Kidney International, Vol. 67 (2005), pp. 2168–2177

T-cell transcriptome analysis points up a thymic disorder in idiopathic nephrotic syndrome

Hicham Mansour, Lydie Cheval, Jean-Marc Elalouf, Jean-Christophe Aude, Maria-Alexandra Alyanakian, Béatrice Mougenot, Alain Doucet, and Georges Deschênes

Institut des Cordeliers, CNRS-UPMC UMR7134, Paris, France; CEA Saclay, Gif sur Yvette, France; INSERM U580, Faculté Necker Enfants Malades, Paris, France; Service d'Anatomie Pathologique, Hôpital Tenon, Paris, France; and Service de Néphrologie Pédiatrique, Hôpital Armand Trousseau, Paris, France

T-cell transcriptome analysis points up a thymic disorder in idiopathic nephrotic syndrome.

Background. Idiopathic nephrotic syndrome is a proteinuric disease secondary to the release of a nonidentified circulating glomerular permeability factor by T cells. Because specificities of T-cell activation in idiopathic nephrotic syndrome remain unknown, we evaluated transcriptional activation of T cells in nephrotic patients during proteinuria.

Methods. Transcriptomes of CD2+ cells were analyzed by serial analyis of gene expression (SAGE) in a nephrotic child during proteinuria relapse and after remission, away from any immunosuppressive treatment. Expression of specific transcripts overexpressed during proteinuria relapse was compared by reverse transcription-polymerase chain reaction (RT-PCR) in CD2+ cells from 11 nephrotic patients during relapse and remission and 11 nonnephrotic patients during infection and after recovery.

Results. Differential analysis of CD2+ cell transcriptome identified >200 mRNA tags overexpressed during proteinuria relapse, including many T-cell markers. RT-PCR analysis of expression of specific transcripts indicated that (*1*) under remission conditions, nephrotic children displayed induction of four transcripts, including *IKBKB*, and repression of *NFKBIA* as compared to nonnephrotic children after recovery, and (*2*) proteinuria relapse was associated with induction of L-selectin and T-lymphocyte maturation–associated protein, two markers of T-cell differentiation and recent emigrant/naive T cells.

Conclusion. Results indicate that circulating T cells from relapsing nephrotic patients include a significant population of low-mature cells while those from nephrotic patients in remission are characterized by constitutive activation of nuclear factor-κB (NF-κB), altogether suggesting a thymic dysregulation of apoptosis in nephrotic patients.

Key words: steroid-sensitive nephrotic syndrome, selection, apoptosis, L-selectin, NFκB, T-cell differentiation protein.

Received for publication September 21, 2004
and in revised form November 12, 2004, and December 13, 2004
Accepted for publication December 20, 2004

Idiopathic nephrotic syndrome, the most frequent renal disease in childhood, is characterized by massive proteinuria (>50 mg/kg/day) leading to hypoalbuminemia (<30 g/L). Histopathologic changes are restricted to effacement of the podocytic foot processes without significant glomerular deposit or inflammatory lesion. In most cases, proteinuria and foot process effacement are completely reversible under steroids, without sequellae. Clinical and experimental data support that this renal disease is primarily a T-cell disease leading to the release of a glomerular permeability factor in the blood circulation: (*1*) idiopathic nephrotic syndrome is sensitive to steroid therapy in >90% of the patients, (*2*) T-cell immunosuppressive drugs (mainly cyclophosphamide and anticalcineurin agents) as well as measles are also able to induce remission of proteinuria, (*3*) lymphoma and thymoma are significantly associated with idiopathic nephrotic syndrome in children and adults, (*4*) massive but transient proteinuria is observed in neonates from nephrotic mothers, and proteinuria resumes soon after renal transplantation in patients with a steroid-resistant form who progressed toward end-stage renal failure, (*5*) conversely, recipients of a kidney from a patient deceased during a relapse of idiopathic nephrotic syndrome rapidly recover from proteinuria, and (*6*) plasma and serum as well as T-cell hybridoma supernatant from nephrotic patients induce proteinuria in rats [1].

T-cell dysfunction during proteinuria relapse in patients with idiopathic nephrotic syndrome is not properly characterized yet, as data from the past decade are rather misleading. Analysis of T-cell subpopulations revealed no reproducible difference in CD3+, CD4+, and CD8+ cells distribution between patients in relapse and patients in remission or healthy children [2, 3]. Th1/Th2 balance studies provided controversial results. The profile of serum cytokines indicated a Th1 activation pattern during relapse [3, 4], whereas mRNA cytokines profile supported a Th2 pattern [5]. In addition, analysis

of intracellular cytokines profile during relapse concluded to a Th2 activation pattern in CD4+ cells [6], a Th1 pattern in CD3+ cells [7], and no significant shift of the Th1/Th2 balance in CD4+ cells [8]. Peripheral blood mononuclear cells (PBMCs) from patients with idiopathic nephrotic syndrome express a truncated c-maf inducing protein (Tc-mip) which, when expressed in T-cell Jurkat, induces a Th2 commitment [9]. Conversely, increased interleukin (IL)-12 release by monocytes from nephrotic patients supports Th1 activation [10]. Thus, the specificities of T-cell activation remain to be described in idiopathic nephrotic syndrome.

Identification of the circulating glomerular permeability factor in idiopathic nephrotic syndrome has failed also despite numerous attempts over the past three decades. Purification of the factor was first based on plasma fractionation and different biologic assays: subcutaneous diffusion of Evans blue [11], heparin desorption [12], induction of proteinuria in rat or rabbit with human plasma [13], and decreased synthesis of glomerular heparan sulfate [14]. Additional works included studies of hybridoma supernatants [15], fractionation of plasma eluates from protein A [16], or anti-IgG affinity columns [17], as well as genomic analysis of PBMCs [18]. The circulating glomerular permeability factor was not characterized either in recurrent focal and segmental glomerulosclerosis on renal transplant [13, 16, 17, 19, 20].

The present study was designed (*1*) to characterize the changes in T-cell transcripts profile during proteinuria in children with idiopathic nephrotic syndrome, (*2*) to compare the mRNA activation profile of T cells in nephrotic and nonnephrotic children, and (*3*) to search, among transcripts induced during proteinuria, for candidates as circulating glomerular permeability factor. Changes in transcriptome were analyzed using serial analysis of gene expression (SAGE) in T cells sampled in a same patient during proteinuria and remission. Specific transcripts expression was compared by quantitative reverse transcription-polymerase chain reaction (RT-PCR) between T cells from nephrotic patients during proteinuria relapse and remission and nonnephrotic patients during infection and after recovery.

METHODS

Patients

SAGE libraries were prepared from T cells from a boy with a history of steroid-sensitive idiopathic nephrotic syndrome, defined by complete remission of proteinuria within 4 weeks of oral prednisone (2 mg/kg/day). A "relapse" library was prepared from blood sampled when the boy was 10.2 years, during a proteinuria relapse (proteinuria 3.05 g/L and serum albumin 16.5 g/L) occurring 21.3 months after stopping prednisone. Blood was sampled before the initiation of steroid therapy, and in absence of any other medication. A "remission" library was prepared from blood sampled during remission (undetectable proteinuria and serum albumin 44.6 g/L) 16.5 months after prednisone withdrawal, no other medication being given to the patient.

T-cell expression of a selection of candidate genes was then evaluated by real-time RT-PCR in a group of 11 patients (four boys and seven girls) with steroid-sensitive idiopathic nephrotic syndrome, which includes the patient selected for transcriptome analysis (patient 3). Patients were aged 6.4 $\pm$ 3.0 and 7.3 $\pm$ 3.2 years at the time of the two blood samplings, respectively. Blood was sampled at the time of relapse (proteinuria 8.8 $\pm$ 7.6 g/L and serum albumin 13.9 $\pm$ 4.9 g/L) at least 4 weeks after withdrawal of any immunosuppressive drug and before initation of immunosuppressive drugs, and at the time of remission (undetectable proteinuria) at least 2 weeks following prednisone withdrawal. Patients did not receive any other medication since at least 4 weeks at the time of both blood samplings. These samples will be referred to as "nephrotic relapse" and "nephrotic remission," respectively. In order to determine the specificity of T-cell activation in proteinuric disease, we used a control group of patients with T-cell activation in response to infectious disease but without history of proteinuria. Eleven patients matched in age and gender (five boys and six girls, aged 7.6 $\pm$ 2.8 years) with high fever and severe infectious diseases were selected. Four had a virus infection (patients 27, 77, 100, and 110), five had acute malaria (patients 103, 104, 105, 106, and 108), and two had acute pyelonephritis (patients 17 and 121). Patients were successively sampled at the time of fever ("nonnephrotic-infection") and at least 1 week following clinical recovery ("nonnephrotic-recovery"). This work was approved by the local ethic committee, and written consents of patients and/or parents were obtained for each couple of samples.

T-cell isolation

Blood was collected from a forearm vein into potassium ethylenediaminetetraacetic acid (EDTA)-containing tubes and kept at room temperature until fractionation (performed within 6 hours after sampling). T cells were isolated using anti-CD2 magnetic beads (Dynal, Oslo, Norway) according to the manufacturer's protocol. CD2 is a specific marker of the whole T-cell lineage. T cells adsorbed to anti-CD2 beads were directly lysed using the Dynal lysis solution, and the lysates were frozen at -80°C until use.

SAGE libraries

SAGE [21] consists in characterizing each cDNA obtained by retrotranscription of tissue mRNAs by a short (10 bp) informative nucleotide sequence, or tag. Several transcript-specific tags are concatenated into long DNA

Table 1. Number of tags differentially represented in relapse (Rel) and remission (Rem) libraries

	cDNAs					
	Known function	Unknown function	Multiple matches	No reliable match	No match	Total
Rel > Rem	179 (79%)	8 (3%)	12 (5%)	24 (11%)	5 (2%)	**228**
Rem >Rel	13 (10%)	3 (2%)	2 (1%)	63 (48%)	51 (39%)	**132**
Total	**192 (53%)**	**11 (3%)**	**14 (4%)**	**87 (24%)**	**56 (16%)**	**360**

EST, expressed sequence tag. Number of tags differentially represented in relapse and remission libraries in the different classes of identification through GenBank match. Tags matching to a cDNA were subdivised into two classes on the basis of the presence or absence of a functional characterization of the protein encoded by the cDNA. Multiple matches: tags with >two reliable identifications in GenBank; No reliable match: tag matching to cDNA(s) or EST(s) without poly(A) signal or poly(A) tail; No match is a tag without any matching sequence in GenBank.

molecules which are cloned and sequenced. Computer-assisted analysis of the sequences permits to extract, classify, and count the different tags, and data base queries allow identification. The method is quantitative as the relative abundance of the different tags in the library reflects the abundance of the cognate transcripts in the biologic sample. SAGE libraries were generated using the adaptation for downsized extracts (SADE) [22]. The main modifications of this protocol consists in the use of oligo$(dT)_{25}$-coated magnetic beads (Dynabeads mRNA Direct Kit) (Dynal) to purify poly(A) RNAs from T-cell lysates, and the use of *Sau*3AI as the anchoring enzyme.

Tags extraction and counting, and comparison of the two libraries were performed using SAGE2000 software [21]. Tags corresponding to linker sequences were discarded, and those originating from duplicate ditags were counted only once.

Identification of tags was first performed through matching to UniGene clusters using SAGEmap (*www.ncbi.nlm.nih.gov/SAGE*) and carried out up to June 2004. Next, the reliability of the UniGene identification was validated by confirming the correct location and orientation of the tag in relation to the 3′-most *Sau3*AI site, as well as the presence of a polyadenylation signal and/or poly(A) tail.

Real-time RT-PCR

RNAs were extracted from T-cell lysates using an adaptation [23] of the method of Chomczynski and Sacchi, and reverse transcribed using the First-Strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostics, Meylan, France), according to the manufacturer's protocol. Real-time PCR was performed on a LightCycler (Roche Diagnostics) with the LightCycler FastStart DNA Master SYBR Green 1 Kit (Roche Diagnostics) according to the manufacturer's protocol, except that the final reaction volume was reduced to 8 μL. Primers (sequences available upon request) were designed using oligo 4.04 (National Biosciences, Plymouth, MA, USA). Samples were submitted to 30 to 40 cycles of three temperature steps (95°C for 10 seconds; 60 to 62°C for 10 seconds; and 72°C for 25 seconds) followed by a fusion curve. In each experiment, a standardization curve was made using serial dilutions (1 to 1/500) of a cDNA stock solution made of a mixture of cDNAs from nephrotic and nonnephrotic samples. The amount of PCR product was calculated as percent of the standard cDNA. Expression of the different genes in each sample was expressed relatively to that of ribosomal protein L28 which remained constant. Results are presented either as mean values ± SE in the four groups of samples (nephrotic remission, nephrotic relapse, nonnephrotic recovery, and nonnephrotic infection), or as individual ratios between activated and resting conditions in nephrotic (nephrotic relapse/nephrotic remission) and nonnephrotic patients (nonnephrotic infection/nonnephrotic recovered).

Statistics

Significant differences in tag abundance between the two libraries were assessed by Monte-Carlo analysis [24], $P < 0.05$ being considered as significant. This method, which is based on large number (100, 000) simulations to determine the probability of obtaining the observed difference in tag abundance, was chosen because it is part of the SAGE2000 software used to analyze SAGE data. In RT-PCR experiments, values statistically different between groups were assessed either by paired *t* test (nephrotic relapse vs. nephrotic remission and nonnephrotic recovered vs. nonnephrotic infection) or by variance analysis (nephrotic remission vs. nonnephrotic recovered), $P < 0.05$ being considered as significant.

RESULTS

T-cell transcriptome in a patient with proteinuria relapse and remission

A total of 24,665 and 24,336 tags were sequenced in the remission and relapse libraries respectively (see complete list in Supplemental Table 1 *http://www.blackwellpublishing.com/products/journals/suppmat/kid/kid322/kid322sm.htm*). The fusion of the two libraries generated 23,863 different tags. This T-cell transcriptome is characterized by the following: (*1*) 38% of all tags (79% of the molecular species) were counted only once; (*2*) 170 tags (7% of the molecular species) were counted ≥25 and their cumulative abundance accounted for 27% of all tags; (*3*) the most abundant tag, which corresponded to translation elongation factor 1 alpha 1, represented >1% of all tags; and (*4*) 70 of the 170 most represented tags encoded ribosomal proteins.

A total of 360 tags were differentially expressed ($P < 0.05$) in the two libraries (*http://www.blackwellpublishing.com/products/journals/suppmat/kid/kid322/kid322sm.htm* Supplemental Table 2), including 228 tags overrepresented in the relapse library. Among the differentially represented tags, 56% were reliably identified as single cDNAs whereas 16% (56 tags) did not match any GenBank sequence (Table 1).

Supplemental Table 3 *http://www.blackwellpublishing.com/products/journals/suppmat/kid/kid322/kid322sm.htm* lists the 179 tags over represented in the relapse library which were identified in GenBank as single cDNAs with known functions. This list includes 21 transcripts coding for T-cell surface markers with distinct functions: class I major histocompatibility complex (MHC), subunits of the T-recognition complex (two variants of the β subunit *TRBC1*, *CD3D* associated with the δ subunit, and the γ subunit *TRG@*), the early activation-dependent surface antigen CD97; other markers of T cells (*SELL, MAL*, and *KLRK1*), components of the immunologic synapse (*CD6*, *CD48*, and *LAT*), and genes involved in T-cell growth balance (*LRRN4* and *IFITM1*) and survival (*SELS*, *IL7R*, *TNFRSF7*, and *LTB*). The largest functional class of transcripts over expressed during relapse encodes proteins involved in protein synthesis (52 ribosomal proteins and 7 translation factors), processing (7 chaperonins) and degradation (7 ubiquitination proteins), demonstrating a massive induction of protein metabolism during relapse. Other important functional classes include actin subunits and actin-interacting proteins (11 transcripts) and proteins involved in energy supply (19 transcripts) and signal transduction (24 transcripts). Genes encoding potent transcription factors (*JUNB*, *JUND*, *FYN*, *ENO1*, and *TCF7*, which is specific for T cells) as well as two thymic hormones inducing T-cell differentiation (*PTMA* and *TMSB10*) were overrepresented during relapse (Fig. 1).

Comparison of T-cell activation in nephrotic and control patients

To validate SAGE data and to characterize a putative specificity of T-cell activation in nephrotic relapse as compared to nonproteinuric infection, T-cell expression of a selection of transcripts was compared between nephrotic patients (during proteinuria relapse and remission) and nonnephrotic patients (during infection and after recovery). The criteria for transcripts selection were the following: (*1*) transcripts with a relapse/remission abundance ratio ≥5, (*2*) transcripts encoding proteins with a signal peptide sequence or a transmembrane domain, as they might be released in the blood by shedding and thereby putatively act as circulating permeability factor (putative transmembrane domains were determined using SMART (*http://smart.embl-heidelberg.de/) and PSORT II (http://psort.nibb.ac.jp/form2.html*) softwares), and (*3*) transcripts from most functional classes of genes, as defined in Figure 1. Thus, the 39 selected genes included (*1*) all genes coding for surface markers, except MHC genes which displayed a high constitutive expression in the remission library, (*2*) genes of unknown function with a putative transmembrane domain (*FLJ21438* and *C20orf52*), (*3*) genes coding for secreted proteins (*TGFB1* and *GPX4*), and (*4*) genes coding for intracellular proteins involved in nucleic acid metabolism (*ADAR*), protein synthesis (*EIF4G2*, *HNRPC*, and *CIRBP*), protein processing (*TAPBP*, *RPN2*, *CCT7*, *SSR4*, and *PSMA6*), cytoskeleton-related proteins (*COTL1* and *CORO1A*), energy metabolism (*NDUFA3* and *NDUFB8*), and signalization (*ARL10A* and *IKBKB*). Although their tag abundance was not altered in the relapse SAGE library, we also included *NFKB1* and *NFKBIA* which, along with *IKBKB*, control a major regulatory pathway in T cells.

Table 2 summarizes both mean mRNA expression (normalized to expression of *RPL28* transcripts) in the four groups of samples, and individual changes in mRNA level during proteinuria relapse (nephrotic relapse/nephrotic remission) and infection (nonnephrotic infection/nonnephrotic recovery) in nephrotic and nonnephrotic patients, respectively, and Figure 2 shows paired data for some transcripts. Note that in nonnephrotic patients, the profile of activation of gene expression during infection is independent of the disease (viral infection, malaria, or pyelonephritis). In nephrotic patients, only five genes (*SELL*, *MAL*, *ADAR*, *EIF4G2,* and *IKBKB*) were statistically significantly induced during proteinuria relapse, whereas 24/39 genes were significantly induced during infection in nonnephrotic patients. Genes induced during infection in nonnephrotic patients include *FLJ21438* (induced in 9/11 patients), which encodes a hypothetical protein with a RasGAP domain likely involved in intracellular signalization. Interestingly, *MAL* was the only gene specifically induced in nephrotic patients but not in controls (Fig. 2). Under resting conditions (remission or recovery), four genes (*CD3D*, *FLJ21438*, *COTL1*, and *IKBKB*) were overexpressed in nephrotic as compared to nonnephrotic patients (nephrotic remission vs. nonnephrotic recovery), whereas six genes were repressed (*SELL*, *KLRK1*, *TGFB1*, *ARL10A*, *CCT7*, and *NFKBIA*).

Analysis of changes in gene expression during T-cell activation (proteinuria relapse or infection) at the individual level indicates that (*1*) there was an heterogeneity in gene expression in both nephrotic and nonnephrotic patients, possibly due in part to the different stages of disease evolution (duration of proteinuria or of infection) at the time of blood sampling; (*2*) in patient 3 whose T cell served to generate the SAGE libraries, RT-PCR confirmed the SAGE analysis except for *LTB* (SAGE relapse/remission 12/1 and RT-PCR relapse/remission 0.9) and for *NFKB1* for which the 15.8-fold induction

Table 2. Expression of a selection of transcripts in T cells from nephrotic and nonnephrotic patients under basal (remission and recovery) and activated conditions (relapse and infection)

		mRNA level: Gene of interest/RPL28					mRNA level: Nephrotic relapse/nephrotic remission										mRNA level: Nonnephrotic infection/nonnephrotic recovery										
	Gene	Nephrotic remission	Nephrotic relapse	Non-nephrotic recovery	Non-nephrotic infection	Tag abundance rel/rem	3	78	44	39	6	64	14	62	70	59	54	27	100	110	77	108	105	104	106	103	121	17
A	*CD3D*	**1.4 ± 0.8**	1.4 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	14/0	2.0	0.6	0.8	0.6	1.1	1.8	1.4	1.0	0.9	0.8	0.9	0.8	0.9	0.6	0.7	2.8	2.8	1.2	8.4	2.0	1.1	1.6
	TRBC1	1.0 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	**1.5 ± 0.2**	43/6	1.6	1.5	3.0	1.3	0.8	1.1	1.5	1.0	1.2	1.0	0.7	1.7	3.8	2.0	0.9	2.4	1.2	1.6	1.2	1.2	2.7	1.9
	TRG@	1.2 ± 0.3	1.0 ± 0.2	0.9 ± 0.4	1.5 ± 0.5	5/0	2.4	0.7	1.2	1.0	4.1	0.6	0.6	0.8	0.5	0.5	2.1	1.2	1.2	0.9	3.7	36.4	3.5	1.5	0.4	0.5	1.7	2.2
	CD97	0.9 ± 0.1	1.2 ± 0.8	0.4 ± 0.1	**1.3 ± 0.2**	13/1	7.3	0.8	1.7	1.2	2.3	0.7	0.9	0.8	1.2	0.9	0.5	1.6	2.2	5.0	3.3	1.8	4.2	3.8	0.7	8.2	6.3	3.9
	LTB	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	**1.2 ± 0.2**	12/1	0.9	1.4	1.6	2.3	2.0	5.1	1.5	0.9	1.1	0.8	0.7	1.6	1.7	1.4	1.4	1.1	0.8	2.4	1.2	2.3	3.9	1.2
	LRRN4	1.3 ± 0.2	2.3 ± 0.4	1.3 ± 0.3	**2.5 ± 0.4**	10/1	2.6	4.7	1.1	1.5	0.5	1.1	2.4	0.9	1.9	2.6	12.8	1.5	1.2	5.7	1.7	6.1	2.2	1.4	1.6	1.7	2.9	0.9
	IL7R	0.7 ± 0.1	1.3 ± 0.5	0.7 ± 0.2	1.3 ± 0.3	9/0	7.3	1.1	5.8	1.2	0.9	0.4	0.7	0.7	0.7	0.9	1.3	2.8	1.3	1.3	5.1	7.4	0.7	0.9	4.5	0.8	0.8	3.9
	CD6	1.1 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	**1.8 ± 0.4**	9/1	2.1	2.0	1.0	1.1	0.9	0.7	0.3	0.8	1.0	1.0	0.6	1.7	2.2	1.5	1.3	2.0	1.5	2.4	3.2	6.9	2.9	0.6
	LAT	0.9 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	1.1 ± 0.2	8/0	2.7	1.8	2.3	0.8	1.4	0.3	0.7	0.3	0.6	2.1	1.3	0.9	0.8	3.2	2.1	7.3	1.1	1.4	4.1	1.6	2.6	0.7
	SELL	**0.7 ± 0.2**	**2.1 ± 0.5**	1.4 ± 0.5	**2.3 ± 0.6**	7/1	4.5	2.9	10.8	1.6	2.1	3.4	2.6	3.1	1.8	2.4	2.7	1.5	NT	1.4	2.2	7.2	1.7	1.6	0.9	1.5	3.2	0.7
	TNFRSF7	0.6 ± 0.1	1.1 ± 0.2	0.5 ± 0.1	**0.8 ± 0.1**	6/0	4.7	0.9	11.1	10.5	22.0	0.7	1.0	1.2	0.8	1.2	2.1	12.8	8.0	1.2	1.0	3.8	2.1	8.1	0.8	0.8	3.7	1.2
	KLRK1	**0.6 ± 0.1**	0.9 ± 0.2	1.8 ± 0.7	2.2 ± 0.8	5/0	2.9	4.6	2.4	2.5	2.6	0.7	0.6	1.4	0.3	1.3	0.8	1.5	1.3	2.5	1.4	0.8	1.4	1.6	0.7	0.7	0.6	1.3
	TNRC5	0.8 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	1.8 ± 0.4	5/0	1.8	1.1	1.0	0.9	2.1	1.3	1.5	1.1	1.4	0.8	0.8	0.8	18.7	1.4	1.2	0.9	1.3	2.7	1.2	3.6	1.8	1.3
	MAL	0.9 ± 0.2	**1.6 ± 0.3**	0.8 ± 0.1	1.0 ± 0.2	5/0	2.1	6.5	5.0	4.6	1.4	1.0	1.3	1.0	6.5	1.1	0.7	1.2	0.5	1.5	1.3	1.3	0.6	4.2	1.0	1.4	1.5	0.8
	CD48	1.4 ± 0.2	1.9 ± 0.8	1.1 ± 0.2	1.9 ± 0.4	15/6	4.4	0.4	11.3	0.8	2.7	0.5	0.6	1.3	0.7	0.7	0.6	0.9	2.2	1.0	0.9	5.7	10.2	0.5	0.5	3.9	0.7	2.9
	IFITM1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	**2.1 ± 0.4**	15/6	1.6	0.8	1.9	1.3	0.9	1.2	0.5	0.8	2.8	0.6	0.6	1.6	6.4	1.0	2.0	2.5	1.6	1.7	1.7	2.7	4.9	1.4
	SELS	1.3 ± 0.3	1.3 ± 0.2	1.6 ± 0.5	**4.6 ± 1.5**	10/2	2.4	1.9	4.9	0.6	1.4	1.0	0.3	1.7	0.5	0.3	0.9	0.5	25.6	1.3	1.4	2.6	9.8	0.6	14.9	1.3	0.7	8.8
D	*FLJ21438*	**1.0 ± 0.2**	0.6 ± 0.2	0.7 ± 0.1	**1.5 ± 0.3**	5/0	3.0	0.7	1.6	0.8	1.3	0.3	0.4	0.3	1.5	0.3	2.1	0.9	4.9	1.7	2.5	3.7	1.6	9.6	2.5	1.6	2.4	0.7
	C20orf52	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	**1.9 ± 0.5**	7/0	3.0	0.7	9.4	0.6	0.7	1.6	1.4	2.0	0.8	0.7	0.7	0.9	0.9	2.7	1.3	3.4	8.5	2.4	0.7	3.6	4.3	1.8
C	*TGFB1*	**1.4 ± 0.3**	1.7 ± 0.4	2.1 ± 0.8	2.8 ± 0.7	12/1	2.0	3.3	0.5	0.9	0.5	0.5	1.7	4.7	0.7	0.6	1.3	1.3	4.2	0.9	4.0	0.6	0.5	3.1	1.2	1.4	0.9	2.0
	GPX4	1.4 ± 0.2	1.6 ± 0.3	1.3 ± 0.2	1.5 ± 0.2	7/0	1.8	3.4	1.2	1.0	1.0	0.8	0.8	0.7	2.1	0.8	1.1	1.2	2.8	15.6	0.7	0.6	1.1	2.2	0.7	2.9	0.9	0.5
D	*HA-1*	1.2 ± 0.2	1.2 ± 0.1	0.6 ± 0.1	**1.1 ± 0.2**	16/1	3.0	3.8	0.9	0.7	1.5	0.7	0.6	0.5	0.9	0.7	1.3	1.9	6.9	0.5	1.6	1.7	0.7	6.1	1.9	0.9	2.3	2.5
	ADAR	1.6 ± 0.4	**4.0 ± 1.8**	1.6 ± 0.4	**3.6 ± 0.8**	5/0	4.4	0.9	4.8	2.4	2.1	1.7	1.0	1.3	1.0	2.6	3.0	2.1	2.2	2.6	3.7	4.5	4.2	0.5	0.6	4.1	2.6	4.9
	EIF4G2	1.1 ± 0.2	**2.0 ± 0.4**	1.1 ± 0.3	**3.3 ± 0.8**	13/1	4.8	2.8	4.6	0.5	1.0	1.9	2.8	2.4	1.0	1.4	1.0	3.2	0.7	26.7	2.8	9.4	3.4	1.8	3.0	4.7	1.0	1.5
	HNRPC	1.1 ± 0.2	1.4 ± 0.2	1.1 ± 0.3	**2.7 ± 0.5**	7/1	1.5	0.6	12.6	1.6	58.0	2.6	0.9	0.3	2.2	0.4	1.2	1.4	1.8	2.7	2.2	9.5	33.4	7.9	0.6	1.8	1.7	4.6
	CIRBP	1.2 ± 0.4	1.9 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	6/0	8.8	2.4	8.2	2.0	1.6	1.4	4.0	3.0	0.9	0.5	0.5	1.2	0.2	0.5	1.1	1.5	1.0	1.3	0.9	0.8	0.8	1.0
	TAPBP	1.0 ± 0.1	1.3 ± 0.2	0.7 ± 0.1	**1.3 ± 0.2**	11/0	3.5	2.0	1.5	1.2	0.7	0.7	2.1	1.2	1.1	0.8	0.6	1.1	3.0	1.4	3.0	1.2	0.6	6.7	2.3	3.2	3.0	1.3
	RPN2	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	**0.8 ± 0.1**	9/0	2.5	0.6	1.9	0.9	1.0	1.9	1.8	0.7	0.5	0.5	1.0	1.1	0.8	15.9	1.4	1.4	1.5	3.1	10.3	8.1	1.2	1.3
	CCT7	**1.0 ± 0.3**	1.4 ± 0.3	1.9 ± 11	**3.3 ± 1.3**	5/0	2.2	1.5	15.4	0.5	3.3	1.5	0.3	2.6	14.9	1.7	0.2	1.6	1.8	1.2	2.0	9.5	33.4	7.9	9.8	0.9	NT	NT
	SSR4	1.0 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.5 ± 0.2	13/1	1.6	2.3	0.8	1.9	1.0	2.4	1.2	0.8	1.2	1.1	0.5	0.9	1.5	0.8	1.3	1.0	1.0	1.4	1.7	1.8	1.4	0.7
	PSMA6	0.8 ± 0.1	1.0 ± 0.2	0.8 ± 0.2	**2.4 ± 0.5**	7/1	2.8	1.7	3.0	0.5	0.5	0.7	2.5	1.8	2.3	0.7	2.2	1.8	6.8	10.9	4.9	8.5	2.1	5.0	0.6	0.9	0.8	5.0
	COTL1	**0.9 ± 0.1**	1.4 ± 0.4	0.6 ± 0.1	**1.6 ± 0.2**	17/2	2.0	1.5	3.4	0.6	0.7	7.8	1.1	1.2	1.3	0.8	0.6	1.6	2.6	11.1	2.6	3.5	1.8	5.2	3.9	3.3	1.2	1.0
	CORO1A	1.1 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	**1.5 ± 0.2**	38/4	2.0	0.8	1.0	0.6	0.6	0.7	0.7	0.9	0.8	0.7	0.7	1.1	5.5	46.3	1.6	6.2	2.4	7.5	3.6	2.1	5.3	0.8
	NDUFA3	1.1 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	**1.5 ± 0.2**	5/0	1.5	1.5	1.5	1.0	0.5	1.6	1.3	1.1	0.8	0.7	0.5	1.2	2.5	5.4	2.7	5.9	0.8	3.3	0.7	0.9	2.5	2.1
	NDUFB8	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.6 ± 0.4	11/1	1.6	1.5	2.3	0.7	0.7	1.5	1.3	1.3	1.1	0.7	0.7	1.2	3.5	34.0	1.0	2.7	1.0	1.5	0.9	11.2	1.1	2.4
	ARL10A	**1.0 ± 0.1**	1.3 ± 0.2	1.2 ± 0.1	**1.9 ± 0.3**	8/0	1.8	4.3	1.2	0.8	0.8	1.3	1.1	1.2	1.0	0.5	1.0	1.1	3.1	1.8	1.5	2.0	0.9	4.6	0.7	1.0	2.7	1.8
E	*IKBKB*	**1.3 ± 0.1**	**2.2 ± 0.3**	0.8 ± 0.2	**1.2 ± 0.2**	5/0	2.2	3.7	1.5	1.5	1.1	1.3	1.4	1.9	2.0	1.3	1.6	0.9	3.3	0.9	1.5	1.9	1.4	1.8	1.1	1.9	NT	1.2
	NFKB1	0.9 ± 0.2	1.5 ± 0.5	1.1 ± 0.4	1.6 ± 0.4	1/0	15.8	34.6	0.9	0.5	3.8	0.5	1.1	0.9	1.1	0.6	0.5	0.6	0.6	3.0	2.3	1.1	6.1	3.1	1.0	7.8	0.9	0.5
	NFKBIA	**0.8 ± 0.1**	1.6 ± 0.4	2.0 ± 0.3	2.8 ± 0.6	1/0	0.9	7.7	2.7	1.5	0.5	0.8	2.0	6.4	5.0	0.8	1.0	2.8	1.1	0.9	2.2	0.3	3.4	1.9	1.1	2.2	0.5	1.4

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) expression of mRNAs in T cells from nephrotic patients during relapse and remission and nonnephrotic patients during infection and after recovery. The first column lists the genes tested, according to the HUGO nomenclature when available (see usual names below) or to Unigene cluster name. The following four columns show the mean RNA expression level, relative to ribosomal protein L28, in the four groups of samples. Values are means ± SE from 11 patients [except for *SELL* ($N = 10$), *CCT7* ($N = 9$), and *IKBKB* ($N = 10$) in nonnephrotic groups]. Values statistically different between groups ($P < 0.05$) are indicated in bold and are overlined in green (induction) or red (repression). Statistical comparison was done between groups nephrotic remission and nonnephrotic recovery (shown in column nephrotic remission), groups nephrotic relapse vs. nephrotic remission (shown in column nephrotic relapse) and groups nonnephrotic infection vs. nonnephrotic recovery (shown in column nonnephrotic infection). Column 6 indicates the abundance of the corresponding tag in the relapse and remission serial analysis of gene expression (SAGE) libraries prepared from patient 3. The last 2 × 11 columns show individual changes in expression during relapse (nephrotic relapse/nephrotic remission) or infection (nonnephrotic infection/nonnephrotic recovery) in each patient (identified by numbers on the top line). Induction and repression ≥1.5-fold are overlined in green and red, respectively, whereas changes of lesser amplitude are in yellow. NT is not tested.

HUGO nomenclature stands for: ***CD3D***, CD3D antigen, delta polypeptide (TiT3 complex); ***TRBC1***, T-cell receptor beta constant 1; ***TRG@***, T-cell receptor gamma locus; ***CD97***, CD97 antigen; ***LTB***, lymphotoxin beta [tumor necrosis factor (TNF) superfamily, member 3]; ***LRRN4***, leucine-rich repeat neuronal 4; ***IL7R***, interleukin 7 receptor (CD127); ***CD6***, CD6 antigen; ***LAT***, linker for activation of T cells; ***SELL***, L-selectin (lymphocyte adhesion molecule-1) (CD62L); ***TNFRSF7***, tumor necrosis factor receptor superfamily, member 7 (CD27); ***KLRK1***, killer cell lectin-like receptor subfamily K, member 1 (NKG2D); ***TNRC5***, trinucleotide repeat containing 5; ***MAL***, mal, T-cell differentiation protein; ***CD48***, CD48 antigen (CD2 accessory ligand); ***IFITM1***, interferon-induced transmembrane protein 1 (9-27) (CD225 or Leu13); ***SELS***, selenoprotein S; ***C20orf52***, chromosome 20 open reading frame 52; ***TGFB1***, transforming growth factor, beta 1 (Camurati-Engelmann disease); ***GPX4***, glutathione peroxidase 4 (phospholipid hydroperoxidase); ***HA-1***, minor histocompatibility antigen HA-1; ***ADAR***, adenosine deaminase, RNA-specific; ***EIF4G2***, eukaryotic translation initiation factor 4 gamma, 2; ***HNRPC***, heterogeneous nuclear ribonucleoprotein C (C1/C2); ***CIRBP***, cold-inducible RNA-binding protein (cold shock protein); ***TAPBP***, TAP binding protein (tapasin); ***RPN2***, ribophorin II; ***CCT7***, chaperonin containing TCP1, subunit 7 (eta); ***SSR4***, signal sequence receptor, delta (translocon-associated protein delta); ***PSMA6***, proteasome (prosome, macropain) subunit, alpha type, 6; ***COTL1***, coactosin-like 1; ***CORO1A***, coronin, actin-binding protein, 1A; ***NDUFA3***, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa; ***NDUFB8***: NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19 kD; ***ARL10A***, ADP-ribosylation factor-like 10A; ***IKBKB***, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; ***NFKB1***, nuclear factor of kappa light polypeptide gene enhancer in B cells 1 (p105); ***NFKBIA***, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; ***FLJ21438,*** hypothetical protein FLJ21438.

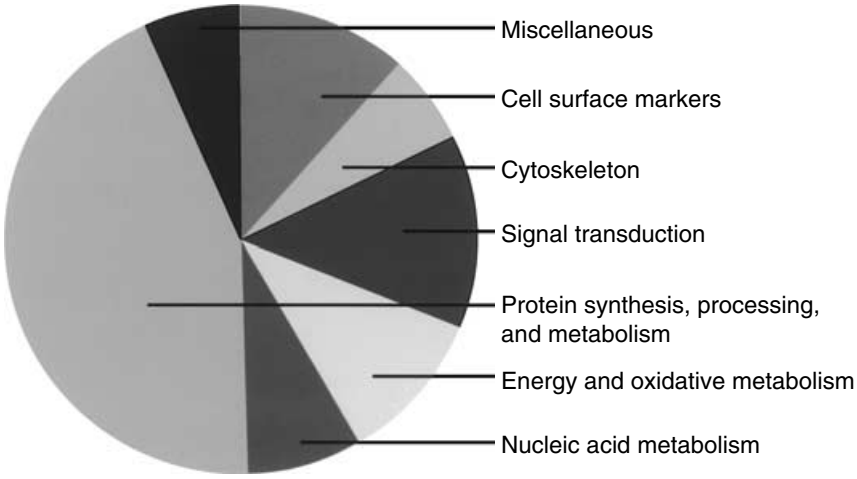

Fig. 1. Functional classes of genes for tags represented at statistically higher level in the relapse than in the remission library. The 179 functionally identified tags overrepresented in the relapse library were clustered into seven functional subgroups. List of genes in each cluster is available in supplemental Table 3.

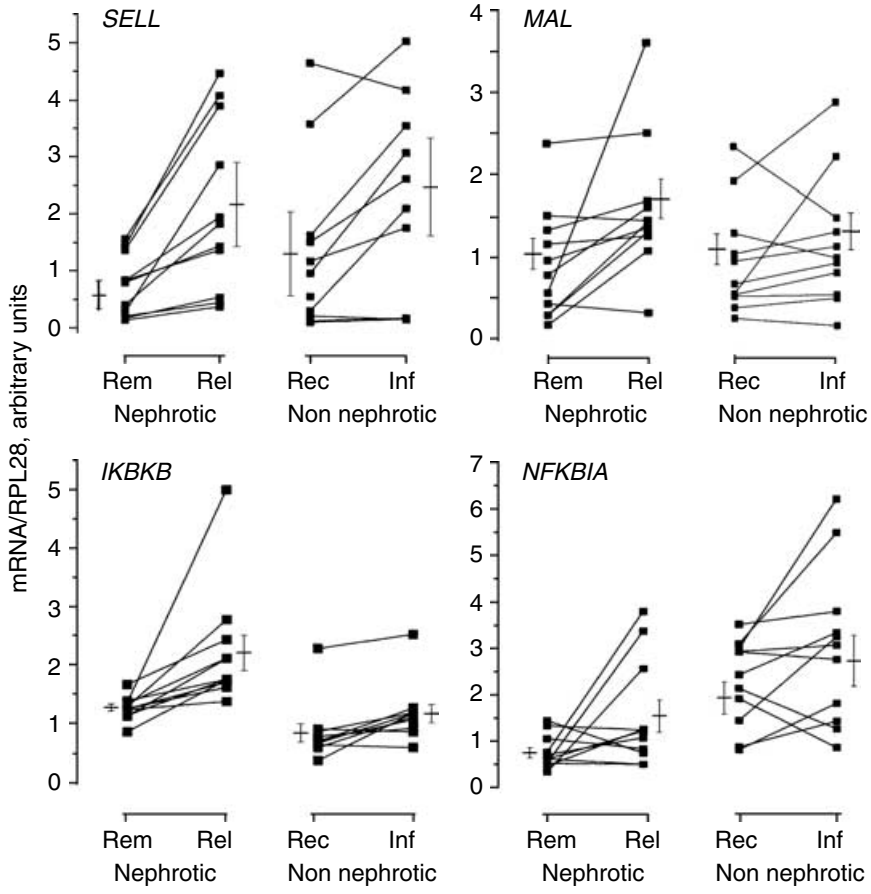

Fig. 2. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNAs expression in T cells from nephrotic patients during proteinuria relapse (rel) and after remission (rem) and non-nephrotic patients during infection (inf) and after recovery (rec). RT-PCR data are expressed relative to ribosomal protein L28 mRNA. *SELL*, L-selectin (lymphocyte adhesion molecule 1) (CD62L); *MAL*, mal, T-cell differentiation protein; *IKBKB*, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; *NFKBIA*, nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, alpha. Lines join values obtained in the same patients under the two pathophysiologic conditions.

observed by RT-PCR could not be revealed by SAGE; (*3*) gene induction was much less frequent in nephrotic patients than in nonnephrotic (175 vs. 249 green boxes in Table 2), whereas the opposite holds for gene repression (66 vs. 28 red boxes); and (*4*) a single gene (*SELL* or *CD62L*) was induced in all nephrotic patients.

L-Selectin as a putative glomerular permeability factor

L-Selectin, the product of *CD62L*, was considered as a potential glomerular permeability factor because (*1*) it was induced in all relapsing nephrotic patients, (*2*) it is a transmembrane protein with an extracellular domain which can be cleaved and released from the cell

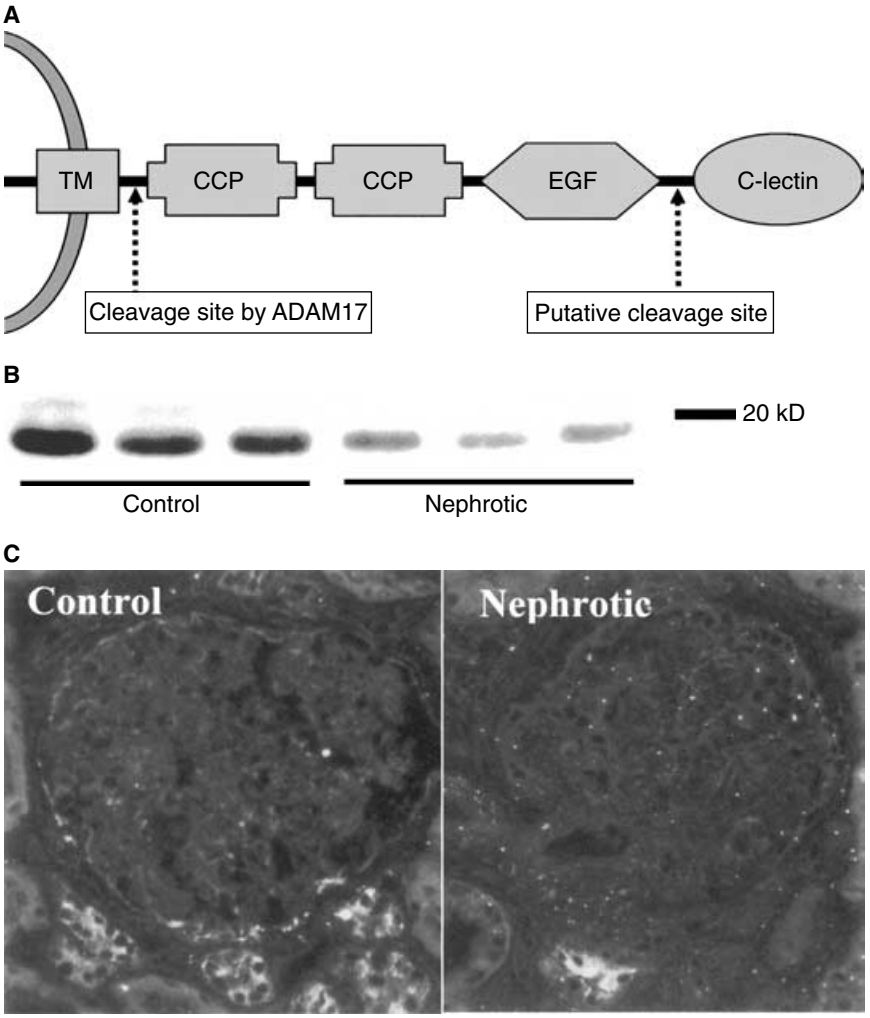

Fig. 3. Search for the presence of L-selectin in plasma and kidney of control and nephrotic patients. (*A*) Schematic representation of L-selectin with its transmembrane domain (TM), complement control protein domains (CCP), epidermal growth factor domain (EGF) and C-lectin domain, as well as its known and putative sites of cleavage by proteases. (*B*) Plasma from three control and three nephrotic patients in relapse were submitted to sodium dodecyl sulfate-polyacrylamide gel elctrophoresis (SDS-PAGE) (10% polyacrylamide gels) under denaturating conditions and to immunoblotting using an anti-L-selectin antibody directed against the C-lectin domain of L-selectin (Santa Cruz Biotechnologies, Santa Cruz, CA, USA). The ≈19 kD protein visualized by chemiluminescence of a secondary antibody corresponds to the C-lectin domain of L-selectin. (*C*) Unprocessed fragments of kidney biopsies from a nonnephrotic patient (control) and a nephrotic patient in relapse were used for L-selectin immunocytochemistry using the same antibody as above. Antigen-antibody complexes were revealed with a Cy3-conjugated goat antirabbit IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA) and sections were examined with a fluorescence microscope. L-Selectin staining was absent in both control and nephrotic glomerulus.

surface through either ADAM17-dependent or independent pathways [25], and (*3*) in high endothelial venules, L-selectin interacts with podocalyxin, a protein also expressed in podocytes where it is essential for the integrity of slit diaphragms structure. The extracellular domain of L-selectin consists of an extracellular C-lectin domain, responsible for the interaction with podocalyxin, linked in tandem to an epidermal growth factor (EGF) domain and several short consensus repeats homologue to complement binding proteins (Fig. 3A). In silico analysis of L-selectin sequence revealed that the short domain linking the C-lectin and the EGF domains contains a consensus sequence for protease similar to that linking the transmembrane and the extracellular domains. In addition, we demonstrated that the C-lectin domain can be released in the blood since, using an anti-C-lectin domain antibody, we identified in the plasma a protein of 19 kD, consistent with the size of the C-lectin domain (Fig. 3B). However, the C-lectin domain was less abundant in the plasma during proteinuria than in remission (Fig. 3B), likely as the result of decreased plasma protein content. In addition, immunohistochemistry on human renal biopsies failed to demonstrate the presence of the C-lectin domain on podocytes of either nonnephrotic or nephrotic patients during relapse (Fig. 3C), whereas the antibody staining was positive on liver sections.

DISCUSSION

Transcriptional activation of T cells was investigated in patients with steroid-sensitive idiopathic nephrotic syndrome, in absence of any steroid or immunosuppressive therapy. The study was carried out on circulating CD2+ cells because CD2 is a specific marker of the whole T-cell lineage expressed from the earliest stages of T commitment in the thymus to mature circulating T cells. Differential analysis of CD2+ cell transcriptomes in a nephrotic patient identified 228 mRNA tags, including a large number of markers of T-cell activation, overrepresented during proteinuria relapse. It cannot be inferred, however, whether overexpression of these genes is a cause or a consequence of proteinuria. RT-PCR analysis of 39 transcripts in nephrotic and nonnephrotic patients revealed that (*1*) >20% of these transcripts

displayed differential expression between nephrotic and nonnephrotic patients under resting conditions, (*2*) only *MAL* was activated in relapsing nephrotic patients but not during infection in nonnephrotic patients, (*3*) transcriptional activation was lesser in relapsing nephrotic patients than in nonnephrotic ones during infection, and (*4*) among genes encoding putatively secreted proteins, only *CD62L* was induced in all relapsing nephrotic patients.

T-cell activation in nephrotic syndrome

Many transcripts over represented in the "relapse" library encode T-cell surface markers, transcription factors involved in T-cell survival, and actin and cytoskeleton-related proteins closely involved in T-cell activation [26]. This validates the procedure for T-cell purification; in particular it demonstrates that the use of CD2 beads did not induce nonselective activation of lymphocytes.

Among transcripts over expressed during proteinuria relapse, *SELL* and *MAL* deserve special attention because *SELL* was the only transcript induced in all relapsing patients and *MAL* was overexpressed in relapsing patients but not during infection in nonnephrotic patients. *CD62L*, which was previously reported as being induced during proteinuria in nephrotic patients [18], is an early marker of T-cell commitment in bone marrow progenitors [27] and is involved in thymic homing [28]. *CD62L* is also expressed in circulating recent thymic emigrant and naive T cells (in which it plays a major role in lymph nodes homing), but its expression is markedly reduced in mature T cells [29]. *MAL* (T-lymphocyte maturation–associated protein) is a proteolipid expressed at T-cell surface which is a marker of late thymic T-cell differentiation [30]. Accordingly, it is strongly expressed in thymus cortex, but it is also found in a population of peripheral CD4+ T cells [31]. Thus, the overexpression of *SELL* and *MAL* in peripheral CD2+ cells reveals a population of low-mature circulating T cells (recent emigrant and naive T cells) in nephrotic patients. This conclusion is also supported by the over expression in relapsing patients of Tc-mip, a signaling factor much more abundant in thymus than in circulating PBMCs [9].

Differences in T-cell expression profile in nephrotic and nonnephrotic individuals in remission

The most striking findings were the changes in expression level under basal condition (remission or recovery) in nephrotic versus nonnephrotic patients of several genes involved in the control of apoptosis. Repression of *NFKBIA* (IKBα) in nephrotic remission versus nonnephrotic recovery patients, which largely accounts for the repression previously reported between relapsing patients and non nephrotic controls [32], joined with the induction of *IKBKB*, which specifically phosphorylates and inactivates IKBα, leads to an hyperactivation of nuclear factor-κB (NF-κB) profile in nephrotic patients in remission. In T-cell lineage, NF-κB plays a central role in the control of cytokine expression, T cell proliferation after T-cell receptor engagement, as well as quality control of thymocytes through the regulation of apoptosis [33]. As a matter of fact, NF-κB displays dual effects in the control of apoptosis. It is required for αCD3-mediated apoptosis of double positive thymocytes [34] but it is also essential for protecting T cells against tumor necrosis factor (TNF)-induced apoptosis [35]. Changes in transcript expression as well as clinical observations indicate that both pro- and antiapoptotic pathways are involved in idiopathic nephrotic syndrome. Activation of antiapoptotic pathway is supported by the repression of *ARL10A* which belongs to the proapoptotic adenosine diphosphate (ADP)-ribosylation factors [36], and by the beneficial effect of thymic proapoptotic factors on the disease. Indeed, first, prednisone and cyclophosphamide induce marked proapoptotic actions on thymocytes [37, 38], which may lead to complete loss of thymic cortex [39] and prolonged remissions in nephrotic patients [40, 41]. Second, similarly, measles lead to thymic apoptosis [42] and induce permanent remission [43]. Third, conversely, cyclosporine which decreases apoptosis in the thymic corticomedullary area [44], the site of negative selection, rarely induces sustained remission in nephrotic patients after treatment withdrawal [45]. Finally, decrease activity of idiopathic nephrosis observed with aging [46] is temporally associated with thymus involution [47]. Conversely, activation of apoptosis is supported by the repression of the antiapoptotic gene *TGFB1* [48] and by the close association between antithymoglobulin therapy which induces peripheral T-cell apoptosis [49] and early relapse of nephrotic syndrome in transplanted patients [50]. Thus, the constitutive activation of NF-κB pathway in peripheral CD2+ cells may reveal a dysregulation of thymus and peripheral T-cell apoptosis in nephrotic patients during remission. This thymic defect, along with the over expression of *CD3D*, which is associated with thymocyte differentiation [51, 52], may be responsible for the presence of a low-mature circulating T-cell population in relapsing nephrotic patients. *CCT7*, which is repressed in nephrotic remission vs. nonnephrotic recovery patients, is a T-complex protein 1 subunit involved in Fas-induced apoptosis of Jurkat T cells [53] which belongs to the SSNS1 (steroid sensitive nephrotic syndrome 1) locus [54].

Glomerular permeability factor

The identification of the glomerular permeability factor in idiopathic nephrotic syndrome has remained unanswered despite an outstanding number of studies. The C-lectin domain of L-selectin appeared as a good candidate because (*1*) expression of L-selectin gene was induced during proteinuria in all nephrotic patients (Table 2) and the C-lectin domain of L-selectin was released in

the plasma (Fig. 3B); (*2*) apoptosis, which is altered in T cells from nephrotic patients, induces L-selectin shedding from T-cell surface [55]; (*3*) the C-lectin domain is responsible for the specific interaction of L-selectin with sulfated sialyl Lewis(x) carbohydrate of podocalyxin in high endothelial venules, permitting lymphocytes homing into lymph nodes [56]; and (*4*) podocalyxin is an important protein for the integrity of the glomerular filter. Indeed, uncoupling of podocalyxin from the actin skeleton leads to the loss of glomerular foot processes in three different experimental models of heavy proteinuria and nephrotic syndrome [57], and transgenic mice lacking podocalyxin exhibit profound defects in kidney development and die within 24 hours after birth [58]. However, the presence of the C-lectin domain in control plasma and the absence of detectable C-lectin domain of L-selectin on podocytes from nephrotic patients makes unlikely its role as the glomerular permeability factor unless its interaction with glomerular podocalyxin is labile, like the interaction of L-selectin with high endothelial venules. In particular, it is possible that the podocalyxin/C-lectin domain interaction is no longer possible once it has induced effacement of the podocytic foot processes.

Alternatively, we may have missed the permeability factor in our screening of T-cell transcriptome. It is worth recalling that although we studied the largest T-cell molecular population ever analyzed in idiopathic nephrotic syndrome (>20,000 different mRNA tags), SAGE is not an exhaustive method. Indeed, first, ≈5% cDNAs lack the anchoring enzyme (*Sau*3AI) site and therefore were excluded from SAGE analysis. Second, transcripts displaying AT-rich elements in their 3′untranslated region (UTR), a common feature for lymphokines and cytokines [59], are submitted to an intense degradation which may compromise their full recollection. Third, analysis of 25,000 tags provides a 95% confidence level for detecting transcripts expressed at ≥35 copies per cell [60], therefore excluding transcripts with low expression level as those coding for a majority of lymphokines and cytokines [59]. In addition, unidentified tags were obviously excluded from the screening of putative secreted proteins.

In view of the accumulation over the past three decades of fruitless attempts to identify a circulating glomerular factor of T-cell origin in nephrotic syndrome, one might question whether circulating T lymphocytes are the actual source for this factor.

CONCLUSION

Circulating T cells from relapsing nephrotic patients are likely including a significant population of low-mature cells closely related to thymocytes or naive T cells freshly coming from the thymus. Constitutive activation of NF-κB, along with other pro- and antiapoptotic markers, during remission might represent a defect in thymocyte apoptosis and selection. Present results provide a molecular basis for the "special role for the thymus" proposed by Shalhoub [43] in the primary pathogenesis of idiopathic nephrotic syndrome some 30 years ago.

ACKNOWLEDGMENTS

We thank Dr. M. Nathanson (Hôpital J. Verdier), Dr. S. Nathanson (Hôpital H. Mignot), Dr. A. May (Hôpital L. Michel) and Dr. V. Guigonis (Hôpital A. Trousseau) and Professor A. Bensman (Hôpital A. Trousseau) for their contribution in the selection of patients and blood sampling. This work was supported by a grant from Institut National de la Santé et de la Recherche Médicale (INSERM 4P005E). We sincerely thank Professor E. Haddad (Hôpital Robert Debré) and Dr. J. Galon (Institut des Cordeliers) for helpful discussions.

Reprint requests to Alain Doucet, UMR 7134, Institut des Cordeliers, 15 rue de l'école de médecine, 75270 Paris cedex 6, France.
E-mail: alain.doucet@bhdc.jussieu.fr

REFERENCES

1. Broyer M, Meyrier A, Niaudet P, Habib R: Minima changes and focal segmental glomerular sclerosis, in *Oxford Textbook of Clinical Nephrology* (vol 1), edited by Davison A, Cameron J, Grünfeld J, Kerr D, Ritz E, CG W, Oxford, Oxford University Press, 1998, pp 493–535
2. Topaloglu R, Saatci U, Arikan M, *et al*: T-cell subsets, interleukin-2 receptor expression and production of interleukin-2 in minimal change nephrotic syndrome. *Pediatr Nephrol* 8:649–652, 1994
3. Daniel V, Trautmann Y, Konrad M, *et al*: T-lymphocyte populations, cytokines and other growth factors in serum and urine of children with idiopathic nephrotic syndrome. *Clin Nephrol* 47:289–297, 1997
4. Lama G, Luongo I, Tirino G, *et al*: T-lymphocyte populations and cytokines in childhood nephrotic syndrome. *Am J Kidney Dis* 39:958–965, 2002
5. Yap HK, Cheung W, Murugasu B, *et al*: Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: Evidence for increased IL-13 mRNA expression in relapse. *J Am Soc Nephrol* 10:529–537, 1999
6. Stachowski J, Barth C, Michalkiewicz J, *et al*: Th1/Th2 balance and CD45-positive T cell subsets in primary nephrotic syndrome. *Pediatr Nephrol* 14:779–785, 2000
7. Zachwieja J, Bobkowski W, Dobrowolska-Zachwieja A, *et al*: Intracellular cytokines of peripheral blood lymphocytes in nephrotic syndrome. *Pediatr Nephrol* 17:733–740, 2002
8. Kaneko K, Tuchiya K, Fujinaga S, *et al*: Th1/Th2 balance in childhood idiopathic nephrotic syndrome. *Clin Nephrol* 58:393–397, 2002
9. Grimbert P, Valanciute A, Audard V, *et al*: Truncation of C-mip (Tc-mip), a new proximal signaling protein, induces c-maf Th2 transcription factor and cytoskeleton reorganization. *J Exp Med* 198:797–807, 2003
10. Matsumoto K, Kanmatsuse K: Increased IL-12 release by monocytes in nephrotic patients. *Clin Exp Immunol* 117:361–367, 1999
11. Lagrue G, Branellec A, Blanc C, *et al*: A vascular permeability factor in lymphocyte culture supernants from patients with nephrotic syndrome. II. Pharmacological and physicochemical properties. *Biomedicine* 23:73–75, 1975
12. Levin M, Gascoine P, Turner MW, Barratt TM: A highly cationic protein in plasma and urine of children with steroid-responsive nephrotic syndrome. *Kidney Int* 36:867–877, 1989
13. Tanaka R, Yoshikawa N, Nakamura H, Ito H: Infusion of peripheral blood mononuclear cell products from nephrotic children increases albuminuria in rats. *Nephron* 60:35–41, 1992
14. Girardin EP, Birmele B, Benador N, *et al*: Effect of plasma from patients with idiopathic nephrotic syndrome on proteoglycan

synthesis by human and rat glomerular cells. *Pediatr Res* 43:489–495, 1998
15. Koyama A, Fujisaki M, Kobayashi M, *et al*: A glomerular permeability factor produced by human T cell hybridomas. *Kidney Int* 40:453–460, 1991
16. Dantal J, Baatard R, Hourmant M, *et al*: Recurrent nephrotic syndrome following renal transplantation in patients with focal glomerulosclerosis. A one-center study of plasma exchange effects. *Transplantation* 52:827–831, 1991
17. Le Berre L, Godfrin Y, Lafond-Puyet L, *et al*: Effect of plasma fractions from patients with focal and segmental glomerulosclerosis on rat proteinuria. *Kidney Int* 58:2502–2511, 2000
18. Sahali D, Pawlak A, Valanciute A, *et al*: A novel approach to investigation of the pathogenesis of active minimal-change nephrotic syndrome using subtracted cDNA library screening. *J Am Soc Nephrol* 13:1238–1247, 2002
19. Savin VJ, Sharma R, Sharma M, *et al*: Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med* 334:878–883, 1996
20. Musante L, Candiano G, Bruschi M, *et al*: Characterization of plasma factors that alter the permeability to albumin within isolated glomeruli. *Proteomics* 2:197–205, 2002
21. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW: Serial analysis of gene expression. *Science* 270:484–487, 1995
22. Virlon B, Cheval L, Buhler JM, *et al*: Serial microanalysis of renal transcriptomes. *Proc Natl Acad Sci USA* 96:15286–15291, 1999
23. Elalouf JM, Buhler JM, Tessiot C, *et al*: Predominant expression of beta 1-adrenergic receptor in the thick ascending limb of rat kidney. Absolute mRNA quantitation by reverse transcription and polymerase chain reaction. *J Clin Invest* 91:264–272, 1993
24. Velculescu VE, Zhang L, Zhou W, *et al*: Characterization of the yeast transcriptome. *Cell* 88:243–251, 1997
25. Walcheck B, Alexander SR, St Hill CA, Matala E: ADAM-17-independent shedding of L-selectin. *J Leukoc Biol* 74:389–394, 2003
26. Samstag Y, Eibert SM, Klemke M, Wabnitz GH: Actin cytoskeletal dynamics in T lymphocyte activation and migration. *J Leukoc Biol* 73:30–48, 2003
27. Perry SS, Wang H, Pierce LJ, *et al*: L-selectin defines a bone marrow analog to the thymic early T-lineage progenitor. *Blood* 103:2990–2996, 2004
28. Lepique AP, Palencia S, Irjala H, Petrie HT: Characterization of vascular adhesion molecules that may facilitate progenitor homing in the post-natal mouse thymus. *Clin Dev Immunol* 10:27–33, 2003
29. Luettig B, Sponholz A, Heerwagen C, *et al*: Recent thymic emigrants (CD4+) continuously migrate through lymphoid organs: Within the tissue they alter surface molecule expression. *Scand J Immunol* 53:563–571, 2001
30. Alonso MA, Weissman SM: cDNA cloning and sequence of MAL, a hydrophobic protein associated with human T-cell differentiation. *Proc Natl Acad Sci USA* 84:1997–2001, 1987
31. Copie-Bergman C, Plonquet A, Alonso MA, *et al*: MAL expression in lymphoid cells: Further evidence for MAL as a distinct molecular marker of primary mediastinal large B-cell lymphomas. *Mod Pathol* 15:1172–1180, 2002
32. Sahali D, Pawlak A, Le Gouvello S, *et al*: Transcriptional and post-transcriptional alterations of IkappaBalpha in active minimal-change nephrotic syndrome. *J Am Soc Nephrol* 12:1648–1658, 2001
33. Esslinger CW, Jongeneel CV, MacDonald HR: Survival-independent function of NF-kappaB/Rel during late stages of thymocyte differentiation. *Mol Immunol* 35:847–852, 1998
34. Hettmann T, DiDonato J, Karin M, Leiden JM: An essential role for nuclear factor kappaB in promoting double positive thymocyte apoptosis. *J Exp Med* 189:145–158, 1999
35. Senftleben U, Li ZW, Baud V, Karin M: IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. *Immunity* 14:217–230, 2001
36. Zhu JW, DeRyckere D, Li FX, *et al*: A role for E2F1 in the induction of ARF, p53, and apoptosis during thymic negative selection. *Cell Growth Differ* 10:829–838, 1999
37. Chow SC, Snowden R, Orrenius S, Cohen GM: Susceptibility of different subsets of immature thymocytes to apoptosis. *FEBS Lett* 408:141–146, 1997
38. Han S, Choi H, Ko MG, *et al*: Peripheral T cells become sensitive to glucocorticoid- and stress-induced apoptosis in transgenic mice overexpressing SRG3. *J Immunol* 167:805–810, 2001
39. Loc NK, Olah I, Takacs L, *et al*: Effect of cyclophosphamide on the rat thymus. *Acta Morphol Acad Sci Hung* 29:319–333, 1981
40. Ehrich JH, Brodehl J: Long versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. Arbeitsgemeinschaft fur Padiatrische Nephrologie. *Eur J Pediatr* 152:357–361, 1993
41. Ueda N, Kuno K, Ito S: Eight and 12 week courses of cyclophosphamide in nephrotic syndrome. *Arch Dis Child* 65:1147–1150, 1990
42. Auwaerter PG, Kaneshima H, McCune JM, *et al*: Measles virus infection of thymic epithelium in the SCID-hu mouse leads to thymocyte apoptosis. *J Virol* 70:3734–3740, 1996
43. Shalhoub RJ: Pathogenesis of lipoid nephrosis: A disorder of T-cell function. *Lancet* 2:556–560, 1974
44. Damoiseaux JG, Defresne MP, Reutelingsperger CP, Van Breda Vriesman PJ: Cyclosporin-A differentially affects apoptosis during in vivo rat thymocyte maturation. *Scand J Immunol* 56:353–360, 2002
45. Niaudet P: Comparison of cyclosporin and chlorambucil in the treatment of steroid-dependent idiopathic nephrotic syndrome: A multicentre randomized controlled trial. The French Society of Paediatric Nephrology. *Pediatr Nephrol* 6:1–3, 1992
46. Fakhouri F, Bocquet N, Taupin P, *et al*: Steroid-sensitive nephrotic syndrome: From childhood to adulthood. *Am J Kidney Dis* 41:550–557, 2003
47. Steinmann GG, Klaus B, Muller-Hermelink HK: The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. *Scand J Immunol* 22:563–575, 1985
48. Wahl SM, Orenstein JM, Chen W: TGF-beta influences the life and death decisions of T lymphocytes. *Cytokine Growth Factor Rev* 11:71–79, 2000
49. Michallet MC, Saltel F, Preville X, *et al*: Cathepsin-B-dependent apoptosis triggered by antithymocyte globulins: A novel mechanism of T-cell depletion. *Blood* 102:3719–3726, 2003
50. Raafat R, Travis LB, Kalia A, Diven S: Role of transplant induction therapy on recurrence rate of focal segmental glomerulosclerosis. *Pediatr Nephrol* 14:189–194, 2000
51. Nal B, Carroll P, Mohr E, *et al*: Coronin-1 expression in T lymphocytes: Insights into protein function during T cell development and activation. *Int Immunol* 16:231–240, 2004
52. Delgado P, Fernandez E, Dave V, *et al*: CD3delta couples T-cell receptor signalling to ERK activation and thymocyte positive selection. *Nature* 406:426–430, 2000
53. Gerner C, Frohwein U, Gotzmann J, *et al*: The Fas-induced apoptosis analyzed by high throughput proteome analysis. *J Biol Chem* 275:39018–39026, 2000
54. Ruf RG, Fuchshuber A, Karle SM, *et al*: Identification of the first gene locus (SSNS1) for steroid-sensitive nephrotic syndrome on chromosome 2p. *J Am Soc Nephrol* 14:1897–1900, 2003
55. Kern PM, Keilholz L, Kalden JR, Herrmann M: Apoptotic UV-irradiated lymphocytes undergo protease mediated shedding of L-selectin in vitro. *Transfus Apheresis Sci* 24:99–101, 2001
56. Lawrence MB: Selectin-carbohydrate interactions in shear flow. *Curr Opin Chem Biol* 3:659–664, 1999
57. Takeda T, McQuistan T, Orlando RA, Farquhar MG: Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. *J Clin Invest* 108:289–301, 2001
58. Doyonnas R, Kershaw DB, Duhme C, *et al*: Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. *J Exp Med* 194:13–27, 2001
59. Stordeur P, Zhou L, Goldman M: Analysis of spontaneous mRNA cytokine production in peripheral blood. *J Immunol Methods* 261:195–197, 2002
60. Clarke L, Carbon J: A colony bank containing synthetic Col El hybrid plasmids representative of the entire *E. coli* genome. *Cell* 9:91–99, 1976