Acta Medica et Biologica
Vol. 35, No. 1, 45-51, 1987

MEMBRANE NEGATIVE CHARGE IN CHILDREN WITH MINIMAL CHANGE NEPHROTIC SYNDROME

MAKOTO UCHIYAMA, TAKAYUKI GOMI, KAORU SAKAI

Department of Pediatrics, Niigata University School of Medicine, Niigata 951

(Received February 19, 1987)

ABSTRACT

Negative charge on erythrocytes and platelets (alcian-blue binding) was measured in children with minimal change nephrotic syndrome (MCNS). The charge was significantly less in children with MCNS in relapse than in those in complete remission and in control children. The reduced charge did not show any significant correlation with serum albumin concentration. These findings suggest that the reduced negative charge on erythrocytes or platelets may be of some relevance to the pathogenesis of MCNS.
Key words: membrane negative charge, alcian-blue binding, erythrocytes, minimal change nephrotic syndrome

INTRODUCTION

Nephrotic syndrome results from an increased permeability of the glomerular basement membrane to albumin. Minimal change nephrotic syndrome (MCNS) is the most common form in childhood, and is characterized by responsiveness to corticosteroid therapy. Its cause still remains unclear, although several immunologic mechanisims have been postulated. MCNS may be related to T-cell dysfunction in which humoral factors, perhaps lymphokines, are produced to alter glomerular basement membrane permeability[1-4].

Recently it has been suggested that a decreased membrane negative charge of the glomerulus might increase urinary albumin excretion and cause MCNS, since the glomerulus functions not only as a size-selective filter, but also as a charge-selective filter which restricts the passage of negatively charged macromolecules such as albumin[5].

The negative charge on the surface of erythrocytes or platelets may reflect the

charge on the glomerular capillaries. Levin et al. have already reported that the negative charge on erythrocytes is decreased in children with MCNS. However, they did not show any results based on nephrotic children in complete remission. In this study, we therefore examined the negative charge on erythrocytes and platelets of children with MCNS in both relapse and complete remission.

Materials and Methods

Twelve children with steroid-responsive nephrotic syndrome, aged 6 to 17, and 21 age-matched normal children were studied. The nephrotic children were considered to have minimal change lesions from the pattern of steroid response, although renal histology was not undertaken in most children. Nine of the nephrotic children were in relapse and three in complete remission.

The method of measurement of negative charge on erythrocytes and platelets is shown in Table 1[5]. The chemical test is based on the binding of the cationic dye alcian-blue 8GX.

Table 1 *ALCIAN BLUE BINDING TECHNIQUE*

Red Cells and platelet preparation

Take whole blood into sodium citrate.
Remove excess plasma by centrifuge at 3000 rpm for 10 minutes.
Wash packed red cells three times in pH 7.4 buffered saline.
Resuspend in saline and count, adjusting count to $1.2 \times 10^{12}/1$.

Platelets

Centrifuge at 140g for 10 minutes to prepare PRP (platelet rich plasma)
Aspirate plasma with plastic pipette.
Check count for red cell and white cell contamination.
Layer PRP onto metrizamide gradient (25% & 10%) and centrifuge at 3500 rpm for 12 minutes.
Remove PRP and metrizamide and carefully resuspend platelet layer in pH 7.4 buffer.
Count platelets and adjust count to 400 x 400 $10^9/1$.

Alcian Blue Working Solution

0.5 ml 1% Alcian blue
0.25 ml 2M $MgCl_2$
19.25 ml pH 7.4 buffered saline

Binding Assay

Take 0.5 ml of alcian blue working solution, add 0.5 ml of test suspension, mix well and incubate for 30 minutes at 37℃. Centrifuge at 3000 rpm for 10 minutes.
Blank control. 0.5 ml alcian blue + 0.5 ml pH 7.4 buffer. Read at 650 wavelength in spectrophotometer using a water zero.
Amount of alcian blue bound = reading of blank control − reading of supernatant
Using the above method 1 ml of solution contains 125 μg of alcian blue.

Results were expressed as mean ± SD, and analyzed using unpaired t-tests. Simple correlation coefficient was calculated between plasma albumin concentration and alcian blue binding in nephrotic children in relapse.

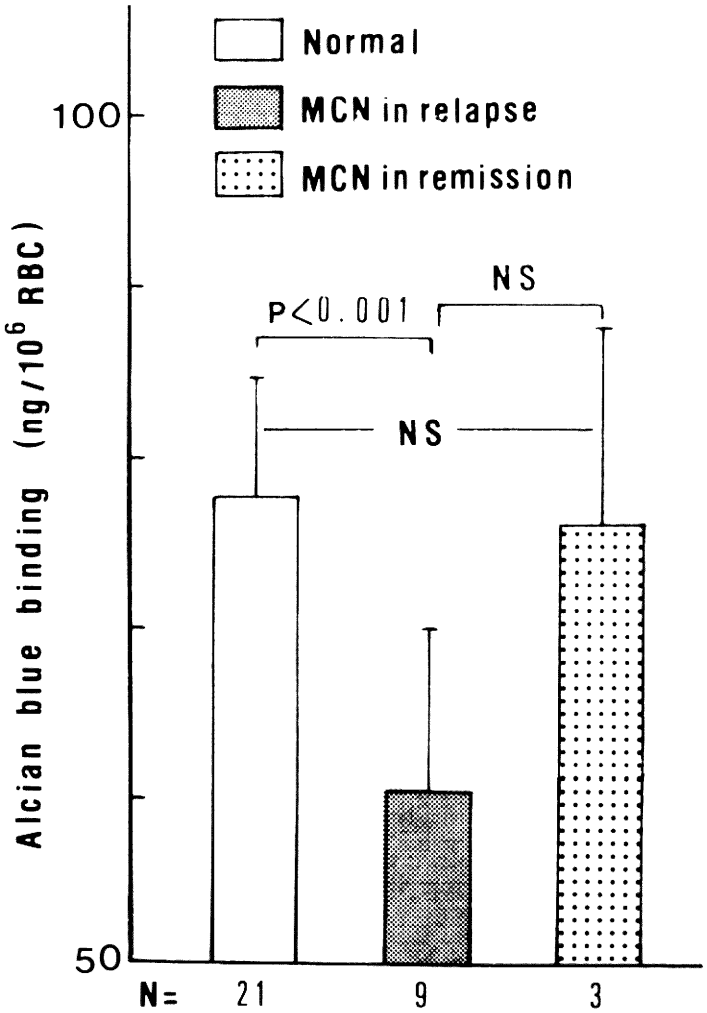

Fig. 1 Alcian blue binding to erythrocytes in normal children, minimal change nephrotic children (MCN) in relapse, and minimal change nephrotic children (MCN) in remission

RESULTS

The results of alcian blue binding to erythrocytes and platelets in the three groups (normal children, nephrotic children in relapse and those in complete remission) are shown in Figs. 1, 2. Alcian blue binding to erythrocytes and platelets was significantly lower in nephrotic children in relapse than in normal control children. Nephrotic children in complete remission showed higher alcian blue binding to erythrocytes and platelets than those in relapse, however this did not reach a statistical significance possibly because of the small numbers of subjects involved.

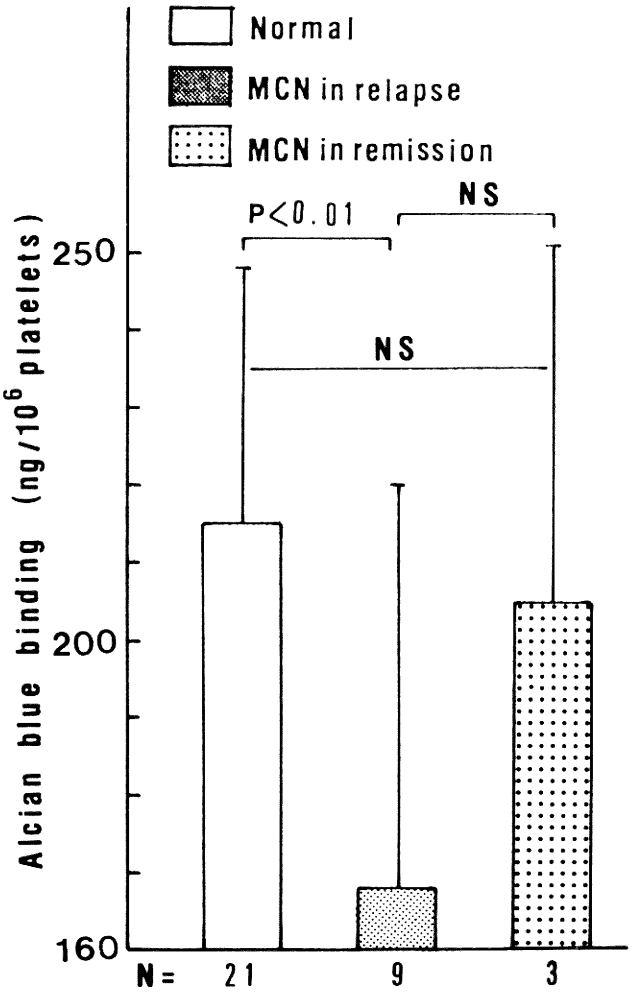

Fig. 2 Alcian blue binding to platelets in normal children, minimal change nephrotic children (MCN) in relapse, and minimal change nephrotic children (MCN) in remission

In nephrotic children in relapse, there was no significant correlation betweeen plasma albumin concentration and the reduction in alcian-blue binding to erythocytes (Fig. 3) or to platelets ($r=0.1171$, $P>0.05$).

Discussion

The cause of MCNS remains unclear, although several immunologic mechanisms have been postulated. T-cell dysfunctions have been reported to have some relevance to the pathogenesis of MCNS[1-4], which produces lymphokines that alters glomerular basement membrane permeability. This hypothesis comes from studies in which 50% of extracts from cultured lymphocytes of patients with MCNS have shown to cause enhan-

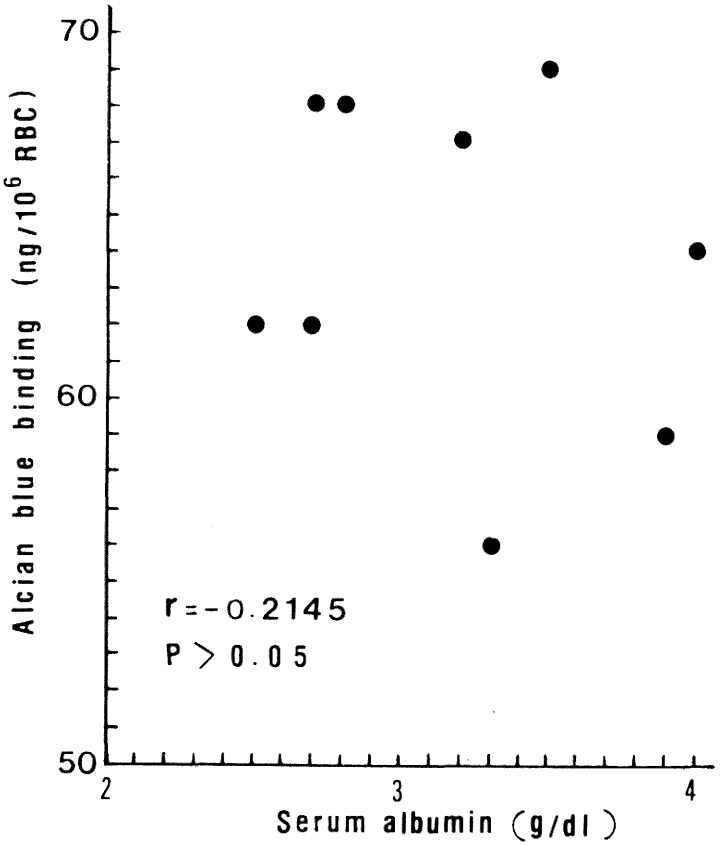

Fig. 3 Relationship between alcian blue binding to erythrocytes and serum albumin concentration in minimal change nephrotic children (MCN) in relapse

ced vascular permeability when injected subcutaneously into guinea pigs[2]. In addition, normal blastogenesis of lymphocytes is inhibited by serum from patients with active MCNS[3,4,6]. Sakai[3,4] has reported the possibility that exogenously-administered glucocorticoid may have an effect on lympocytes and induce remission in nephrotic children. This hypothesis on T-cell dysfunction has still received much support from the foregoing findings.

On the other hand, the glomerulus functions not only as a size-selective filter, but also as a charge-selective filter. Negatively charged macromolecules are not permeable to the glomerular capillary as compared with neutral or positively charged macromolecules of the same effective molecular radius[5,7]. Recently, interest has turned to the possibility that MCNS may be related to a specific loss of this charge-selective funcion of the glomerulus[8,10]. The glomerular anionic sites have been reported to be reduced in

MCNS[10]. Vehaskari[11] observed the following findings; an infusion of polycation neutralized glomerular anionic sites, which was proved histochemically, and increased albumin excretion.

Levin et al.[5] have developed an assay for measuring cell-surface negative charge based on the binding of alcian-blue to anionic groups on the cell surface. They found a reduced alcian-blue binding to erythrocytes and platelets of nephrotic children in relapse, suggesting that there might be a generalized disorder of membrane negative charge in MCNS.

Their method is simple enough to be undertaken in any laboratory, and its outline is shown in Table 1. Using this method, we found a significant decrease in alcian-blue binding to erythrocytes and platelets of nephrotic children in relapse as compared with normal children, which confirmed the previous report by Levin et al.[5]. Levin et al. did not examine nephrotic children in remission: however, we found no decrease of alcian-blue binding in them. Boulton-Jones et al. reported a decreased value in nephrotic adults in remission, although it did not reach a statistical significance[12].

The negative charge on erythrocytes is largely due to sialic acid residues[13]. However, Levin et al.[5] reported normal sialic acid content in the cell membrane of erythrocytes in spite of the reduced alcian-blue binding in nephrotic children. These findings suggest that a generalized loss of membrane negative charge occurs in MCNS and that this is due to neutralization rather than the absence of anionic groups.

We did not examine T-cell dysfunction in this study. Elucidation of the relationship between T-cell dysfunction and cell membrane negative charge was not our aim, but we hope to investigate this at a future date.

References

1) Shalhoub, R. J.: Pathogenesis of lipoid nephrosis. A disorder of T-cell dysfunction. *Lancet,* **2**: 557, 1974.
2) Sasdelli, M., et al.: Lymphocyte subpopulations in minimal change nephropathy. *Nephron,* **25**: 72, 1980.
3) Sakai, K., et al.: Cell-mediated immunity in lipoid nephrosis in children. *Igaku no Ayumi,* **119**: 351, 1981(in Japanease).
4) Sakai, K., et al.: Treatment of long term steroid dependent nephrotics. Proceeding of 7 th International Congress of Paediatric Nephrology, Elsevier Sci. Publishers, Amsterdam, 1987 (in press).
5) Levin, M., et al.: Steroid-responsive nephrotic syndrome: A generalised disorder of membrane negative charge. Lancet, ii: 239. 1985.
6) Iitaka, K., West, C. D.: A serum inhibitor of blastogenesis in idiopathic nephrotic syndrome transferred by lymphocytes. Clin. Immunol. *Immunopathol.,* **12**: 62, 1979.
7) Deen, W. M., et al.: The glomerular barrier to macromolecules: Theoretical and experimental consideration. In: Brenner, B. M., Stein, J. H. eds. Contemporary issues in nephrology. *New York, Churchill Livingstone,* **9**: 1-29, 1982.
8) Robson, A. M., et al: Normal glomerular permeability and its modification by minimal change nephrotic syndrome. *J. Clin. Invest.,* **54**: 1190, 1974.
9) Carrie, B. J., et al.: Minimal change nephropathy: An electrochemical disorder of the glomerular membrane. *Am. J. Med.,* **70**: 262, 1981.

10) Blau, E. B., Haas, J. E.: Glomerular sialic acid and proteinuria in human renal disease. *Lab. Invest.*, 28: 477, 1973.
11) Vehaskari, V. M., et al.: Glomerular charge and urinary protein excretion: Effect of systemic and intrarenal polycation infusion in the rat. *Kindney Int.*, 22: 127, 1982.
12) Boulton-Jones, J. M., et al.: Variation in charge on red cells of patients with different glomerulopathies. Lancet, ii: 186, 1986.
13) Eylan, E. H., et al.: The contribution of sialic acid to the surface charge of the erythrocyte. *J. Biol. Chem.*, 237: 1992, 1962.