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Effect of Concanavalin A on Nephrotic Peripheral Blood Mononuclear Cells Mediated Increased ^{35}Sulfate Uptake in Rat Glomerular Basement Membrane[1]

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ABSTRACT. We have previously shown a significant increase in ^{35}sulfate uptake in rat glomerular basement membrane (GBM) when glomeruli were cocultured with peripheral blood mononuclear cells (PBMC) from patients with idiopathic minimal lesion nephrotic syndrome (IMLNS) in relapse, but an uptake not different than normal controls if glomeruli were incubated with PBMC of patients in remission. In the present study we examined ^{35}sulfate uptake by GBM after PBMC from 12 IMLNS patients in remission were stimulated with Concanavalin A (Con A) (10 μg/ml of culture media). There was a significant increase in ^{35}sulfate GBM uptake when glomeruli were cocultured with Con A-stimulated IMLNS PBMC (geometric mean), 331 cpm/mg dry glomerular weight) as compared to glomeruli cocultured with IMLNS PBMC (geometric mean, 200) ($p = 0.048$); glomeruli alone stimulated with Con A (geometric mean, 182) ($p = 0.008$) or glomeruli alone (geometric mean, 146) ($p = 0.002$). No significant differences were seen between the groups when glomeruli were cocultured with PBMC from 12 normal adults. These data show that Con A stimulated PBMC from IMLNS patients in remission alter the sulfate metabolism of rat GBM. The stimulation of PBMC with Con A reproduces the increase in ^{35}sulfate uptake observed when glomeruli are cocultured with PBMC from IMLNS in relapse. Sulfated compounds in the GBM may play a role in glomerular permeability. Since stimulated nephrotic PBMC alter the metabolism of GBM sulfated compounds, these findings may have pathogenic significance. (*Pediatr Res* 20: 321–323, 1986)

Abbreviations

IMLNS, idiopathic minimal lesion nephrotic syndrome
GMB, glomerular basement membrane
PBMC, Peripheral blood mononuclear cells
GM, geometric mean
Con A, Concanavalin A

The pathogenesis of IMLNS is unknown though current observations suggest both immune and genetic mechanisms (1–3). We have previously shown that PBMC from IMLNS patients in relapse induced an increased incorporation of ^{35}sulfate in rat GBM (4). This increase in ^{35}sulfate incorporation was not observed when PBMC from IMLNS in remission were cocultured with rat glomeruli. Since the sulfate groups are an important source of anionic sites in the GBM (5), and the GBM negative charges seem to play a role in glomerular permeability (6), a change in sulfate metabolism may have pathogenic significance.

The onset and relapses of IMLNS usually follow viral infections of the upper respiratory tract (7). In some patients, relapses have been preceded by immunization (7) or exposure to allergens (8, 9). Thus, some type of activation of the immune system has occurred prior to the appearance of proteinuria.

The purpose of this study was to test the hypothesis that stimulation of PBMC from IMLNS patient in remission could reproduce the increased uptake of sulfate in the rat GBM observed when PBMC from IMLNS patients in relapse were cocultured with rat glomeruli.

MATERIALS AND METHODS

Patients. Twelve patients with IMLNS as defined by the International Study of Kidney Disease in Children were included in the study (10). Six were males and six were females. Their ages ranged from 5 to 21 yr with a median age of 14 yr. Twelve normal adults served as controls.

All patients were studied in remission. Remission was defined as serum albumin within normal limits and normal urinary protein excretion (trace or negative by Albustix or < 5 mg/m^2/h). At the time of the study, patients had been off prednisone for periods of 2 wk to 6 yr.

Isolation of PBMC. Peripheral blood mononuclear cells were isolated from heparinized blood by density centrifugation on a Ficoll Hypaque gradient. After washing three times with Hanks' balanced salt solution, viability was assessed by Trypan Blue exclusion (95% in all preparations).

Isolation of rat glomeruli. Glomeruli from Sprague Dawley rats (100–350 g) (Charles River, Wilmington, MA) were isolated by the method described by Fong and Drummond (11). At the end of the procedure, a drop of the preparation on a glass slide was examined for purity by counting and expressing as a percentage the total number of glomeruli and nonglomerular fragments. Preparations that were more than 5% contaminated with tubular fragments were discarded.

The glomeruli obtained from each rat were divided into four aliquots (Fig. 1). One was cocultured with PBMC, another was cocultured with PBMC from the same source (patient or normal control) and the addition of Con A at the concentration of 10 μg/ml culture media, the third was cultured only with Con A at the same concentration, and the fourth was cultured alone.

Glomeruli and PBMC were incubated in 5 ml of Basal Me-

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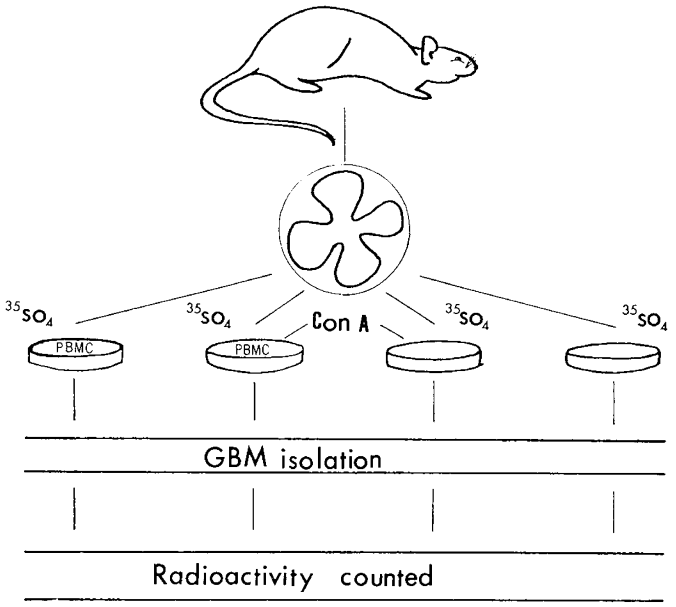

Fig. 1. Experimental design.

dium Eagle (without sulfate, Catalog no. 424-1300, Grand Island Biological Company, New York, NY). The culture medium was supplemented with 200 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 20% decomplemented fetal calf serum, and 80 μCi of ^{35}sulfate (specific activity, 209–335 mCi/mM; New England Nuclear, Boston, MA). Glomeruli and PBMC or glomeruli alone were incubated at 35°C in Petri dishes (50 × 50 mm) in a humidified atmosphere of 5% CO_2 and 10% O_2 for 48 h. At the end of incubation the samples were centrifuged, the supernatants were collected, and the pellet was lyophilized and kept at −35° C until the GBM was isolated.

Isolation of GBM. GBM was prepared from lyophilized, isolated glomeruli by the method of Meezam *et al.* (12) with minor modifications. The washed precipitate was dried and resuspended in Atomlight (New England Nuclear); radioactivity was counted for 1 min in a Beckman liquid scintillation counter. Results were expressed in cpm per milligram of dry glomerular weight.

To evaluate a direct effect of Con A or GBM ^{35}sulfate uptake, rat glomeruli, after being divided into aliquots, were cultured with different concentrations of the lectin (5, 10, and 20 μg/ml of culture media).

Serum albumin was determined by the brown-creosol-green method using a Technicon Autoanalyzer II. Urinary protein was measured by the sulfosalicylic method.

Statistical analysis was performed on an IBM PC computer using NWA Statpak (Northwest Analytical Inc., Portland, OR). Programs included two-way analysis of variance, randomized, block assignment, and multiple *t* tests. Analysis of variance and differences between means were performed on the logarithms of the measured values of ^{35}sulfate uptake by the GBM.

RESULTS

^{35}Sulfate incorporation in rat GBM was significantly higher when glomeruli were cultured with PBMC from 12 IMLNS patients in remission and stimulated with Con A (GM), 331 cpm/mg dry glomerular weight) than it was when glomeruli were cultured with the same PBMC without Con A (GM: 200 cpm/mg; $p = 0.048$) or were cultured alone with Con A (GM: 182 cpm/mg; $p = 0.0083$) or without Con A [GM 146 cpm/mg; $p = 0.0028$ (Table 1)].

The GBM ^{35}sulfate uptake after glomeruli were cultured with PBMC from 12 normal adult controls and stimulated with Con A (10 μg/ml) did not differ significantly from that observed when glomeruli were incubated with the same PBMC, but not stimulated with Con A, or when glomeruli were incubated alone with or without Con A. (ANOVA: F 1.36%; degree of freedom 3,33; p = NS) (Table 2).

Following incubation of glomeruli with different concentrations of Con A, no significant differences in ^{35}sulfate incorporation by GBM were seen between the experimental groups (Table 3).

Table 1. *^{35}Sulfate uptake by rat GBM after incubation of glomeruli with PBMC from IMLNS patients in remission and stimulated with Con A (10 μg/ml)**

^{35}Sulfate uptake (cpm/mg dry glomerular wt)			
A) Glomeruli + IMLNS PBMC	B) Glomeruli + IMLNS PBMC + Con A	C) Glomeruli alone + Con A	D) Glomeruli alone
192	173	144	89
213	380	251	473
110	195	92	82
127	138	53	85
722	444	188	396
75	105	128	97
129	147	52	119
257	287	231	142
286	1738	243	385
60	474	94	80
569	595	571	704
590	1222	158	437
GM incorporation			
200	331	182	146
95% prediction interval			
41–968	60–1840	36–924	36–586
ANOVA (F, df, p) (7.06; 3.33; 0.01)			

* Significance of differences: A *vs* B $t = 2.22$, $p = 0.048$; A *vs* C $t = 0.72$, p = NS; A *vs* D $t = 1.81$, p = NS; B *vs* C $t = 3.21$, $p = 0.0083$; B *vs* D $t = 3.83$, $p = 0.0028$; C *vs* D $t = 1.54$, p = NS.

Table 2. *^{35}Sulfate uptake by rat GBM after incubation of glomeruli with PBMC from normal controls and stimulated with Con A (10 μg/ml)**

^{35}Sulfate uptake (cpm/mg dry glomerular wt)			
A) Glomeruli + Normal PBMC	B) Glomeruli + Normal PBMC + Con A	C) Glomeruli alone + Con A	D) Glomeruli alone
74	170	151	35
51	135	135	100
214	98	120	83
79	120	52	100
240	112	54	65
339	257	55	74
132	59	120	89
100	50	78	158
37	44	26	132
275	282	170	204
100	170	166	98
123	81	32	76
GM incorporation			
117	110	81	91
95% prediction interval			
32–435	34–354	23–287	39–215
ANOVA (F; df; p) (1.368; 3.33; NS)			

* Significance of differences; ANOVA test p = NS.

Table 3. *^{35}Sulfate uptake by GBM after incubation of glomeruli from six rats with Con A*

Con A (μg/ml culture media)	^{35}Sulfate uptake (cpm/mg dry glomerular wt)
0	427 (144–1260)*
5	324 (84–1253)
10	389 (158–959)
20	417 (154–1125)

* GM (95% prediction interval).

DISCUSSION

In this study, the stimulation of PBMC from IMLNS patients in remission was associated with an increase in the ^{35}sulfate uptake by GBM of rat glomeruli cocultured with the PBMC. The effect seems restricted to IMLNS nephrotic PBMC since PBMC from normal controls stimulated with the same concentration of Con A in the culture media did not show any effect on ^{35}sulfate GBM incorporation. We did not stimulate PBMC from nephrotic patients with glomerular lesions other than minimal lesion because on previous studies we have shown that, like normal controls, PBMC of these patients in relapse did not induce an increase in rat GBM ^{35}sulfate uptake (13).

Con A is known to stimulate a variety of cells (14). However, the augmented ^{35}sulfate GBM incorporation was not due to a direct effect on glomerular cells because the incubation of glomeruli with different concentrations of Con A showed no significant differences in ^{35}sulfate uptake between stimulated and control glomerular cultures. Thus, the observed effect is likely due to stimulation of nephrotic PBMC. Con A is known to induce in humans the production of lymphokines by T and B cells (14). Con A does not stimulate the production of monokines by macrophages (15). However, although the lymphocyte is the source of the lymphokine, the presence of macrophages may augment its production, presumably by presenting the antigen or mitogen to the lymphocyte (16, 17). Therefore in this study, T or B cells with or without the cooperation of macrophages can be the cell responsible for the lymphokine that increases ^{35}sulfate uptake in the rat GBM.

In this study, we have shown that Con A stimulation of PBMC from IMLNS patients in remission reproduces the effect observed when PBMC from IMLNS patients in relapse are cultured with glomeruli. Although, the effect on GBM ^{35}sulfate uptake is similar, it needs to be proven whether the supernatant factor secreted in these two experimental conditions represents the same factor. If this is confirmed, these data suggest that the supernatant factor is a lymphokine.

In previous reports, we have advanced the hypothesis that IMLNS PBMC secrete cytokines that by altering the GBM sulfate metabolism, induce proteinuria (4, 13). In the GBM, the ^{35}sulfate is incorporated into glycopeptides and glycosaminoglycans. Glycosaminoglycans seem to play a role in glomerular permeability (5). Its removal is associated with an increase in GBM anionic sites (6). Heparan sulfate has been shown to be decreased in the GBM of patients with congenital nephrotic syndrome and in rats with aminonucleoside nephrosis (18, 19). Furthermore, a decrease in the number of anionic sites (thought to be composed of glycosaminoglycans) in the lamina rarae interna of the GBM of patients with IMLNS has been reported (20).

The lymphokine may have pathogenic significance by augmenting the catabolism of the GBM sulfated compounds. The observed increased sulfate uptake by the GBM would be the result of a subsequent increase in the synthesis of the sulfated compounds. However, if the enhanced synthesis could not compensate for the augmented catabolism, this would result in a decrease in the net ionic charge of the GBM. Studies on the metabolic kinetics of GBM glycosaminoglycans and on the number of anionic sites after glomeruli are cultured with the lymphokine are needed to support this hypothesis. Furthermore, the stimulation of nephrotic PBMC in remission with Con A will allow us to produce the lymphokine in large quantities. Thus, studies using large concentrations of the supernatant factor and tracer molecules to assess changes in glomerular permeability can be performed. These studies may give clues into the pathogenesis of IMLNS.

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