Different erythrocyte and platelet surface electric charge in various types of glomerulonephritis

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Abstract

Background. Some preliminary observations suggest that predisposition to a particular type of glomerulonephritis (GN) may be connected with the genetically determined charge of the glomerular capillary wall. A correlation between erythrocyte surface and the glomerular capillary wall charges has also been observed. The purpose of this study was to verify and extend previous investigations. Therefore we measured erythrocyte and platelet surface charge from patients with idiopathic membranous and mesangial GN as well as idiopathic membranoproliferative GN and lupus nephritis.

Methods. The erythrocyte and platelet surface charge was determined by the binding of the cationic dye, alcian blue (AB). A fresh alcoholic AB solution was made for each experiment, which were run in batches of four, each including cells from a healthy person and from patients each with a different type of GN.

Results. In patients with idiopathic membranous and membranoproliferative GN, a significant decrease in the erythrocyte and platelet charges was observed irrespective of their clinical state (remission or nephrotic syndrome). Erythrocyte charge was decreased despite the normal amount of membranous sialic acid. In contrast, patients with idiopathic mesangial GN, in complete or partial remission, exhibited normal erythrocyte and platelet surface charges. Exclusively in this type of GN, the appearance of nephrotic proteinuria was associated with a slight decrease, the erythrocyte charge, which was not statistically significant ($P > 0.1$). A reduction in the negative erythrocyte charge in lupus nephritis was less in magnitude than in idiopathic membranous or membranoproliferative GN, and occurred independently of the level of daily proteinuria, whereas the platelet charge was normal.

Conclusion. The decrease of the erythrocyte and platelet charge in idiopathic membranous and mebranoproliferative GN seems to be a pre-morbid feature.

Key words: erythrocytes; platelets; surface charge; idiopathic glomerulonephritis; lupus nephritis

Introduction

Preliminary observations by Boulton-Jones et al. [1] in experimental rat models suggest that predisposition to a particular type of glomerulonephritis (GN) may be associated with genetically determined differences in the charge of the glomerular capillary wall. They observed that Lewis rats, which are susceptible to the induction of Heymann’s nephritis (the model of human membranous GN) have fewer glomerular anionic sites than the DA strain which is resistant to this type of experimental GN. Both strains also differ in terms of cationic protein binding. Immunization with cationic human serum albumin led to membranous deposits in Lewis rats and mesangial deposits in DA rats. Furthermore, polycation infusion produced proteinuria in Lewis rats, but not in the DA strain. There also appeared an intriguing parallelism between the erythrocyte surface and glomerular capillary wall charges in those rats. The overall RBC charge is lower in healthy Lewis rats than the equivalent one in DA rats. Complementary data were obtained by Bernard et al. [2,3], who observed a decrease in the erythrocyte charge associated with a loss of glomerular negative charges in cadmium treated rats. These studies in animal models suggested that the erythrocyte surface electric charge may reflect both the normal glomerular polyanion charge and the changes occurring in GN. Indeed, Levin et al. [4] showed a reduction in surface electric charge on red blood cells and platelets in nephrotic children with submicroscopic GN and focal segmental glomerulosclerosis. Afterwards, Boulton-Jones et al. [5] found that erythrocyte surface charge was significantly lesser in patients with submicroscopic and idiopathic membranous GN in remission than in respective patients with IgA nephropathy.

The purpose of this study was to extend the investigations to other types of idiopathic GN and patients with renal manifestation of systemic lupus erythematosus.
Subjects and methods

The surface electric charge on red blood cells (RBC) and platelets was measured in five groups:

(a) control group, 40 healthy volunteers (20 females, 20 males, age 21–46 years, mean ± SEM 26 ± 3.2);
(b) 15 patients with idiopathic membranous GN (7 females, 8 males, age 21–66 years, mean 43.7 ± 3.6);
(c) 19 patients with idiopathic type I membranoproliferative GN (13 females, 6 males, age 17–64 years, mean 35.8 ± 3.1);
(d) 17 patients with idiopathic mesangial GN, including IgA nephropathy, 8; IgM nephropathy, 5; C3 and IgG deposits, 4; (10 females, 7 males, age 16–65 years, mean 31.5 ± 3.4);
(e) 15 patients with renal involvement in systemic lupus erythematosus (13 females, 2 males, age 18–43 years, mean 33.1 ± 2.5); a histological pattern of membranous or membranoproliferative GN appeared in the lupus nephritis group.

RBC charge was determined for all subjects and the platelet charge measured in some individuals in each group.

All patients were tested in a period of normal renal function.

Laboratory methods

The charge on RBC and platelets was measured by means of cationic dye, AB (alcian blue 8 GX, Aldrich) binding.

Eighteen millilitres of venous blood was drawn into 2 ml of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was obtained according to the method described by Mustard et al. [6]. The platelets were suspended in phosphate-buffered saline (PBS) at a concentration of 4 × 10^8/ml. After the removal of the PRP, RBC were washed three times in PBS and resuspended in the same buffer with the final concentration of 1.2 × 10^8/ml.

Preparation of AB solution

Fifty milligrams of AB was completely dissolved in 1 ml of 100% ethanol, according to the recommendations published by Winkel et al. [7]. A fresh alcohol AB solution was made for each experiment. The dye was subsequently filtered through Whatman No. 1 paper and diluted 100 times in PBS containing 25 mmol/1 MgCl₂, resulting in a final concentration of 500 μg/ml AB and 1% ethanol. Immediately before the use of this solution each time, the extinction was measured with a spectrometer (Spectromet 195D) at an optical density of 650 nm. If the E₀.5 values were outside the range of 0.830–1.130 (mean 0.950 ± 0.024), the solution was discarded and a new one prepared.

A 0.5-ml suspension of the RBC (1.2 × 10⁸/ml) or platelets (4 × 10⁹/ml) was mixed with 0.5 ml of the final AB solution (500 μg/ml). The mixture was then incubated for 30 min. at 37 °C. After the removal of the cells by centrifugation, the remaining AB was measured with a spectrometer at an optical density of 650 nm.

The amount of AB bound per RBC or platelet was calculated from the difference between the initial optical density of the AB solution and that of the supernatant. It was expressed in ng per 10⁸ RBC or 10⁹ platelets. To avoid any analytical bias, the experiments were done in batches of four, always including the blank AB solution, the cells from a healthy person and from patients, each with a different type of GN. No change in the optical density of AB solution before and after incubation was observed. Intra-assay coefficient of variation, based on six parallel samples from one healthy person, was 8.1% for RBC and 9.8% for platelets. Interassay coefficient of variation, calculated from eight repetitions of the test in one healthy person, was 4.6% for RBC and 6.2% for platelets.

To examine the dependency of AB binding upon sialic acid residues on the cell surface, cells from five healthy persons were desialylated by incubation with 1 U/ml neuraminidase (SEVBA) and the AB binding was then compared with that for untreated cells. At the dye concentration of 500 μg/ml, neuraminidase treatment reduced AB binding to RBC and platelets to approximately 30 and 50% respectively of that for untreated cells.

In addition the sialic acid content in the erythrocyte membrane was measured in five healthy persons and in seven patients with idiopathic membranous or membranoproliferative GN. One millilitre of the RBC suspension (10⁷/ml) was incubated with neuraminidase (1 U/ml) for 1 h at 37 °C, and after centrifugation of the cells the concentration of sialic acid in the supernatant was determined with the modified thiobarbituric acid assay according to Skoza and Mohos [8].

Statistics

Student’s t test was used for statistical analysis. P values below 0.05 were considered as significant. Data are expressed as mean ± SEM.

Results

The AB binding on RBC in control and GN groups is shown in Figure 1. No difference in RBC surface charge between female and male controls were observed; mean values were 342.1 ± 8.7, and 363.5 ± 9 ng/10⁶ RBC respectively. AB binding to erythrocytes from all GN patients was less than in controls. The greatest drop in surface charge was observed in RBC from patients with idiopathic membranous and membranoproliferative GN. Within the mesangial GN group, no significant difference in the RBC charge was revealed between those with IgM deposits and the remaining patients (298.2 ± 16.2 versus 322.7 ± 14.0 ng/10⁶ RBC). Considering all GN groups together, no correlation was visible between RBC charge and the magnitude of proteinuria (Figure 2); mean values for nephrotic subjects and patients in remission were 310.3 ± 7.5 and 311.4 ± 6.7 ng/10⁶ RBC respectively. More detailed data concerning the particular types of GN (Figure 3) show that the decrease of AB binding to RBC from the patients with idiopathic membranous or membranoproliferative GN in remission was the same as in the whole group encompassing the nephrotic patients and those in remission. Lupus nephritis patients also maintained the decreased erythrocyte charge during the remission. A correlation between the extent of proteinuria and the erythrocyte charge was only evident in the mesangial GN group. Patients in remission from this group exhibited higher AB binding to RBC than those nephrotic ones; respective mean values 333.0 ± 14.6.
versus 299.7 ± 15.1 ng/10⁶ RBC. This tendency did not reach statistical significance (P > 0.1). However, the RBC charge in mesangial GN patients in remission was not different from the normal subjects (Figure 3).

A decrease in erythrocyte charge occurred in idiopathic membranous and membranoproliferative GN despite a normal quantity of membrane sialic acid; mean values of the sialic acid content in the erythrocyte membrane in the patients and controls were 5.3 ± 1.0 and 5.0 ± 0.5 mg/10⁷ RBC respectively.

The AB binding on platelets from the control and GN groups is presented in Figure 4. In the control group, as in the case of RBC, no difference in the surface charge was found between females and males, mean values of the AB binding were 188.2 ± 16.9, and 209.6 ± 14.0 ng/10⁸ platelets respectively. A significant decrease in platelet anionic charge appeared exclusively in patients with idiopathic membranous and membranoproliferative GN, whereas the values in mesangial and lupus nephritis groups did not differ significantly from the controls. Similar to the RBC charge, no significant relationship was observed between AB binding to the platelet membrane and the magnitude of daily proteinuria in the patients; mean values for the
Fig. 3. The surface electric charge on RBC from glomerulonephritis patients in complete (absent proteinuria) or partial (proteinuria below 2 g/day) remission. **P<0.01; ***P<0.001.

Fig. 4. The surface electric charge on platelets from glomerulonephritis patients with nephrotic and subnephrotic proteinuria *P<0.05; **P<0.01.

nephrotic patients and those in remission were 156.2±47.1 versus 169.7±48.2 ng/10⁸ platelets respectively.

Discussion

The erythrocyte and platelet surface anionic charge varied among different types of GN. A statistically significant decrease in AB binding to RBC and platelets was observed in idiopathic membranous and membranoproliferative GN. This fall in erythrocyte and platelet negative charges was independent of the magnitude of daily proteinuria, appearing despite the normal sialic acid content in the RBC membrane. In contrast, patients with idiopathic mesangial GN, in complete or partial remission, exhibited normal erythrocyte and platelet surface anionic charges. Exclusively in this type of GN, the appearance of nephrotic proteinuria was associated with a slight decrease in the erythrocyte charge. However, it did not reach statistical significance (P>0.1). In the lupus nephritis group a reduction in the negative erythrocyte charge, smaller in magnitude than in idiopathic membranous and membranoproliferative GN, occurred irrespective of the clinical state (remission or nephrotic syndrome), whereas the platelet charge was normal. It should be mentioned that the histological pattern of membranous and membrano-
proliferative GN was found in the lupus nephritis patients, too.

In addition to the slight decrease of RBC charge in all the GN groups and a significant reduction in platelet charge in idiopathic membranous and mem-

brane proliferative GN, some essential similarities were noticed. In the latter two GN groups, the decrease in erythrocyte anionic charge was greater in magnitude than in patients with idiopathic mesangial GN and lupus nephritis.

The greater differences in the platelet membrane charge among the types of GN may be caused by a lesser dependence on sialic acid compared with the RBC membrane. Neuraminidase treatment reduced the AB binding on RBC to 30% and on the platelets to 50%.

Several authors have questioned the validity of the AB assay. The major objections concern the incomplete AB dissolution in PBS buffer and its tendency to precipitate with time [9,10]. We could confirm the observation made by Winkel et al. [7] that 50 mg of AB dissolves completely in 1 ml of 100% ethanol. A fresh alcohol AB solution was prepared for each experiment. Each experiment was considered valid when no change in the optical density of the blank AB solution occurred over the duration of the experiment. In addition, the experiments were done in batches of four with cells from a healthy person and those from patients with each form of GN. Therefore any flaw in the methodology would not affect only one group and thus distort the results. The extent of AB binding to RBC various among reports in the literature. The values obtained in our study are higher than the [14] used differential clearance of isoforms of amylase in glomerular charge may predispose individuals to with IgA nephropathy. The values of the RBC charge normal population. all the GN groups and a significant reduction in glomerular handling of antigen between Lewis and DA rats.

We confirmed that idiopathic membranous GN patients in remission have lower erythrocyte anionic charge than patients with IgA nephropathy. The values of the RBC charge in both groups were within the normal range, but were at the lower and upper end for membranous GN and IgA nephropathy respectively, causing a significant difference. With closer analysis, therefore, our data concerning idiopathic membranous and mesangial GN in remission are similar to those of Boulton-Jones et al.

We believe that our data support the notion that a reduction in erythrocyte and platelet charges in the idiopathic membranous and membranoproliferative GN is a premorbid feature, perhaps connected with the properties of the glomerular capillary wall, which predispose to these types of GN.

References


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