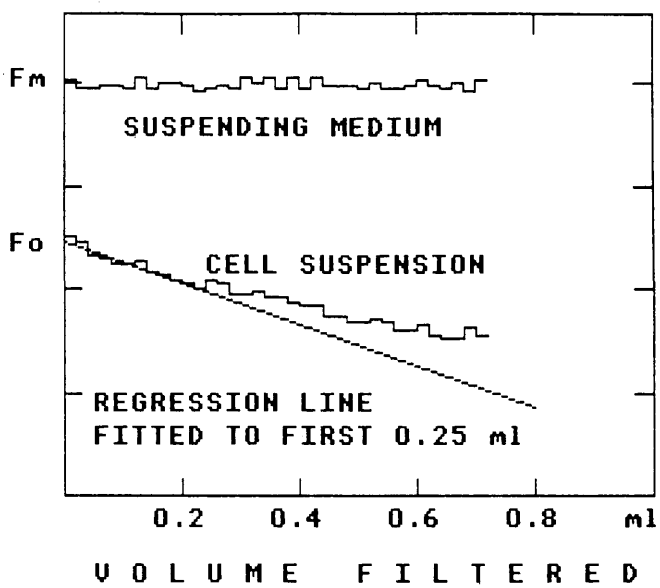

FIG. 2. Evaluation of results: filtration rate (*vertical axis*) vs. volume filtered (*horizontal axis*). Constant filtration rate of buffer (F_m) individually determined for each filter. When cell suspensions are filtered, filtration rate shows continuous decay. This reflects progressive clogging of filter and is quantified by slope of regression line against initial (virtually linear) part of filtration curve. Initial filtration rate of cell suspensions (F_0) is derived from regression line. $F_{0.25}$, filtration rate when 0.25 ml is filtered.

TABLE 1
Filtration results and cell counts in filtered samples from diabetics and controls

	Diabetics (N = 23)	Controls (N = 23)	Statistically significant differences (%)
Red and white cell suspension			
IRCV (fl)	1092 ± 62	1005 ± 52*	9
CP ($10^3/\mu l$)	0.22 ± 0.04	0.13 ± 0.02†	59
White cells ($10^3/\mu l$)	0.40 ± 0.02	0.41 ± 0.02	
Red cells ($10^3/\mu l$)	1010 ± 20	1020 ± 15	
Platelets ($10^3/\mu l$)	15 ± 3	16 ± 4	
Red cell suspension			
IRCV (fl)	1070 ± 46	1007 ± 50*	7
CP ($10^3/\mu l$)	0.04 ± 0.01	0.02 ± 0.01*	50
White cells ($10^3/\mu l$)	0.020 ± 0.008	0.015 ± 0.004	
Red cells ($10^3/\mu l$)	1020 ± 14	1010 ± 10	
Platelets ($10^3/\mu l$)	2 ± 1	3 ± 1	

Values are means ± SD. IRCV, incremental red cell volume (measure of red cell rigidity); CP, clogging particles (measure of filter blockage). *$P < .01$; †$P < .001$.

buffer. The mean of six such tests was calculated and analyzed statistically.

Statistics. Differences between diabetics and controls were evaluated by the test of Wilcoxon and Wilcox. The null hypothesis was rejected at $P < .01$.

RESULTS

With diabetes there is an increase in IRCV when filtering RWCS or RCS samples. CP in diabetic RWCS and RCS samples is increased by 59 and 50%, respectively. Concentrations of red cells, white cells, and platelets are adequately controlled in both cell suspensions (Table 1). Table 2 shows the results of diabetics treated with diet compared with those on oral antidiabetic drugs. There are no significant differences in any of the parameters tested, except for CP when filtering RWCS. However, the group on medication shows a nonsignificant trend toward higher values for IRCV in both suspensions filtered. There was no significant correlation between glucose levels and IRCV ($r = -.09$) or CP ($r = .11$). Red cell platelet suspensions showed filterability similar to that of RCS in both patients and controls (Table 3).

DISCUSSION

The IRCV has been shown to be a measure of red cell deformability when devoid of white cell or other pore-blocking artifacts.[7,9] Therefore, our results can be interpreted as demonstrating reduced overall red cell deformability in diabetes. Quantitatively this abnormality is not impressive (~7%), which confirms results obtained with other techniques, i.e., micropipette studies.[10] Hence, our investigation implies that a small but statistically significant red cell rigidification does exist in type II diabetes mellitus.

CP is significantly increased in RCS when only few platelets or white cells are present. In other words, there is temporary pore blocking in the absence of relevant amounts of white cells or platelets. Thus, this blocking effect is probably caused by red cells, which suggests that a subpopulation of red cells, more rigid than the average diabetic erythrocyte, exists in diabetes. The shortening of red cell survival in poorly controlled diabetics[11] might be partly linked to this finding.

Furthermore, results reveal that CP is increased by 59%

TABLE 2
Filtration results and sample cell counts in diabetics treated with diet and those on oral medication

	Diet (N = 12)	Medication (N = 11)	Statistically significant difference (%)
Red and white cell suspension			
IRCV (fl)	1081 ± 56	1113 ± 59	
CP ($10^3/\mu l$)	0.19 ± 0.03	0.25 ± 0.04*	32
White cells ($10^3/\mu l$)	0.41 ± 0.02	0.41 ± 0.02	
Red cells ($10^3/\mu l$)	1000 ± 10	1020 ± 20	
Platelets ($10^3/\mu l$)	17 ± 5	13 ± 3	
Red cell suspension			
IRCV (fl)	1061 ± 48	1085 ± 52	
CP ($10^3/\mu l$)	0.03 ± 0.01	0.04 ± 0.01	
White cells ($10^3/\mu l$)	0.018 ± 0.007	0.022 ± 0.009	
Red cells ($10^3/\mu l$)	1020 ± 10	1020 ± 15	
Platelets ($10^3/\mu l$)	3 ± 1	1 ± 1	

Values are means ± SD. IRCV, incremental red cell volume (measure of red cell rigidity); CP, clogging particles (measure of filter blockage). *$P < .01$.

TABLE 3
Comparison of filtration data with platelet-poor and platelet-rich red cell suspensions (RCS)

	Diabetics (N = 5)	Controls (N = 5)
Platelet-poor RCS		
IRCV (fl)	1060 ± 55	1010 ± 40
CP ($10^3/\mu l$)	0.05 ± 0.02	0.01 ± 0.01
White cells ($10^3/\mu l$)	0.016 ± 0.006	0.012 ± 0.005
Red cells ($10^3/\mu l$)	1000 ± 16	990 ± 19
Platelets ($10^3/\mu l$)	3 ± 1	2 ± 1
Platelet-rich RCS		
IRCV (fl)	1085 ± 63	1019 ± 47
CP ($10^3/\mu l$)	0.04 ± 0.02	0.02 ± 0.01
White cells ($10^3/\mu l$)	0.021 ± 0.005	0.014 ± 0.006
Red cells ($10^3/\mu l$)	1016 ± 19	1018 ± 13
Platelets ($10^3/\mu l$)	20 ± 5	23 ± 4

Values are means ± SD. IRCV, incremental red cell volume (measure of red cell rigidity); CP, clogging particles (measure of filter blockage). Differences are not statistically significant.

in diabetes when filtering RWCS. Absolute values for RWCS CP are about six times higher than those of RCS CP. Hence, the RWCS CP is probably attributable to white cell effects. In principle, platelet effects might account for part of the differences in CP. Platelet function is known to be altered in diabetes.[12] Filter blockade by platelet aggregates, however, is unlikely for several reasons: thrombocyte counts were kept low compared with the number of pores in the filter; EDTA was used as anticoagulant, which, in contrast to heparin, prevents filter occlusion by platelet aggregates[13,14]; filter blockage by single platelets is unlikely for simple geometric reasons; and most important, filtration of platelet-poor and platelet-rich erythrocyte suspensions from diabetics and controls does not imply a significant platelet effect (Table 3). Hence, a direct influence of platelets on our findings may be excluded. In principle, interactions of platelets and/or their metabolic products in plasma with white cells are possible and might contribute to our findings. Such interactions would have to change leukocyte filterability indirectly to account for our results, implying that diabetic blood is less filterable, probably due to leukocytes.

Comparison of diabetic subgroups also shows a significant difference in RWCS CP (Table 2). Hence, the abnormality seems to be more marked in cases requiring medication than in those treatable with diet alone. However, interpretation of these results must be cautious. Direct effects of antidiabetic drugs might account for the difference. However, data from diabetics switching from diet to drugs suggest that the difference is not due to a drug effect. Moreover, no significant correlations between glucose levels and filtration data were found. However, the possibility of diabetic control influencing the findings cannot be totally excluded. The increased CP could be explained by diabetic leukocytes being less deformable, more adhesive, or both.

Our findings do not determine which leukocyte subgroup may be altered. Furthermore, it is uncertain which mechanism is involved in altering white cell rheology in diabetes.

Leukocytes have been shown to temporarily occlude exchange vessels and to influence the distribution of nutritive flow.[15] Animal experiments suggest that tissue ischemia can be induced by leukocyte plugging.[16] Epidemiologic data indicate that leukocyte counts may be of prognostic value in ischemic diseases.[17–20] It has long been appreciated that diabetics are at higher risk of myocardial infarction.[1] It was demonstrated recently that the size of the myocardial necrosis is generally larger in diabetic patients than in controls.[21] Our findings suggest that altered leukocyte rheology in diabetes might be an important link in the chain of events leading to the impairment of the microcirculation.

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