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Kidney International, Vol. 40 (1991), pp. 453–460

A glomerular permeability factor produced by human T cell hybridomas

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A glomerular permeability factor produced by human T cell hybridomas. T cell hybridomas derived from the T cells of a patient with minimal change nephrotic syndrome (MCNS) made a glomerular permeability factor (GPF). Sufficient quantities of GPF were available for further analysis and characterization. We obtained four stable clones of human T cell hybridomas which produced a glomerular permeability factor. When this factor was injected intravenously into rats, significant proteinurias were induced, and in normal human lymphocyte culture, GPF enhanced Concanavalin-A (Con-A) induced lymphocyte blastogenesis by greater than ten fold. GPF was cytotoxic to tumor cell lines of epithelial origin, but only cytostatic to tumor cells of hematopoietic origin. Electron microscopy studies, with polyethyleneimine (PEI) staining, indicated that GPF induced the changes in the arrangement of PEI particles and partial fusion of glomerular epithelial cells in the rats given this factor intravenously. The molecular weight of GPF were estimated to be between 60,000 and 160,000 daltons. The molecular weight of the factor and its TNF like activity, we speculated that the factor was a lymphokine, like lymphotoxins.

Minimal change nephrotic syndrome (MCNS) is a common cause of nephrotic syndrome in children. However, the pathogenesis of this disease is still unknown. The glomerular capillaries from patients with MCNS have an abnormal permeability to serum proteins, mainly albumin. Histological studies do not indicate any glomerular injury to explain the observed abnormal permeability, except for foot processes fusion of epithelial cell [1].

Another feature of MCNS is abnormality of the T cell functions [2–13]. Several reports of immediate recurrence of nephrotic syndrome in patients with steroid resistant MCNS after kidney transplantation suggest the presence of humoral factor enhancing the glomerular permeability [14]. In addition, it has been reported that lymphocyte cultures obtained from MCNS patients can be stimulated with Con-A to produce a GPF, which causes urinary protein excretion in rats [15]. Based on these observations, it has been postulated that GPF is a lymphokine produced by T cells, and that it mediates abnormal permeability in patients with nephrotic syndrome.The biological profile of GPF resembles that of the skin reactive factor, which has been extensively studied in guinea pigs [3, 4, 14]. The skin reactive factor is thought to be a lymphokine produced by T cells, which induces a rapid increase in vascular permeability at the intradermal site of injection, followed several hours later by a local inflammatory response. However, there have been no observations of an equivalent human lymphokine exhibiting similar biological activity to the skin reactive factor.

In an animal model of MCNS, injection of puromycin aminonucleoside, leads to massive proteinuria and minor glomerular lesion, except for the foot processes fusion of the epithelium of the glomerular basement membrane (GBM). These changes are similar to those of MCNS. This drug is thought to be toxic to the epithelium of GBM, rather than mesangial cells [16].

The present paper describes the production of a GPF by T cell hybridomas derived from T lymphocytes obtained from patients with MCNS. The biological profile of this GPF was consistent with that described in the literature. In addition, we have further characterized the GPF by physical and biological means.

Methods

Production of human T cell hybridomas

Peripheral blood was collected in heparin from three normal volunteers and four patients with MCNS (cases; T, K, N, S), who were nephrotic but had received no corticosteroid or immunosuppressive agents and no histological changes by light microscopy. The blood sample was mixed with equal volume of phosphate buffered saline (PBS), overlaid on top of Percoll (d = 1,080; Pharmacia LKB Biotechnology, Sweden) and centrifuged at 880 × g for 20 minutes. The lymphocyte fraction was isolated from the gradient and the cells were washed twice with RPMI-1640 medium (Gibco Laboratories, Grand Island, USA). To obtain the T cell hybridomas, about 1×10^8 purified lymphocytes were fused with 5×10^7 CCRF-HSB-2 cells with polyethylene glycol 4000 [17]. After fusion, the cells were suspended in hypoxanthine/aminopterin/thymidine (HAT) medium containing 20% fetal calf serum (FCS) and added to 96-well plates at a density of 1×10^6 cells per well. Thymus cells from Balb/c mice served as feeders. The fused cells were allowed to select in HAT or hypoxanthine/thymidine (HT) media at 37°C in 5% CO_2. After stable selection, the hybridomas were then adapted to grow in RPMI-1640 containing 20% FCS.

Received for publication October 29, 1990
and in revised form April 30, 1991
Accepted for publication May 1, 1991

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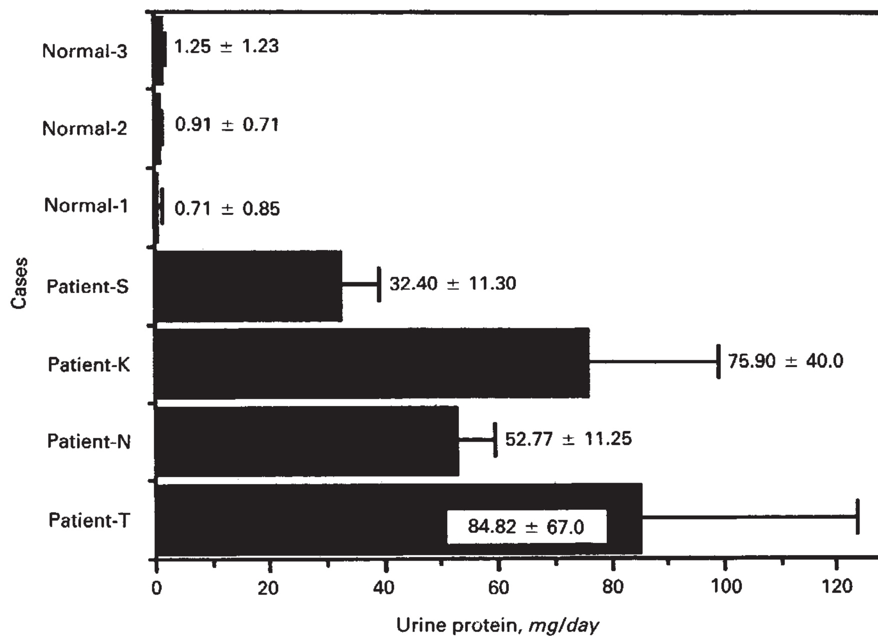

Fig. 1. *Induction of urinary protein excretion by the injection of the crude supernatant of T cell hybridomas.* Before limiting dilution, one ml of the crude hybridoma supernatants from three normal volunteers and four patients with MCNS were injected intravenously into 3 female Wistar rats to confirm the presence of GPF in the culture supernatants. The data represent mean ±SD, in the rats (N = 3) given crude supernatants at the first day after the injections. The levels of urine protein excretion in rats given the supernatants from patients with MCNS are significantly higher than those in the rats given supernatants from normal human volunteers (S, $P < 0.02$; K, $P < 0.01$; N, $P < 0.05$; T, $P < 0.1$).

In vivo assay of GPF

Before limiting dilution, one ml of the crude supernatants from T cell hybridomas were injected intravenously into three female Wistar rats (200 g each) to confirm the presence of GPF in the culture supernatants; also one ml of the samples, which were after limiting dilution, were injected intravenously into three rats. Twenty-four hour urines were collected in the metabolic cages. Amounts of urinary proteins were determined by the sulfosalicylic acid method. The crude supernatants of hybridomas from normal human volunteers were used as controls.

Interleukin 2 assay by the MTH mouse IL-2 dependent cell line and other cytokine assays

The MTH mouse interleukin 2 (IL-2) dependent cell line was used for this assay. Five thousand cells were added to each well of a 96-well plate. The test samples (50 μl) were serially diluted in RPMI-1640 and added to the cells. After the cultures were incubated at 37°C for 28 hours, 0.2 μCi of tritiated thymidine (^{3}H-TdR: specific activity: 2 Ci/mmol) was added to each well. The cells were then labeled at 37°C for four hours and the radioactivity incorporated was determined by liquid scintillation spectroscopy. As positive controls, serial diluted recombinant human IL-2 (rIL-2) was used. Medium only and GPF negative supernatants of hybridoma were used as controls. The percent uptake was expressed as follows: % uptake = (cpm of samples − cpm of medium control)/(cpm of positive control − cpm of medium control). The human cytokine assays were performed by the enzyme-linked immunosorbent assay (ELISA) kits. TNF-alpha, IL-1-alpha, IL-1-beta, and IL-2 in the culture supernatants were measured by the ELISA kits (Otsuka Pharmaceutical Co., Japan) and human IL-4 and IL-6 were measured by the Intertest-4 and -6 kits (Genzyme Corporation, Massachusetts, USA). TNF-beta and IL-2 were

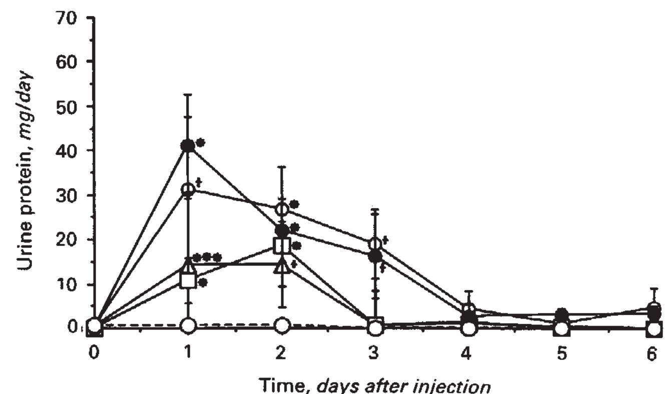

Fig. 2. *Induction of urinary protein excretion by the injection of the supernatant of T cell hybridomas after limiting dilutions.* One ml of samples from four clones (—○—, N3C-A1; —△—, N3D-C1; —□—, N3C-B1; —●—, N3D-D2), which were after limiting dilutions, were injected intravenously into 3 rats. The rats given the supernatants from CCRF-HSB-2 cell culture were used as negative control (N = 5; --○--). Urine proteins were significantly induced by the injection of these supernatants. The data represent mean ± SE (N = 3). $+P < 0.1$, $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

detected by the immunoblotting method [18], using rabbit anti-human TNF-beta and IL-2 antibodies (Hayashibara Biochemical Laboratories Inc., Japan).

Con-A induced lymphocyte blastformation assay

Normal human peripheral lymphocytes were prepared by Ficoll-Conrey (d = 1.077) gradient centrifugation. One hundred thousand cells in 0.2 ml of RPMI-1640 containing 10% FCS are added to each well of a 96-well plate. Then, 20 μl of Con-A (1 mg/ml) were added to each well. After the cells were incubated for 12, 24 or 48 hours in the presence of Con-A, serial diluted test samples were added. Three days later, ^{3}H-TdR was added

Table 1. Effect of supernatants of T cell hybridomas on Con A-induced blast formation in normal lymphocytes

Supernatant	Sample addition time[a]	Enhancement ratio of blast formation[b] supernatant dilution					
		1/320	1/160	1/80	1/40	1/20	1/10
N3D-D2	12 hr	1.07	0.93	1.00	1.45	2.90	2.17
	24 hr	0.97	1.31	1.44	2.20	4.35	5.00
	48 hr	1.38	1.98	4.47	13.4	12.0	12.2
N3D-C1	12 hr	1.10	1.15	1.56	2.18	3.96	5.76
	24 hr	1.10	1.15	1.10	1.33	2.88	3.91
	48 hr	1.15	1.94	5.15	8.44	6.26	6.67
N3D-B1	12 hr	—	—	0.99	1.43	2.79	3.01
	24 hr	—	—	1.11	1.26	3.92	4.79
	48 hr	—	—	4.64	11.6	11.5	11.8

[a] After the cells were incubated for 12, 24 or 48 hours in the presence of Con-A, serial diluted supernatants of the clones, N3D-D2, N3D-C1 and N3C-C1 were added. Three days later, ^{3}H-TdR was added to the cell cultures. The uptake of ^{3}H-TdR after 4 hours of incubation was determined. Supernatant from CCRF-HSB-2 culture was used as the control.

[b] The enhancement ratio of blast formation is expressed as CPM in sample/CPM in control.

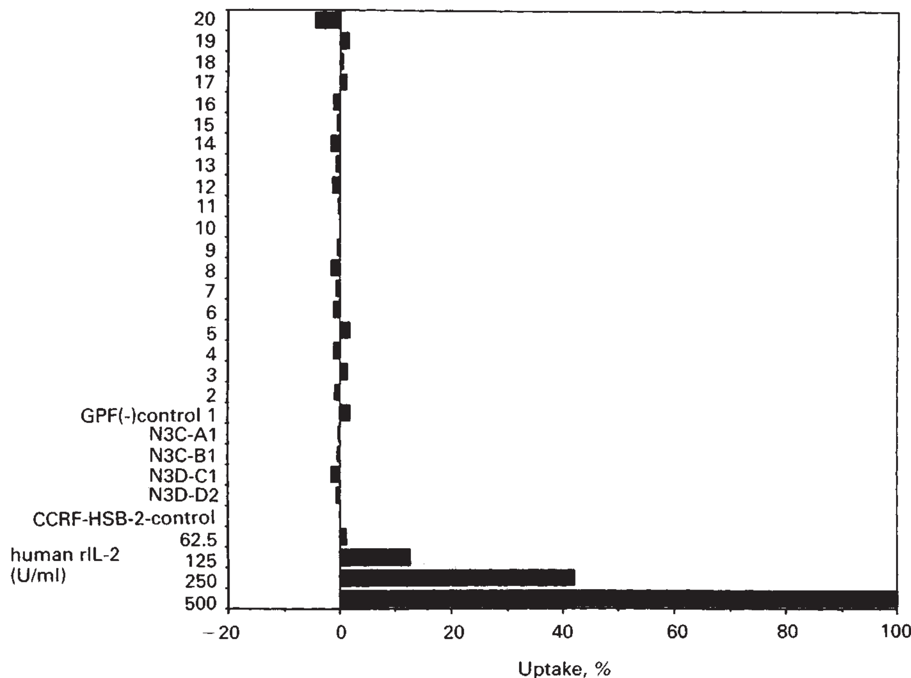

Fig. 3. *Measurement of IL-2 in the supernatants of T cell hydbridomas.* Positive control was serial diluted human rIL-2; negative control was supernatant from CCRF-HSB-2 culture. GPF-positive supernatants were: N3D-D2, N3D-C1, N3C-B1, and N3C-A1. GPF negative supernatants (No1-No20). % Uptake = (CPM of samples − CPM of negative control)/(CPM of positive control − CPM of negative control). The values in GPF positive and many negative samples were below 62.5 U/ml.

to the cell cultures. The uptake of ^{3}H-TdR after four hours of incubation was determined by liquid scintillation spectroscopy. Supernatants from CCRF-HSB-2 culture were used as controls. The enhancement ratio of blastformation was expressed as CPM in sample/CPM in control.

Antiproliferation and cytotoxicity assays

To examine the antiproliferative effects of GPF on tumor cells of human haematopoietic origin, the ability of these cells to take up ^{3}H-TdR was determined after the incubation of these cells in the presence of the factor. Raji, Molt 3 and K562 cells were used for these studies. Three hundred target cells were added to each well of the 96-well plate and the serial dilutions of the test samples were added to each well. The cells were then incubated for 20 hours after which the cells were labelled with ^{3}H-TdR for four hours. The uptake of the ^{3}H-TdR was determined by liquid scintillation spectroscopy.

To examine the effect of GPF on human tumor cells of epithelial origin, the semi-quantitative cytotoxic test was performed by the modified Takasugi and Klein's method [19]. The cell lines examined include carcinoma cells from the stomach, the gall bladder, the prostate gland, the kidney, and squamous cell carcinoma from the penis. Ten thousand cells were added to each well of the 96-well plate and incubated in RPMI medium containing 10% FCS for three days. The supernatant medium was removed and fresh medium was added to the cell culture. To these cells, serial diluted test samples were added. After a 48 hours incubation, the cells were fixed and stained with Giemsa stain. The morphology of the cells were evaluated semiquantitatively using the following criteria: 1) cytotoxic effect: (−) =

Table 2. Effect of the supernatants of T cell hybridomas on the cell lines from epithelial origin

	Supernates dilution											
	1/10		1/20		1/40		1/80		1/160		1/240	
Supernates	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP	CS	CP
Prostatic carcinoma												
Control	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D3	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D2	−	±	−	−	−	−	−	−	−	−	−	−
N3D-C1	−	±	−	−	−	−	−	−	−	−	−	−
N3D-A1	+	+	±	+	−	±	−	−	−	−	−	−
N3C-B1	+	+	±	+	±	+	±	+	−	±	−	−
N3C-C1	+	++	+	+	±	+	−	−	−	±	−	−
Renal cell carcinoma												
Control	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D3	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D2	−	±	−	−	−	−	−	−	−	−	−	−
N3D-C1	+	+	±	+	−	±	−	±	−	−	−	±
N3D-A1	++	++	+	+	±	±	−	±	−	±	−	±
N3C-B1	++	++	+	++	±	+	±	±	−	±	−	−
N3C-C1	++	++	++	++	+	++	±	+	±	+	±	±
Squamous cell carcinoma												
Control	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D3	−	−	−	−	−	−	−	−	−	−	−	−
N3D-D2	++	++	+	++	+	++	+	++	±	+	−	−
N3D-C1	++	++	++	++	++	++	+	++	+	+	±	±
N3D-A1	++	++	++	++	++	++	++	++	++	++	+	+
N3C-B1	++	++	++	++	++	++	+	+	±	±	−	−
N3C-C1	++	++	++	++	++	++	++	++	++	++	+	+

The supernatants exhibited cytostatic and/or cytopathogenic effects in cell lines of adenocarcinomas, especially squamous cell carcinoma.
1) cytotoxic effect: (−)= no cytotoxic effect; (±) = less than 20% cytotoxic effect; (+) = 20 to 50% cytotoxic effect; (++) greater than 50% cytotoxic effect; and 2) cytopathic effect: (−) = no significant morphological change; (±) = unclear; (+) = necrosis indicated; (++) = massive necrosis.

Table 3. Computer assisted morphometric analysis of glomerular anionic site distribution after injection of GPF-positive and negative supernatants

Group	GPF positive	GPF negative	P
Anionic sites /10,000 Å/GBM	20.25 ± 1.5 (N = 4)	19.5 ± 0.577 (N = 4)	0.391
Diameter (Å) of PEI particles	161.19 ± 36.19 (N = 70)	174.84 ± 44.74 (N = 70)	0.056
Density of PEI particles	117.18 ± 10.11 (N = 174)	122.86 ± 11.34 (N = 174)	0.001
Interspacing (Å)	457.84 ± 163.78 (N = 90)	557.82 ± 179.11 (N = 78)	0.006

Data are presented as mean ± SD.
N in anionic sites is the number of the portion (10,000 Å) in GBM; N in diameter, density and interspacing is the number of PEI particles counted in lamina rara externa of GBM.

no cytotoxic effect; (+/−) = less than 20% cytotoxic effect; (+) = 20 to 50% cytotoxic effect; (++) greater than 50% cytotoxic effect; and 2) cytopathic effect: (−) = no significant morphological change; (+/−) = unclear; (+) = necrosis indicated; (++) = massive necrosis.

Molecular weight determination

To determine the molecular weights of the protein, the GPF from the T cell hybridoma cultures was fractionated by sucrose density gradient (5 to 35%; linear sucrose density gradient in PBS, pH 7.2, total volume; 15 ml) ultracentrifugation. The supernatants (0.5 ml) were overlaid on the top of gradient and centrifuged at 100,000 g for 24 hours in a Beckman L-4 series ultracentrifuge (Beckman Instruments Inc., Palo Alto, California, USA) in a SW 27.1 rotor. Fractions (0.5 ml each) were collected from the bottom of the tubes. The ability of the protein(s) in each fraction to induce proteinuria in the rat was determined by the injection of the fractionated supernatants (one ml) into rats. The molecular weights of the protein in fractions exhibiting the proteinuria induction activity were estimated in a parallel gradient using bovine albumin and immunoglobulins as the molecular markers.

Histological examination

Histological evaluations of the renal tissues from rats injected with the GPF positive and negative supernatants were carried out by light microscopy, immunofluoresensce microscopy and electron microscopy by the intravenous injection of PEI (1,800 daltons) [20, 21]. At one hour after the injection of PEI, animals were killed. A computer-associated morphometric analysis were performed (Table 3) [21]. For immunofluorescent microscopy, fluorescein-conjugated goat anti-human IgG, anti-human IgA, anti-human IgM, anti-rat IgG, C3 and albumin (Cappel, Organon Teknika Co., Pennsylvania, USA) were used.

Statistical analysis

Quantitative results are expressed as mean ± SD and analyzed by one-way analysis of variance and Student's t-test for paired and unpaired data.

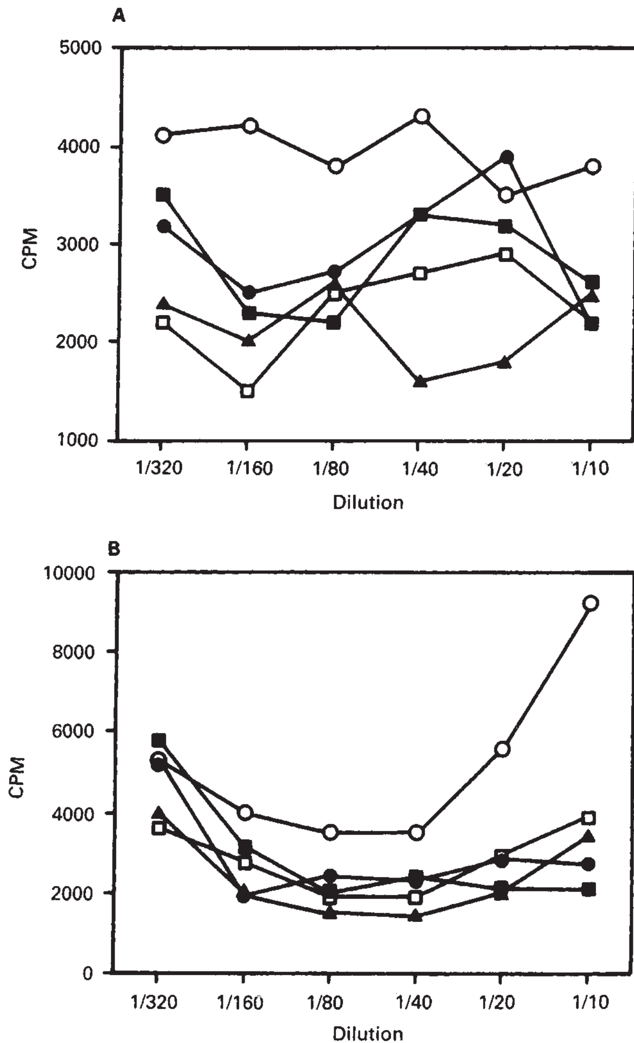

Fig. 4. *Effect of the supernatant of cell growth in haematopoietic origins.* The proliferation of Raji and Molt 3 cells are inhibited in the presence of the supernatants by hybridoma clones: N3D-C1, (—□—), N3C-A1 (—▲—), N3D-D2 (—●—) and N3C-B1 (—■—). Supernatant of K562 cell line (—○—) represent the controls.

Results

Production of human T cell hybridomas

Human T cell hybridomas were made from three normal volunteers and four patients with MCNS. In all hybridoma cells from normal volunteers, the GPF activity could not be detected in the crude supernatants, which were before limiting dilution. On the other hand, in the crude supernatants from patients, the GPF could be detected in all four patients (Cases; N,T,K,S) (Fig. 1). However during limiting dilutions, the GPF activities were lost, except for one patient (Case N).

Production of GPF

To verify the production of GPF, culture media from nearly 50 hybridoma clones from a patient with MCNS (case N) were examined for their ability to induce proteinuria in rats. Of all of the hybridomas, media from four clones were shown to elevate the levels of urinary proteins (higher than 15 mg/day) in rats significantly, when compared to the normal level; below 2 mg/day (Fig. 2). This effect continued for two to three days after injection of the media, and suggested that these four hybridoma lines produce GPF.

Characterization of GPF

Effects of the supernatant on Con-A-induced lymphocyte blastformation in normal human lymphocytes. The presumptive GPF produced by hybridoma clones N3D-D2, N3D-C1 and N3C-B1 enhanced the rate of Con-A-induced lymphocyte blastformation by 12.0-, 6.99- and 11.7-fold respectively, when compared to the rate of induction in the presence of supernatant from CCRF-HSB-2 cells, as indicated in Table 1. The enhancement rate of induction was concentration dependent and this effect was maximized when the presumptive GPF lymphokine was added to the lymphocyte culture at late logarithmic proliferative phase.

Antiproliferation and cytotoxicity assays. The GPF exhibited an antiproliferative effect on human tumor cells of hematopoietic origin (Fig. 3). The proliferation of Raji, Molt 3 and K562 cells were inhibited in the presence of GPF produced by hybridoma clones N3D-C1, N3C-D2, N3D-D2 and N3C-B1. In addition, the GPF exhibited cytostatic and/or cytopathogenic effects in epithelial cell lines of adenocarcinomas, especially squamous cell carcinoma (Table 2).

Interleukin 2 assay by the MTH mouse IL-2 dependent cell line and other cytokine assays. To prove that the lymphokine produced by these hybridoma clones was not IL-2, its ability to induce the proliferation of IL-2 dependent cells was examined. As indicated in Figure 4, these factors failed to support the growth of the IL-2 dependent cells, confirming that the GPF lymphokine produced by these hybridomas is not IL-2 and that the GPF does not have IL-2-like activity, and IL-2 could not be detected in the supernatants by the western blotting and ELISA assay (at least below 0.31 ng/ml). IL-1-alpha and IL-1-beta could not be detected in these supernatants: The concentration of IL-1-alpha and IL-1-beta were below 7.8 pg/ml and 15.6 pg/ml, respectively. Those of IL-4 and IL-6 were below 0.19 ng/ml and 0.15 ng/ml, respectively.

The factor showed also TNF-like activity. Therefore, TNF-alpha was assayed in these supernatants by ELISA. The concentration of TNF-alpha was at least below 1.56 pg/ml. TNF-beta could not detect in these supernatants by the immunoblotting method.

Detection of human immunoglobulins and anti-GBM antibodies. No human immunoglobulins were detected in the supernatants by ELISA and anti-GBM antibody could not be detected by immunofluorecence microscopy.

Histological changes

Histopathological changes in the glomerular basement membrane were examined in rats injected with GPF. Partial fusions of epithelial foot processes were observed by electron microscopy. Computer-assisted morphometric analysis of glomerular anionic sites in lamina rara externa revealed that though anionic sites in lamina rata externa in both groups were almost same, the site-site interspacing of PEI particles was significantly decreased in the rat given GPF positive supernatant (457.84 ± 163.78 Å), compared to the rat given GPF negative supernatant (557.82 ± 179.11 Å). The density and diameters of the PEI

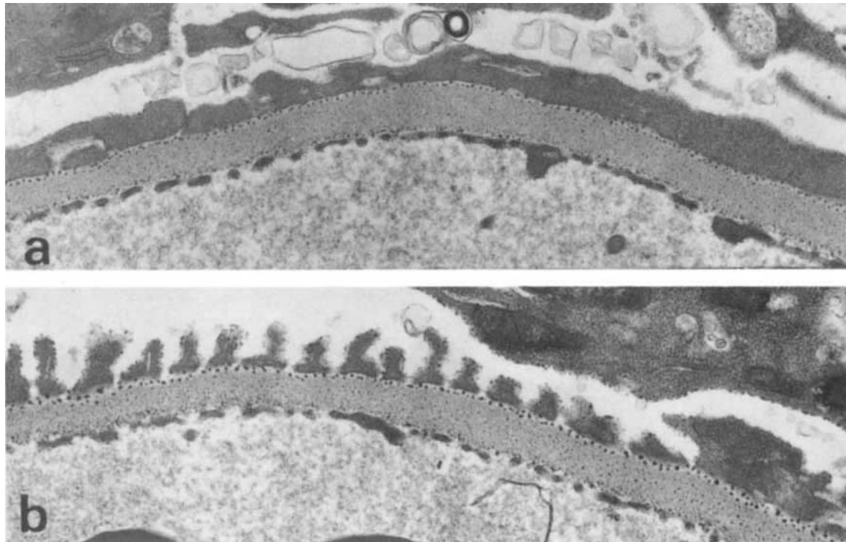

Fig. 5. *Electron microscopic findings of rats intravenously injected with the supernatant (N3D-D2) of T cell hybridoma.* (**a**) Partial fusions of epithelial foot processes and changes in the arrangement and sizes of PEI particles are observed in the rat given N3D-D2 supernatant. (**b**) Regular arrangement of PEI particles are observed in the rat given GPF negative hybridoma supernatant (control).

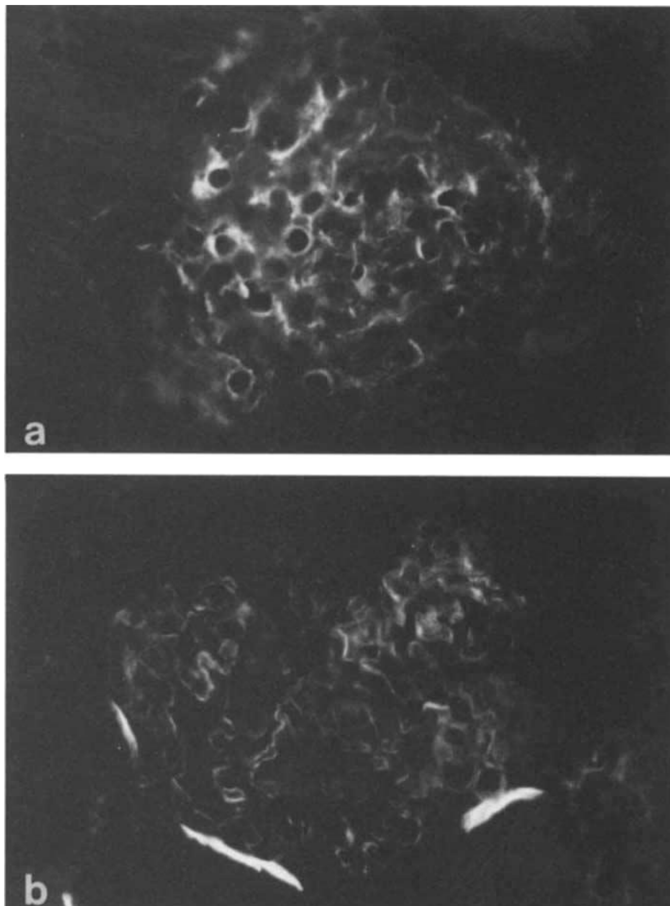

Fig. 6. *Immunofluorescence findings of the rats given the supernatants of T cell hybridomas (N3D-D2).* Albumin (**a**) and C3 (**b**) are observed along the peripheral capillary walls in the rat given N3D-D2 supernatant.

particles in the rat given GPF positive supernatant were also significantly decreased, compared to those in the control rat (Table 3 and Fig. 5). These findings may suggest the changes in

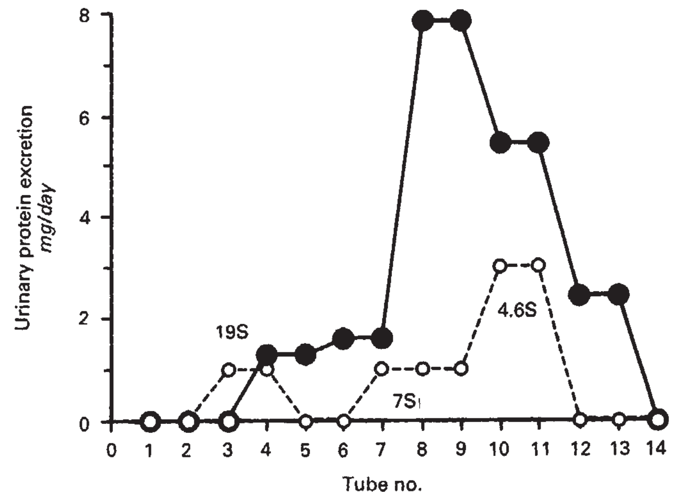

Fig. 7. *Molecular weight of vascular permeability factor.* The supernatant of N3D-D2 was fractionated by sucrose density gradient (linear: 5 to 35%) ultracentrifugation. The ability of the protein(s) in each fraction to induce proteinuria in the rat was determined. The molecular weight of the protein in fractions exhibiting the proteinuria induction activity was estimated in a parallel gradient using bovine albumin and immunoglobulins as the molecular markers. Symbols are: (—●—) urinary protein after injection of supernatant; (--○--) markers.

the characteristics of the negative charge barrier may occur by the injection of GFP. Immunofluorescence studies indicated that rat immunoglobulins could not be detected. However, rat albumin and C3 were detected along the peripheral capillary walls (Fig. 6), suggesting an increased the glomerular permeability. There was no human immunoglobulins in the glomeruli.

Molecular weight of GPF

The molecular weights of the GPF were estimated by sucrose density gradient ultracentrifugation. The fractions of the gradient which induced proteinuria in rats as well as the deposition of C3 and albumin along the peripheral capillary walls were observed in the range of 60,000 to 160,000 daltons (Fig. 7).

Discussion

MCNS is a unique disease, with several T cell abnormalities. Factor(s) in the culture supernatants or sera from nephrotic patients suppressed phytohemagulutinin (PHA)-induced blast formation of the patients' lymphocytes. The massive proteinuria may be due to the damage of negative charge barrier of the GBM, which might be caused by lymphokine(s) [19, 20]. Therefore it is speculated that T cell lymphokines, which were produced in patients with MCNS under the abnormal cellular immunity, caused epithelial damages and massive proteinuria.

In this study we demonstrate that the factor caused significant urinary protein excretion, which continued for two or three days. Histological examination showed minor lesions with partially fused foot process. The changes of PEI particles (density, interspacing and diameter) in the GBM, which may be caused by the injection of GFP suggest the changes in the characteristics of negative charge barrier of the GBM [20–22].

Boulton-Jones et al demonstrated that the supernatants of cultures of stimulated lymphocytes of patients with the nephrotic syndrome containing autologous sera, when injured into the renal arteries of rats caused patchy fusion of the foot processes of epithelial cells and a reduction of charge on GBM [23]. In the rats given fractions of the supernatants of T cell hybridomas, linear staining of albumin and C3 were observed in those fractions which caused urinary proteins. We speculated that this linear patterns of albumin and C3 might be nonspecific and due to the increase of permeability of the GBM, as is seen in diabetic nephropathy [24]. Another mechanism of the glomerular injury may be in part due to complement mediated mechanisms, such as membrane attack complex [25, 26].

There have been many reports that suppression of PHA-induced blastformation occurs in lymphocytes from patients with MCNS [9–13]. The factor showed tenfold more enhancement of Con A-induced blast formation induced in normal human lymphocytes. We have assayed IL-2 activity using the MTH mouse IL-2-dependent cell. This factor could not enhance the growth of this cell, but rather inhibited its growth and also suppressed the proliferation of the hematopoietic cell lines.

There is discrepancy between lymphocyte function in patients with MCNS and the effects of our factor on normal and abnormal lymphocytes in vitro. The reasons for this are not clear. We speculate that lymphocyte function from patients with MCNS may be suppressed, because lymphocytes have already been activated by the factor, or suppressed blastogenesis may be caused by the addition of autologous sera, which contains the factor. The factor may cause the T cell abnormalities in MCNS.

It is of interest that the factor showed tumor necrosis factor-like activity to tumors cells from epithelial cell origin. The origin of the glomerular epithelium is not clear, but like Bowman's capsule and tubular epithelial cells is thought to originate from mesenchymal cells. The factor caused epithelial cell necrosis, both in renal cell carcinoma and other carcinoma cell lines. We could not clarify the exact sites of cell damage. We speculate that the factor may be toxic to glomerular epithelial cells. Recently Thomson et al reported that lymphotoxin (LT)-like substance, with MW 60,000–160,000 daltons, could be detected in MCNS, as a suppressor of lymphocyte-blastformation in the sera form patients with MCNS [27].

In our study, the molecular weight of the urinary protein causing activity ranged between 60,000 to 160,000 daltons. Though the molecular weight of the factor was roughly estimated, it was larger than the other lymphokines, such as ILs and interferons, but similar to those of LTs (molecular wt of TNF-beta 60,000 to 70,000) [27–30]. Of the molecular weight of the factor and its TNF like activity, we speculated that the factor was like LTs. However, we could not detect TNF-alpha, TNF-beta, IL-1-alpha, IL-1-beta, Il-2, IL-4, or IL-6 by ELISA and immunoblotting methods.

In conclusion, we have succeeded in producing a GPF from four stable human T cell hybridomas and have partially characterized this factor by physical and biological means. Further studies are needed to clarify that this factor has both the effects of enhancement and suppression of cell growths, and to elucidate whether this factor is the etiological agent for MCNS.

Acknowledgments

This investigation was supported in part by Research Grant from "Disease Control Division", Health Service Bureau, Ministry of Health and Welfare, Japan, Research Grant from Tsukuba University, and Research Grant (60570284) from the Ministry of Education, Science and Culture, Japan (1987). We thank Professor Guy Neild, St. Philip's Hospital, UCL, who provided us with invaluable guidance, encouragement and support.

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