Recovery of Anionic Sites of the Glomerular Basement Membrane after Their Disappearance by the Cationic Probe Molecule

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Dear Sir,

The negative charge barrier of the glomerular basement membrane (GBM) plays an important role in regulating the selective permeability of the GBM to protein [1, 2]. It is speculated that transient injuries to glomerular anionic sites by various cationic molecules derived from platelets or other cells may contribute to proteinuria in the absence of glomerular structural lesions [3,4]. There is no information about the true period required for recovery of the negative charge barrier of the GBM following transient injuries. In the present study, we examined the reappearance of anionic sites associated with read-ministration of polyethyleneimine (PEI), as a cationic tracer. PEI was readministered after disappearance of PEI particles from the initial injection in order to clarify the rate of 'recovery' of the negative charge barrier following transient PEI-induced injury in the normal kidney.

Twenty-five female Sprague-Dawley rats, weighing 200–250 g, were injected in the tail vein 0.3 ml of 0.5% PEI (molecular weight = 1,800) solution adjusted to pH 7.4 and 400 mosm. Left nephrectomy was done on 5 rats each at 15 min, 1, 2, 3 and 5 h after injection. Renal tissues from all rats were studied to visualize PEI particles. The number of PEI particles per 1,000 nm of lamina rara externa (LRE) in the GBM were counted on all specimens in at least five fields by electron microscopy. Reasonable amounts of PEI particles with a regular interspacing were detected on tissues obtained 15 min (18.8 ± 0.5 , mean $\pm$ SEM) and 1 h (18.1 ± 1.1) after injection. In the following hour, the number of PEI particles significantly decreased as compared with those obtained 15 min after injection (2 h = 16.2 ± 1.2 , $p < 0.05$; 3 h = 12.6 ± 1.0 , $p < 0.01$; 5 h = 8.0 ± 1.0 , $p < 0.01$). Shifting of PEI particles to the slit pores or just below the foot process are prominent at 3 and 5 h after injection indicating the loss of anionic sites, because visualization of PEI was possible by binding with anionic sites. Therefore, the loss of anionic sites began at least 2 h after binding with cationic molecules. From these results, we did the next experiment: an initial injection with 0.3 ml of PEI

solution was performed on 10 rats, and left nephrectomy was done 3 and 5 h after injection on all 5 rats. Immediately after left nephrectomy, readministration of the same dose of PEI solution was done on each rat, and the right kidney was removed 15 min after reinjection. Each tissue sample from both kidneys was subjected to electron-microscopic observation as above. The number of PEI particles per 1,000 nm of LRE from left kidneys was 13.2 ± 1.0 at 3 h and 9.0 ± 1.4 at 5 h, but those from the right kidney were in the normal range after reinjection (18.8 ± 0.5 and 21.2 ± 0.7 ; fig. 1). These results indicated that the binding ability of anionic sites in the LRE to cationic molecules recovered within 3 h. A metabolic study has convincingly identified a fairly rapid turnover of glomerular proteoglycans [5], the principal component of anionic sites of the GBM. An increase in the permeability of macromolecules through the GBM after charge alterations was demonstrated in experimental models and in man. It may also be expected

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Fig. 1. Electron-microscopic localization of PEI particles. Bar = $0.2 \mu\text{m}$. $\times 45,000$. a The remarkable decrease of PEI particles in the LRE of the left kidney is already observed 3 h after initial injection of PEI. Shifting of PEI particles to slit pores is visible (arrows). b The regular arrangement with interspacing of PEI particles in the LRE of the right kidney is demonstrated after reinjection of PEI following left nephrectomy in the same rat as in a.

that the negative charge barrier of the GBM in normal kidneys may be transiently injured when cationic molecules, released from platelets and other cells in several conditions, bind to anionic sites. Experimentally, neutralization of glomerular polyanions in vivo due to infusion of cationic molecules resulted in heavy proteinuria [2, 6]. The rapid synthesis of heparan sulfate proteoglycans following a transient reduction in glomerular anionic sites in the normal kidney may serve to restore the glomerular charge barrier and reduce excessive protein loss in the urine.

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Hunsicker LG, Shearer TP, Shaffer SJ: Acute reversible proteinuria induced by infusion of the polycation hexadimethrine. *Kidney Int* 1981;20:7–17.

Camussi G, Tetta C, Meroni M, Torri-Tarelli L, Roffinello C, Alberton A, Deregibus C, Sessa A: Localization of cationic proteins derived from platelets and polymorphonuclear neutrophils and local loss of anionic sites in glomeruli of rabbits with experimentally induced acute serum sickness. *Lab Invest* 1986; 55:56–62.

Kreisberg JJ, Wayne DB, Karnovsky MJ: Rapid and focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis. *Kidney Int* 1979; 16: 290–300.

Cohen MP, Surma M: Renal glomerular basement membrane. In vivo biosynthesis and turnover in normal rats. *J Biol Chem* 1980;255:1767–1770.

Bastford SR, Sasaki M, Takamiya H, Vogt A: Cationic macro-molecule-induced nephrotic syndrome in rabbits: Lack of immune complex involvement. *Lab Invest* 1983;49:260–269.

References