Validity of the Alcian Blue Binding Test as an Indicator of Red Blood Cell and Glomerular Membrane Negative Charges

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Dear Sir,

A controversy presently exists concerning the possibility of assessing the glomerular polyanion charge on the basis of the Alcian blue (AB) binding to red blood cell (RBC) membrane. Studies by Levin et al. [1] and Boulton-Jones et al. [2] have suggested that the binding of the cationic dye AB to RBC surface reflects the loss of glomerular polyanion in patients with nephrotic syndrome. The enhanced protein excretion in these patients would be due at least partly to a reduced electrostatic repulsion by the glomerular filter of anionic macromolecules like albumin. This hypothesis is supported by our observations showing that the microalbuminuria of diabetes or chronic cadmium poisoning is associated with a parallel decrease of AB binding to RBCs [3, 4].

Several authors, however, have questioned the validity of the AB binding test to RBCs. Feehally [5] could not confirm the reduction of AB binding to RBCs from patients with nephrotic syndrome. Sewell et al. [6] have put forward reservations on the analytical validity of the AB binding test because of the instability of the dye in solution and the possible interference of contaminating albumin. More recently, Ghiggeri et al. [7] studied the physi-cochemical interaction between AB and proteins and concluded that the binding of AB to proteins is hydrophobic and therefore cannot be used for monitoring variations in surface charge due to sialic acid residues.

This conclusion, however, is refuted by the experimental evidence presented here that the AB binding to RBC and glomerular membrane correlates with the sialic acid content of these structures. These data were obtained in rats given 100 ppm cadmium in the drinking water for 10 months and in their controls. The AB binding

to RBC or glomerular membrane was determined by the method of Levin et al. [1] with the following modifications. RBCs were washed 4 times with phosphate-buffered saline. The AB solution (Alcian Blue 8GX, 54% purity, Sigma, St. Louis, Mo.) was prepared just before use by dissolving the dye (4 mg/ml) in a solution containing 25 mM MgCl₂ and 0.15 M NaCl. This solution was vigorously vortex-mixed for 10 min and the undissolved dye removed by centrifugation (2,000 g × 10 min) and filtration on Whatman No. 1 filter. The final AB concentration was determined from the extinction coefficient (Ei0°m 68.4 for the crude AB

preparation). The results presented here were obtained with a saturating AB concentration of 1.5 mg/ml and were corrected for the purity of the dye. The sialic acid content of RBC and glomerular membranes was determined by the method of Hammond and Papermaster [8]. RBC membranes were lysed in distilled water. The pellet obtained by centrifugation at 14,000 g for 10 min was washed with 0.15 M NaCl until it was white. Glomeruli were isolated by a graded sieving technique [9] and their basement membrane (GBM) was purified according to the method of Meezan et al. [10].

The cadmium treatment resulted in a significant reduction of AB binding to RBCs: $118 \pm (\text{SD}) 52$ ng/106 RBCs in cadmium-treated rats ($n = 9$) versus 184 ± 49 ng/ 106 RBCs in controls ($n = 10$). As shown in figure 1, the AB binding to RBCs closely correlates with the sialic acid content of RBC membrane, which suggests an ionic interaction between the cationic dye and anionic residues of sialic acid. This interpretation is consistent with the fact that the AB binding to RBCs is inhibited by desialylation with neuraminidase and by the polycationic protein, protamine sulfate [1]. A negative correlation was also found between the AB binding to RBCs and the albumin-

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185

$n = 19$

$r = 0.83$ $p < 0.001$

250

200

150 -

100-

50

1 1

3.0

0 1.0 2.0

Sialic acid in RBC membrane, ng/106 RBCs

Fig. 1. Correlation between the AB binding to RBC surface and the sialic acid content of RBC membrane in 9 rats given 100 ppm cadmium in the drinking water for 10 months and in 10 controls.

uria ($r = -0.55$, $n = 19$, $p < 0.001$), which presumably reflects the parallelism between RBC membrane negative charges and the glomerular charges involved in the glomerular filtration of albumin.

Direct evidence of this parallelism was obtained by examining in a separate experiment the relationships between RBC charges and the glomerular polyanion in 26 cadmium-treated rats and 11 controls. The AB binding to RBCs from these animals was correlated with the AB binding to isolated GBM ($r = 0.38$, $p < 0.025$) and the latter was correlated with GBM sialic acid ($r = 0.41$, $p < 0.025$). Significant negative correlations were also found between these three parameters and the albuminuria ($r = -0.62$, -0.74 and -0.39 , respectively).

We conclude that the AB binding test, if carried out according to a welldefined protocol, is a reliable method for assessing directly or indirectly (i.e. on the basis of RBCs) the charge selectivity of the glomerular filter.

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