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In vitro decrease of glomerular heparan sulfate by lymphocytes from idiopathic nephrotic syndrome patients

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Background. Lymphocytes are involved in the physiopathologic mechanism of idiopathic nephrotic syndrome (INS). We have recently demonstrated that plasma from patients with INS decreases human glomerular epithelial cell (GEC) glycosaminoglycans (GAGs), particularly heparan sulfates (HS) in vitro. In this study we investigate the effect of peripheral blood lymphocytes (PBL) from INS patients on glomerular cell GAG and HS.

Methods. Human GECs were cultured with total peripheral blood mononuclear cells (PBMCs), PBL, and monocytes from patients and controls. The amounts of GAG and HS were assessed using a cationic membrane after metabolic labeling.

Results. In coculture with GECs, mononuclear cells from controls decreased total epithelial cell GAG (−30% with PBMC, $P < 0.05$; −25% with PBL, $P < 0.02$; −19% with monocytes, $P < 0.05$). Particularly HSs were decreased (−36% with PBMC, $P < 0.05$; −27% with PBL, $P < 0.02$; and −19% with monocytes, $P < 0.05$). When GECs were in coculture with PBL from INS patients, the decrease in GAG and HS was significantly greater in comparison to control PBL (−10%, $P < 0.02$; −10%, $P < 0.02$, respectively, for GAG and HS). Moreover, supernatants of stimulated PBMCs from patients decreased also GAG and HS in comparison with controls (−13%, $P < 0.02$; −15%, $P < 0.02$, respectively, for GAG and HS).

Conclusion. These data provide direct evidence that PBLs from INS patients are able to decrease GEC HS as previously shown with plasma from patients. This might be instrumental in the onset of albuminuria.

Lymphocyte function seems to be involved in the physiopathology of idiopathic nephrotic syndrome (INS) [1]. Subsequently, the association of INS with thymoma or Hodgkin's disease [1–4] and with allergic phenomena [2, 5] was described. Various studies have shown modifications of lymphocyte counts and lymphocyte functions in patients with INS. Modifications of subclasses of lymphocytes were described by some authors [6–9], particularly a decrease in CD4 versus CD8 cells, and a modification of the equilibrium between Th1 and Th2 [9], although these findings contradicted a previous study [10]. Moreover, lymphocytes from INS patients exhibit a decreased proliferative response to allogeneic cells and to phytoheamagglutinin (PHA) [11–13]. Humoral-mediated immunity is also affected with a decrease in plasma IgG [2, 14] caused by modifications in the IgM-IgG switch [15]. Finally, the effectiveness of immunosuppressive drugs such as corticosteroids and cyclosporine A to induce remission demonstrates that changes in the immune system contribute to the physiopathology of INS [16–19].

The involvement of a circulating factor in INS is suggested by transient proteinuria in newborns from women with INS [20] and by proteinuria in rats after injection of sera from INS patients [21, 22]. The relapse of proteinuria after renal transplantation and the remission after immunoadsorption of plasma from patients support this hypothesis [23]. Moreover, serum from patients increases glomerular permeability to albumin in an in vitro model [24]. The cellular origin of this factor is unknown. Nevertheless, proteinuria can be induced in rats by a factor synthesized by T-cell hybridomas [25] and by supernatants of mononuclear cells from INS patients [26, 27]. These results strongly suggest that lymphocytes may be the cells that secrete this factor(s). Furthermore, a soluble immune response suppressor factor [28] and a vascular permeability factor that may modify the glomerular permeability [29–32] are derived from lymphocytes from INS patients.

Previous studies have demonstrated a decrease in the polyanion heparan sulfate (HS) chains on kidney biopsies of patients with INS [33], and we have recently reported that plasma from children with INS is able to decrease the synthesis of secreted and cellular glycosami-

Key words: glomerular epithelial cell, albuminuria, thymoma, Hodgkin's disease, proteinuria, circulating factor, mononuclear cells.

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noglycans (GAGs) in vitro, particularly the synthesis of glomerular cell HS [34]. The circulating factor in plasma from patients might have an effect on glomerular cells by decreasing HS and could contribute to albuminuria.

The present study was carried out to determine whether mononuclear cells from INS patients and from controls could modify human glomerular epithelial cell (GEC) HS and chondroitin/dermatan sulfates (CS), as previously shown with INS plasma. We studied the effect of mononuclear cells from INS patients in coculture with human GECs and the effect of peripheral blood mononuclear cell (PBMC) supernatants on the cell-bound fraction and the secreted fraction of epithelial cell HS and CS.

Table 1. Clinical data

Patient	Diagnosis	Treatment	Urinary prot/creat *g/mmol*	Urinary protein *g/l*	Plasma albumin *g/l*
1	INS-Rel	WT	0.52	3.33	9.43
2	INS-Init	WT	1.93	16.0	10.85
3	INS-Rel	WT	3.33	27.0	18.6
4	INS-Init	WT	1.33	4.8	14.6
5	INS-Init	WT	0.98	3.5	12.6
6	INS-Rel	WT	0.66	10.0	30.8
7	INS-Rel	Pred	0.83	3.06	30.7
8	INS-Rel	Pred	0.3	1.87	NA
9	INS-Rel	Pred	0.89	4.2	12.5
10	INS-Rel	Pred	0.14	1.1	32
11	INS-Rel	Pred	0.6	3.0	15.1
12	INS-Rel	Pred	0.8	1.12	NA

Abbreviations are: INS, idiopathic nephrotic syndrome; Init, initial episode; Rel, relapse; WT, without treatment; Pred, treated with prednisolone; NA, not available.

METHODS

Patients

The definition of INS and of response to steroid therapy was those used by the International Study for Kidney Disease in Children [35]. Mononuclear cells were obtained from 12 children with INS, at initial episode for 3 children and at relapse for 9 children. They all had a typical presentation of the disease and responded to the steroid treatment. None had received any other immunosuppressive drug, particularly cyclosporine A. On account of the small volume of blood samples, mononuclear cells of each child were studied either in coculture or in preparation of supernatants. Table 1 summarizes the clinical features of the INS patients. PBMCs were collected from patients 1, 4, 8, 9, and 12. Peripheral blood lymphocytes (PBLs) were collected from patients 1, 2, 3, 5, 6, 7, and 12, and monocytes were collected from patients 3, 5, 6, 7, and 12. Supernatants were prepared from PBMCs from patients 1, 2, 3, 4, 8, 9, 10, and 11.

Blood samples

Blood samples were collected on ethylenediaminetetraacetic acid (EDTA). Control blood samples were obtained from healthy volunteers (20 to 36 years) without proteinuria or medication. Preliminary studies showed that age had no influence on the action of mononuclear cells on glomerular cell GAG, CS, and HS.

The study was approved by the ethics committee of our institution.

Glomerular cell culture

Glomerular epithelial cells were obtained from the normal cortex of kidneys removed from patients who had unilateral nephrectomy for malignant neoplasm or urologic malformation, as previously described [36]. Briefly, the renal cortex was homogenized and pushed through 80 and 140 mesh sieves. The glomeruli were incubated with 300 U/mL collagenase type I (Sigma, St. Louis, MO, USA) at 37°C. After 30 minutes, they were pushed through a 500 mesh sieve. GECs were recovered in RPMI 1640 culture medium (GIBCO, Grand Island, NY, USA) supplemented with 10 mmol/L HEPES (Sigma), 2 mmol/L L-glutamine (GIBCO), 50 UI/mL penicillin-50 μg/mL streptomycin (GIBCO) and 20% heat-inactivated fetal calf serum (FCS; GIBCO). GECs were incubated in bovine gelatin (Sigma)-coated culture flasks at 37°C, 5% CO_2. GECs were identified by their typical morphology on light microscopy (Fig. 1), positive staining with cytokeratine and vimentine, and negative staining for factor VIII and actin [37–39]. Immunohistological techniques were also assessed, and a positive staining was observed with monoclonal antibodies against common acute lymphocytic leukemia antigen (CALLA) and Wilms' tumor (WT-1) [40, 41]. Finally, cGMP synthesis was increased when cells were stimulated by atrial natriuretic peptide [42]. Subcultures of third and fourth passages were used.

Mononuclear cell preparation

Peripheral blood mononuclear cells were isolated from venous blood by Ficoll density centrifugation (Ficoll-Paque; Pharmacia Biotech, Quebec, Canada). The cells were collected and washed in RPMI 1640 supplemented with 10% heat-inactivated FCS.

Peripheral blood lymphocytes were separated from the PBMC population by removing the monocytes that adhered to plastic: The PBMCs were resuspended at 4×10^6 PBMC/mL in medium supplemented with 10% FCS and incubated for one hour at 37°C, 5% CO_2 in a plastic culture flask. The nonadherent PBL were collected by gentle washing with medium. The cell population obtained in these conditions contained less than 0.2% nonlymphocyte cells by fluorescence-activated cell sorter analysis after incubation with monoclonal antibodies (data not shown).

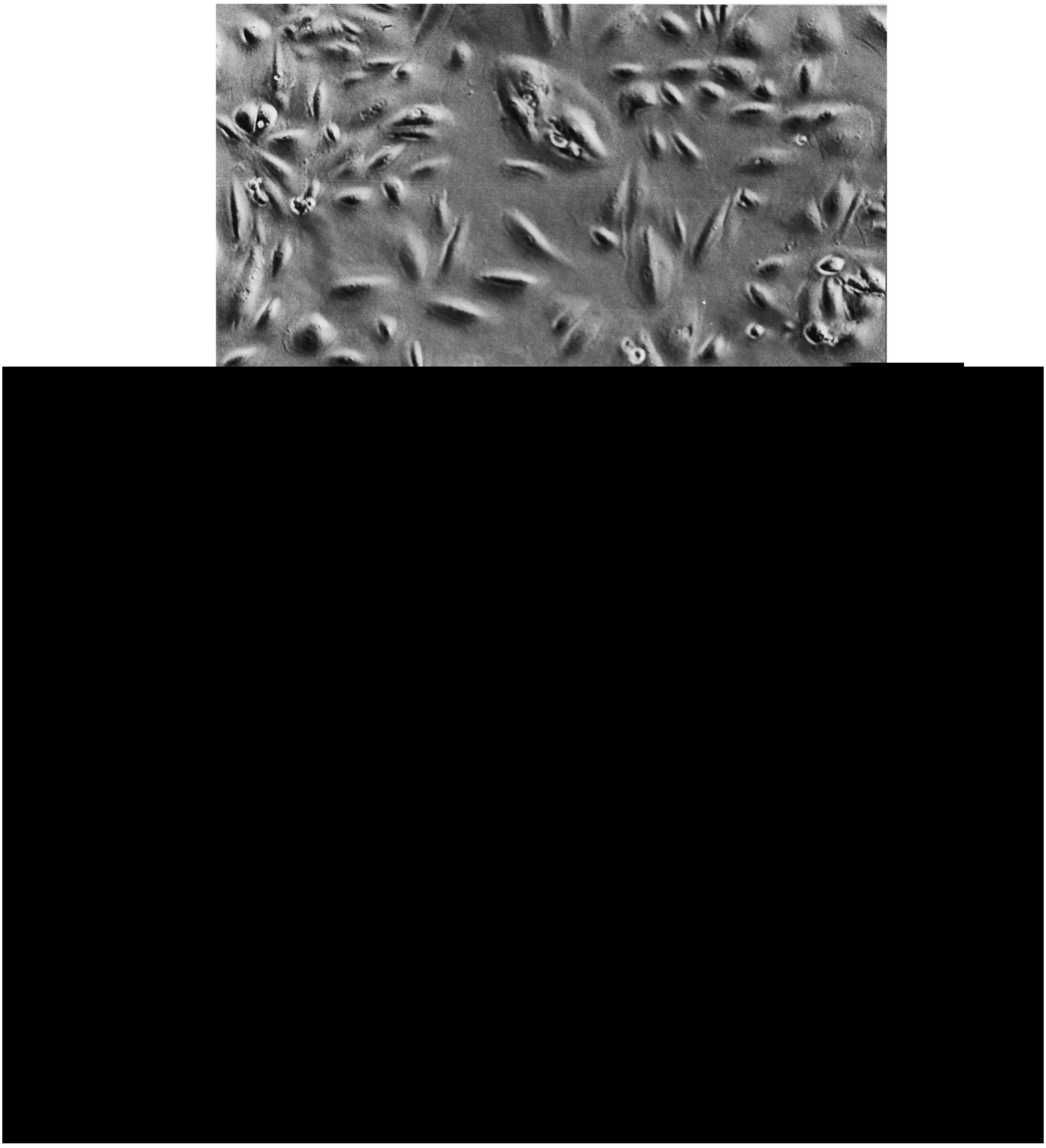

Fig. 1. Human glomerular epithelial cells (GECs). (*A*) General aspect of a confluent culture on light microscopy. (*B*) After immunohistologic staining with monoclonal antibodies against CALLA on isolated cells. (*C*) After immunohistologic staining with monoclonal antibodies against WT-1 on isolated cells. Negative controls showed no staining. (A ×400; B and C ×700).

Monocytes were detached from the plastic by incubation of the adherent cells in medium containing EDTA 5 mmol/L and 20% FCS for 15 minutes at 4°C.

Supernatants of PBMCs from patients or controls were obtained by incubation of 2×10^6 PBMC/mL in medium (RPMI 1640) containing 10% FCS and 1 μg/mL PHA (Sigma) at 37°C, 5% CO_2. After 48 hours, cells were centrifuged, and the supernatants were removed and stored at −80°C until processed. Supernatants were heat inactivated before added to the cultures.

Quantitation of GAG and HS

Glomerular epithelial cells were cultured to confluent monolayers in 24-well plates. The confluent monolayers of GECs were at the bottom of the plates, whereas the mononuclear cells were incubated in inserts (Falcon, Meylan, France) with pores of 0.4 μm to prevent a direct contact between the two cell types and to allow the passage of molecules. PBMCs or PBLs (0.8×10^6/well, 2×10^6 cells/mL) and monocytes (0.05×10^6/well, 0.125×10^6 cells/mL) were incubated in inserts. The cocultures were incubated in SO_4-free medium containing 50 μCi/mL $Na_2{}^{35}SO_4$ (specific activity: 1050 to 1600 Ci/mmol; Amersham, Arlington Heights, IL, USA) and 5% FCS. For some experiments, GECs were incubated with 5 and 10% supernatants of PBMCs in SO_4-free medium containing 50 μCi/mL $Na_2{}^{35}SO_4$, without FCS. All experiments were conducted in duplicate.

After 24 hours, the secreted and the cell-bound fractions of HS and GAG were quantitated as previously described [34, 43]. Briefly, GAGs were extracted in Tris buffer containing 8 mol/L urea and 0.1% Triton X-100, heated at 90° for 20 minutes, and bound to a cationic nylon membrane (Zeta-probe; Bio-Rad Laboratories, Hercules, CA, USA) using a dot-blot technique. Two fractions of each sample were pulled through the blot by vacuum. The first was incubated in Tris buffer containing 0.65 mol/L NaCl for one hour, washed, and dried. The other was washed and incubated for 2×90 minutes with nitrous acid in order to depolymerize and release HS chains [44], and then treated as the first. In this condition, only GAG-bearing CS chains remained bound to the membrane. The blots were then cut for scintillation counting. The amount of HS was evaluated by the difference between total cpm and cpm obtained after nitrous acid treatment. The method was validated by comparison with Diethylaminoethyl (DEAE)-Sephacel chromatography and gel filtration [34, 45]. Radiolabeled GAGs were isolated by DEAE-Sephacel chromatography after β-elimination and phenol extraction to remove the core proteins. GAGs were than treated either with chondroitinase ABC (Sigma), either with heparitinase (Seikagaku, Tokyo, Japan), either with both enzymes. We showed that glomerular cell GAGs were only HS and CS, and the proportion of CS and HS was similar with both techniques. Moreover, supernatants of GEC cultures were also treated with chondroitinase ABC or heparitinase or both enzymes. We found that there was no nonspecific binding on the cationic membrane; indeed, after treatment with both enzymes, no binding of ^{35}S-labeled molecules was detected, and the amounts of HS and CS were the same than with the dot-blot technique. For all experiments, cells were grown in parallel in exactly the same experimental conditions. Cells were scraped and dissolved in Tris buffer containing 2 mol/L urea. The amounts of cell protein for each well were measured using the Bio-Rad protein assay.

The results were expressed as cpm/μg of cell protein for each test condition divided by cpm/μg of cell protein for the control condition.

Preliminary studies showed that secreted and cell-bound HS and CS fractions of mononuclear cells after 24 hours of incubation in medium or in coculture with glomerular cells were insignificant. Mononuclear cells in inserts were then analyzed with the supernatants of GECs.

Study of GAG chains depolymerization

Glomerular epithelial cells were cultured without mononuclear cells or in coculture with control PBL in inserts or with patient PBL in inserts in 24-well plates in medium with $Na_2{}^{35}SO_4$ for 24 hours, in the same conditions as for quantitation of GAG. Culture medium of each condition was collected and centrifuged. Supernatants were treated by β-elimination to cleave GAG chains from the core proteins, and proteins were removed by phenol extraction [46].

Polyacrylamide gel electrophoresis

The labeled samples were loaded on a polyacrylamide gradient gel [47]. Each sample was loaded in duplicate. The gel was run at 400 V for three hours. The ^{35}S-labeled GAG chains were revealed by autoradiography. The standard samples showed that GAGs with molecular weight above 5000 D were detected on the gel.

Gel filtration

Glycosaminoglycan chains' size distribution was analyzed on a Sephadex G-50 column (Pharmacia) eluted in 10 mmol/L NH_4HCO_3 buffer. The column was loaded with the labeled GAG chains; 0.5 mL fractions were collected, and GAG chains were quantitated by scintillation counting. The samples were studied before and after treatment with heparitinase (Sigma).

Statistics

Data are expressed as the mean ± SE. *N* represents the number of experiments. The Wilcoxon signed-rank test was used to evaluate the significance of the results.

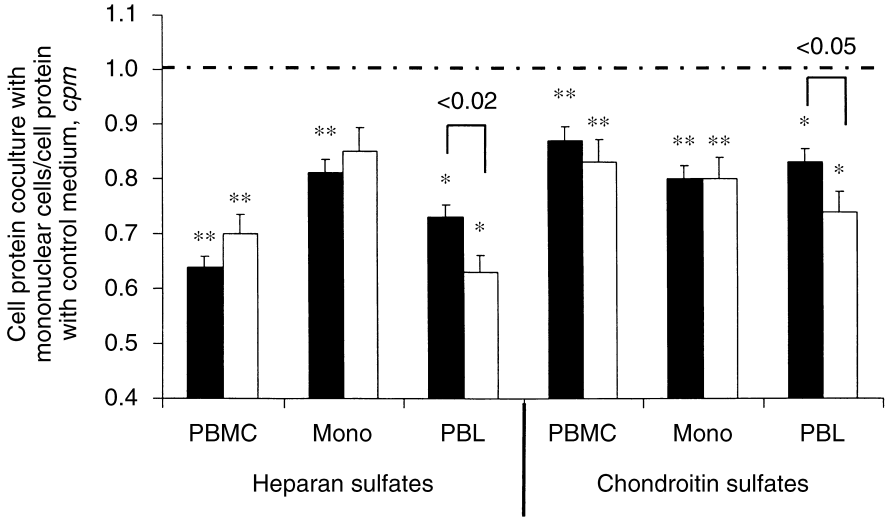

Fig. 2. Effect of mononuclear cells from normal controls (■) and idiopathic nephrotic syndrome (INS; (□) patients on glomerular epithelial cells (GEC) heparan sulfate (HS) and chondroitin/dermatan sulfates (CS). GECs were cocultured with PBMCs, monocytes, or PBLs for 24 hours in medium with ^{35}S for metabolic labeling of GAG. HS and CS were quantitated as described in the **Methods** section. The results are expressed as mean ± SE of the ratios of the cpm/μg cell protein when GECs were in coculture with mononuclear cells divided by cpm/μg cell protein when GECs were in medium without mononuclear cells, of each experiment. $*P < 0.02$; $**P < 0.05$, in comparison to the situation with medium without mononuclear cells whose ratio is 1 (horizontal dashed line). $N = 5$ for PBMCs (patients 1, 4, 8, 9, and 12) and monocytes (patients 3, 5, 6, 7, and 12), $N = 7$ for PBLs (patients 1, 2, 3, 5, 6, 7, and 12).

RESULTS

Effect of mononuclear cells from controls and INS patients on glomerular epithelial cell GAG, HS, and CS

When GECs were cocultured with control mononuclear cells, the glomerular cell GAG decreased significantly (0.70 ± 0.02, $P = 0.04$ with PBMCs; 0.81 ± 0.02, $P = 0.04$ with monocytes; and 0.75 ± 0.03, $P = 0.02$ with PBL in comparison with medium without mononuclear cells). When GECs were in coculture with PBL from patients, the decrease in GAG was significantly more important than when GECs were in coculture with control PBL (0.65 ± 0.05 with PBL from patients, $P < 0.02$ in comparison to medium without mononuclear cells and $P < 0.02$ in comparison with coculture with control PBL).

As shown in Figure 2, when GECs were in coculture with PBMCs from controls and from patients, HS decreased significantly (0.64 ± 0.03, $P = 0.04$, and 0.70 ± 0.04, $P = 0.04$, respectively). When GECs were in coculture with monocytes, HS decreased only with monocytes from controls, whereas monocytes from patients induced no significant change in HS (0.81 ± 0.02, $P = 0.04$, and 0.85 ± 0.05, NS, respectively, with control monocytes and with patient monocytes). In coculture with PBMCs and monocytes, the decrease in glomerular cell HS was similar with control mononuclear cells and with patient mononuclear cells.

When GECs were in coculture with PBLs from controls and from patients, HS deceased significantly (0.73 ± 0.03, $P = 0.02$, and 0.63 ± 0.05, $P < 0.02$, respectively). In coculture with patient PBLs, glomerular cell HS decreased significantly more than in coculture with control PBLs ($P < 0.02$; Fig. 2). For each experiment, GECs were in coculture with PBLs from one INS patient in comparison with PBLs from one control, and in each case, we observed that PBLs from INS patient decreased more GAG and HS than PBLs from control. We did not observe a difference of effects of mononuclear cells from INS patients with steroid treatment or without treatment, and no difference between patients during the initial episode of disease or in relapse.

Chondroitin/dermatan sulfates decreased significantly when GECs were in coculture with PBMCs from controls and from patients (0.87 ± 0.04, $P = 0.04$, and 0.83 ± 0.05, $P = 0.04$, respectively) and with monocytes from controls and from patients (0.80 ± 0.04, $P = 0.04$, and 0.80 ± 0.04, $P = 0.04$, respectively; Fig. 2). In coculture with PBMCs and with monocytes, the decrease in glomerular CS was similar with control mononuclear cells and with patient mononuclear cells. When GECs were in coculture with control PBL and with patient PBL, CS decreased significantly (0.83 ± 0.03, $P = 0.02$, and 0.74 ± 0.06, $P < 0.02$, respectively). In coculture with PBLs, CS decreased significantly more with PBLs from patients in comparison with PBLs from controls ($P < 0.05$; Fig. 2).

With control or patient PBMCs and PBLs, the effect was more important on HS than on CS, whereas with control or patient monocytes the effect was similar on HS and CS, but smaller than the effect of PBLs and PBMCs on HS. With patient PBMCs and PBLs, glomerular cell HS and CS decreased significantly, whereas with monocytes only CS decreased significantly.

Effect of mononuclear cells from controls and INS patients on the secreted and cell-bound fractions of GEC HS and CS

As shown in Figure 3A, the effect on the secreted fraction of glomerular cell HS induced by control and INS patient PBMCs, monocytes, and PBLs was very similar to the effect on the total HS (Fig. 2). The effect on HS induced by PBMCs and PBLs was more important

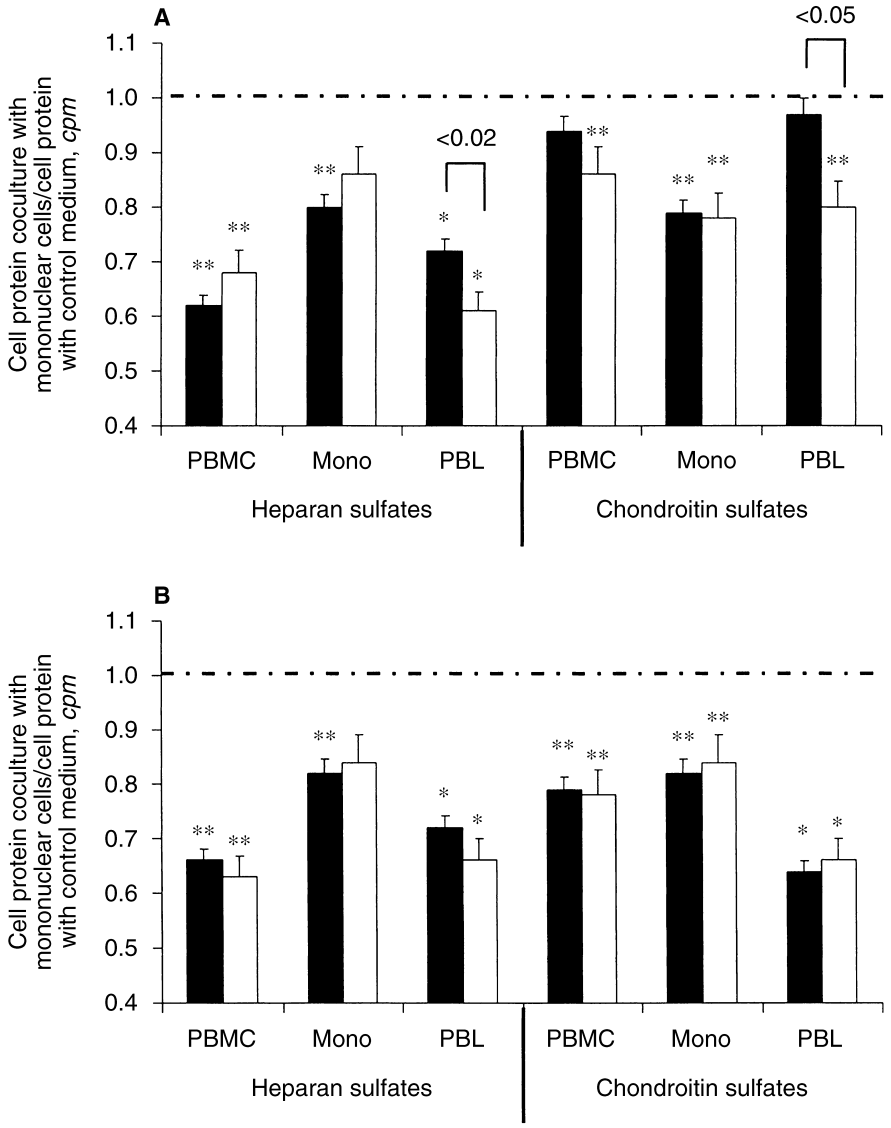

Fig. 3. Effect of PBMCs, PBLs, and monocytes from normal controls (■) and INS patients (□) on the secreted (*A*) and the cell-bound (*B*) fractions of GEC HS and CS. GECs were cocultured with PBMCs, monocytes, or PBLs for 24 hours in medium with ^{35}S for metabolic labeling of GAG. The secreted (A) and the cell-bound (B) fractions of HS and CS were quantitated as described in the **Methods** section. The results are expressed as mean ± SE of the ratios of the cpm/μg cell protein when GECs were in coculture with mononuclear cells divided by cpm/μg cell protein when GECs were in medium without mononuclear cells, of each experiment. $^{*}P < 0.02$; $^{**}P < 0.05$, in comparison to the situation with medium without mononuclear cells whose ratio is 1 (dashed horizontal line). $N = 5$ for PBMCs (patients 1, 4, 8, 9, and 12) and monocytes (patients 3, 5, 6, 7, and 12), $N = 7$ for PBLs (patients 1, 2, 3, 5, 6, 7, and 12).

than the effect induced by monocytes, as it was shown for the total HS. When the effect of mononuclear cells from patients was compared with the effect of mononuclear cells from controls, we observed that PBLs from patients decreased the secreted fraction of glomerular cell HS significantly more than PBLs from controls, whereas there was no significant effect of monocytes from patients on HS in comparison with monocytes from controls.

The effect on the secreted fraction of glomerular cell CS in coculture with control mononuclear cells was only significant with monocytes. Control PBMCs and PBLs did not induce any modification on this fraction. In contrast, PBMCs, PBLs, and monocytes from patients all decreased the secreted fraction of CS (Fig. 3A).

The effect on the cell-bound fraction of glomerular cell HS and CS was different from the effect observed on the total and secreted HS and CS (Fig. 3B). Although the effect of PBMCs and PBLs was again more important than the effect of monocytes, the range of the decrease in HS and in CS was comparable regardless of the mononuclear cells. Moreover, there was no difference between the effect of mononuclear cells from controls and from patients, except for monocytes from patients, which did not induce a significant decrease in the cell-bound fraction of HS.

Effect of supernatants of stimulated PBMCs from INS patients or controls on glomerular epithelial cell GAG, HS, and CS

When GECs were cultured with medium containing 5% supernatants of PHA-stimulated PBMC from INS patients, total GAG, HS, and CS decreased significantly in comparison with supernatants of PHA-stimulated

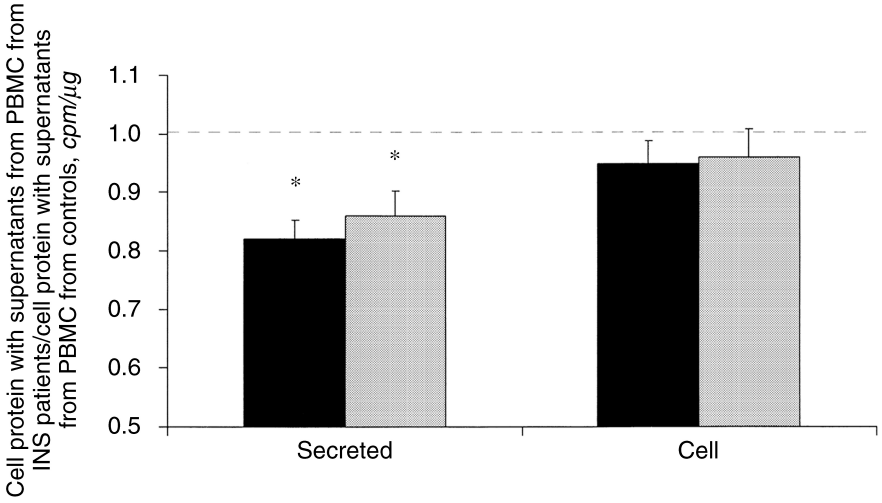

Fig. 4. Effect of supernatants from stimulated PBMCs from INS patients on the secreted and cellular fractions of GEC HS (■) and CS (▒). PBMCs from INS patients or controls were cultured for 48 hours in medium with 10% FCS and 1 μg/mL PHA. Supernatants were removed and added at 5% to medium with ^{35}S for metabolic labeling of GAG. GECs were cultured for 24 hours in this medium. HS and CS were quantitated as described in the **Methods** section. The results are expressed as mean ± SE of the ratios of the cpm/μg cell protein when GECs were cultured with supernatants of PBMCs from INS patients divided by cpm/μg cell protein when GECs were cultured with supernatants of PBMCs from controls, of each experiment. $*P < 0.02$ for the situation with supernatants from patients in comparison to controls. No difference is a ratio of 1 (dashed horizontal line). $N = 8$ (patients 1, 2, 3, 4, 8, 9, 10, and 11).

PBMCs from normal volunteers (0.87 ± 0.03, $P < 0.02$, 0.85 ± 0.05, $P < 0.02$, and 0.89 ± 0.02 $P < 0.02$, respectively, for GAG, HS, and CS; $N = 8$). As shown in Figure 4, these modifications were prevalent on the secreted fractions of HS and CS, whereas the decrease of the cell-bound fractions of HS and CS was not significant. For each experiment, GECs were in coculture with the supernatant of activated PBMCs from one INS patient in comparison with the supernatant of activated PBMCs from one control, and in each case, we observed that the supernatant of activated PBMCs from INS patient decreased more GAG and HS than the supernatant of activated PBMCs from control. No difference of effects of PBMC supernatants from INS patients with steroid treatment or without treatment was observed, nor was there a difference between patients during the initial episode of disease or in relapse.

When GECs were cultured with medium containing 10% supernatants of PHA-stimulated PBMCs, secreted HS and CS decreased in the same manner as with 5% supernatants (data not shown).

Study of GAG chains depolymerization

Polyacrylamide gel electrophoresis. When supernatants of GEC cultures were studied by polyacrylamide gel electrophoresis (PAGE), we observed a similar migration of GAG chains in the three conditions (Fig. 5): without mononuclear cells (lanes I), in coculture with control PBLs (lanes II), and in coculture with patient PBLs (lanes III). All smears were on the same level, indicating that there was no depolymerization of HS chains.

Gel filtration. Elution profiles on the Sephadex G-50 column of ^{35}S-labeled GAG chains were similar in the three conditions, and no degradation products were observed. In contrast, when the samples were previously treated with heparitinase, the profiles were changed with the presence of degradation products.

DISCUSSION

In this study, we demonstrated that mononuclear cells decreased glomerular cell HS and CS when both cell types were in coculture. This effect was induced by control mononuclear cells, but especially by INS patient cells.

First, when GECs were in coculture with PBMCs, glomerular cell HS and CS decreased. This effect was greater on HS. The decrease of HS was induced in particular by PBLs and less by monocytes. The decrease of CS was induced by PBLs and monocytes in a similar manner. Finally, the decrease of HS involved equally the cell-bound and the secreted fractions, whereas the decrease of CS involved only the cell-bound fraction, except for monocytes, which induced a decrease in both fractions. These results indicated that mononuclear cells from normal allogeneic controls were able to decrease glomerular cell GAG, particularly HS. It has been previously shown that activated lymphocytes are able to release and degrade HS of endothelial cells and of the extracellular matrix [48, 49], but to the best of our knowledge, this is the first study that emphasizes the direct effect of mononuclear cells, especially PBLs on glomerular cell HS. Studies of GAG chains on polyacrylamide gradient gel and gel filtration showed that there was no depolymerization of GAG chains when GECs were in coculture with control PBL in comparison with medium without mononuclear cells, indicating that the decrease in glomerular cell HS induced by control PBL was not induced by an increased degradation of these chains. Finally, our data did not permit explanation of the more pronounced effect on HS particularly by PBMCs and

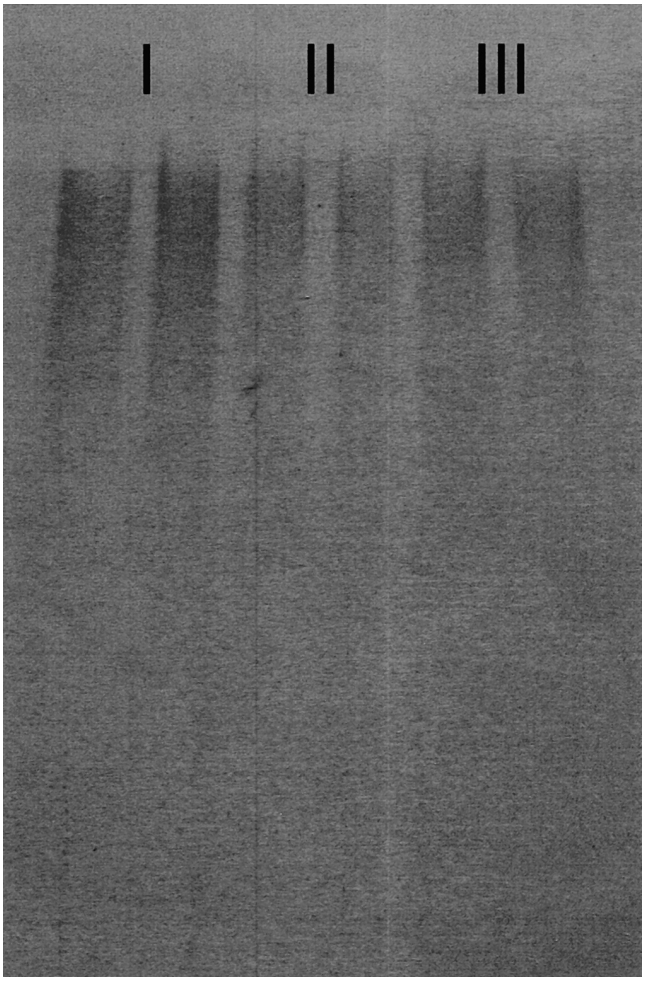

Fig. 5. Polyacrylamide gradient gel of glomerular cell GAG chains. GECs were cultured for 24 hours in medium with ^{35}S for metabolic labeling of GAG. After removing of proteins from the supernatants of the cultures, the samples were loaded on a polyacrylamide gradient gel, each sample in duplicate: culture in medium without mononuclear cells (lanes I), coculture with control PBL (lanes II), and coculture with patient PBL (lanes III). GAG chains were revealed by autoradiography.

PBLs in comparison with the effect on CS. However, this effect was not induced by an increased degradation.

More importantly, mononuclear cells from patients varied from those of controls by at least three ways. First, PBLs from patients decreased the secreted fraction of HS more importantly than PBL from controls. Second, PBMCs and PBLs from patients but not from controls decreased the secreted fraction of glomerular cell CS. Finally, in agreement, supernatants of PHA-stimulated PBMCs from patients decreased significantly more the secreted HS and CS than supernatants of PHA-stimulated PBMCs from controls.

The effect of PBL and of supernatants of stimulated PBMC from patients is comparable to the effect of plasma from INS patients, which we have shown previously [34]. Indeed, we demonstrated that glomerular cell HS and CS are decreased when GECs are incubated with INS plasma in comparison with control plasma, and in these conditions, the cell-bound and the secreted fractions of HS and CS are decreased. Finally, PAGE and gel filtration showed that there was no depolymerization of GAG chains. Thus, the decrease in glomerular cell HS induced by patient PBL was probably induced by a decrease in synthesis of HS chains, as it was shown with plasma from INS patients.

In this study, the decrease in HS and CS in our in vitro model was induced by one or various factors secreted or released by PBLs, since there was no direct contact between PBLs and GECs, and supernatants of stimulated PBMCs had the same action. This is in agreement with studies in which supernatants of mononuclear cells from INS patients increased glomerular permeability to albumin in animal models [26, 27]. Moreover, mononuclear cells from INS patients induced a decrease in polyanions in vitro on rat kidney tissue sections [50]. This factor(s) could be the plasma-borne circulating factor(s) whose involvement was suggested in INS by transient proteinuria in newborns from women with INS [20], by proteinuria in rats after injection of sera from INS patients [21, 22], by relapse of proteinuria after renal transplantation and a remission after immunoadsorption of the plasma [23], and by an increase in glomerular permeability induced by serum from patients in an in vitro model [24].

The decrease in GAG might be involved in the physiopathologic mechanism of proteinuria in INS. Indeed, HS had a role in glomerular permeability to albumin. HS is the major GAG of the glomerular basement membrane [51, 52] and is present in the polyanionic glycocalyx of the apical plasma membrane of GEC [53]. A decrease of polyanions has been demonstrated during albuminuria states in various studies [54, 55], particularly in INS in relapse, whereas normalization has been observed in remission [56–59]. The role of HS with regard to other polyanions is most prevalent in the glomerular permeability [60]. A decrease in HS in the glomerular basement membrane has been described in disease states in which glomerular permeability to albumin is increased [61], especially in INS [33] and in experimental nephrotic syndrome in animal models [62, 63]. Moreover, a monoclonal antibody against glomerular basement membrane HS was shown to induce acute selective proteinuria in rats [64]. Thus, our data showing that PBL from INS patients decrease GAG and particularly HS indicate that PBLs contribute to the increase of glomerular permeability to albumin. Further experiments will state which are the factors involved in the cross-talk between the two cells and the exact implication of this phenomenon in the pathogenesis of INS.

The plasma factor(s) was secreted by lymphocytes, but

we cannot rule out an origin other than mononuclear cells. Indeed, plasma from some INS patients might induce modifications in lymphocyte functions from normal volunteers, particularly an impairment of mitogenic response to PHA, as it was shown with PBLs from patients [12, 13]. Preliminary studies in our in vitro model showed that PBLs from normal volunteers incubated with plasma from some INS patients induced a larger decrease in glomerular cell HS and CS in comparison to PBLs from normal volunteers incubated with control plasma (data not shown). Thus, the plasma from some INS patients seems to contain factors able to induce modifications in PBLs from normal volunteers so that these control PBLs had the same action as PBLs from INS patients on glomerular cell HS. The plasma-borne factor(s) was secreted by PBLs from patients, but we could not exclude an other origin of either the same factor(s) or perhaps of the other factor(s).

In conclusion, we have shown that mononuclear cells from controls and from INS patients decreased glomerular cell HS and CS. Moreover, this effect was more prominent when GECs were in coculture with PBLs from INS patients in comparison with control PBLs. A similar effect was previously shown with plasma from INS patients. These in vitro results were not a direct reflection of the in vivo situation, but we hypothesize that one or several factors released in the plasma from INS patients by PBLs might modify GECs and glomerular basement membrane HS, and thus could increase glomerular permeability to albumin. Further studies will characterize the modifications in the PBL function of these patients and better determine which plasma-borne factors are involved in this disease.

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APPENDIX

Abbreviations used in this article are: BSA, bovine serum albumin; CS, chondroitin/dermatan sulfates; FCS, fetal calf serum; GAG, glycosaminoglycans; GEC, glomerular epithelial cells; HS, heparan sulfates; INS, idiopathic nephrotic syndrome; PAGE, polyacrylamide gel electrophoresis; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin.

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