Identification of Nucleated Cells in Urine Using Lectin Staining

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• **Microscopic examination of urinary sediment is an integral component in the evaluation of nephropathies. However, identification and differentiation of the nucleated nonsquamous cells in urine is often difficult using such conventional techniques as phase contrast or bright field microscopy, even after Papanicolaou staining, and requires a lot of experience. We now report a method to differentiate urinary cell types using lectin staining. Twenty-five lectins were examined with respect to their binding pattern on cryosections of the human kidney and urinary tract, as well as binding to blood cells. The specificity of lectin binding to a cell type both in situ and in urine was confirmed by double labeling with specific antibodies directed against various sections of the nephron or nucleated blood cells. For urine cytologic examinations, acetone-fixed cytopreparations of urinary sediments were incubated with a combination of a fluorescein isothiocyanate (FITC)-coupled and a rhodamine-coupled lectin, followed by staining of the nuclei with 4',6-diamidino-2-phenylindole. Specimens were examined in triple immunofluorescence (FITC/rhodamine/UV). Cell types could be identified by their characteristic lectin-binding pattern. For example, the lectin combination of *Sophora japonica* agglutinin (aggl; SJA) and *Erythrina cristagalli* aggl (ECA) permitted a differentiation between cells of the proximal tubules (SJA positive [SJA+], ECA+), distal tubules (SJA negative [SJA–], ECA+), collecting ducts (SJA+, ECA–), and lymphocytes (SJA–, ECA–). In preliminary studies, examination of urinary sediment in various chronic nephropathies by this technique showed differences in their cellular excretion pattern. In summary, staining urinary sediments with combinations of lectins provides a rapid and relatively inexpensive method for a facilitated and reliable differentiation of the various nucleated cell types in urine.**

• **INDEX WORDS: Kidney; urine cytology; sediment; nephropathy; diagnosis; monitoring.**

MICROSCOPIC examination of the urinary sediment is considered essential in the evaluation of nephropathies.[1] It includes the analysis of formed elements, casts, and crystals and the morphological characteristics of bacteria, erythrocytes, and nucleated cells. Analysis of the excretion pattern of nucleated cells in urine might provide information about the severity, pathogenesis, and course of a nephropathy. However, identification and differentiation of the nucleated nonsquamous cells by such conventional techniques as phase contrast or bright field microscopy, even after staining the specimens by such techniques as Papanicolaou or Giemsa, is often difficult and requires very skilled examiners.[1-3] To evaluate the significance of the presence of the various nucleated cell types in the urinary sediment, their easy, accurate, and systematic identification by a quick and routinely applicable method is desirable.

The aim of the present study is to evaluate the use of lectin staining to differentiate nucleated cell types in urine. Lectins are oligomeric proteins with several sugar-binding sites per molecule that interact with monosaccharides and oligosaccharides by noncovalent binding. They are therefore regarded as carbon hydrate–specific antibodies. They have been used to show the distribution of cellular glycoconjugates in human tissues, such as the kidney,[4-9] but also to identify cells in culture[10] and for cell isolation.[11] We examined the binding patterns of 25 lectins to nucleated cell types that can potentially occur in urine. Staining urinary sediments of healthy volunteers and patients with chronic nephropathies with lectins proved a rapid and feasible method that facilitated the differentiation of cell types in urine. This procedure might significantly enhance the diagnostic value of urine cytologic examination as a noninvasive tool in the basic diagnosis of both acute and chronic kidney diseases.

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PATIENTS AND METHODS

Patients

Urine samples were collected from healthy volunteers (n = 20) and patients with the following kidney diseases: mesangioproliferative glomerulonephritis (hereafter referred to as the glomerulonephritis group, n = 13), diabetic nephropathy (n = 12), hypertensive nephropathy (n = 10), and lupus nephritis (n = 5). Most patients had moderately impaired renal function (serum creatinine > 1.3 mg/dL and < 2 mg/dL; serum creatinine clearance < 75 mL/min) or proteinuria greater than 500 mg/d of protein. Patients with lupus nephritis had stable kidney function at the time of examination. Diagnosis was usually established by renal biopsy, but also by clinical data, particularly in the case of diabetic nephropathy.

Urine Cytologic Specimens

Urine samples were collected from spontaneously voided morning urine and prepared within 4 hours. Cytopreparations of urinary sediments were obtained by concentrating the cellular elements in 10 mL of urine by centrifugation at 460*g* for 10 minutes. The pellet was resuspended in 100 μL of phosphate-buffered saline (PBS). Sixty microliters of this suspension was spun by a cytocentrifuge (Cytospin 2; Shandon, Astmoor, England) onto a glass cover slide (164*g* for 10 minutes) and further processed, as described next.

Cryosections

Eight-micron cryosections were prepared from human kidney (cortex and outer and inner medulla), ureter, and bladder. Material was obtained from patients who underwent nephrectomy or cystectomy, eg, for cancer. After removal, tissue was immediately frozen in isopentane cooled by liquid nitrogen. Specimens were prepared from tissue regions that were histologically free from tumor.

Blood mononuclear cells (blood groups A, B, and O) were prepared from buffy coats and enriched by density centrifugation at 820*g* for 25 minutes (Lymphoprep; Nycomed Pharma AS, Oslo, Norway). The cells were cytocentrifuged onto glass slides, as reported for the urine specimens.

Immunohistochemistry

Urine cytologic specimens, as well as frozen sections, were fixed with acetone (–20°C for 10 minutes), washed with PBS, and incubated with a combination of a fluorescein isothiocyanate (FITC)-conjugated and a rhodamine-conjugated lectin dissolved in PBS at a concentration of 75 μg/mL for 15 minutes at room temperature in the dark. After a washing step with PBS, nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma, St Louis, MO; 5 μg/mL of stock solution in methanol, 1:50 diluted with PBS before use) for 7 minutes at room temperature. To prevent bleaching, specimens were finally embedded with Mowiol (Calbiochem, La Jolla, CA), 10% (wt/vol) in 25% (vol/vol) glycerol, pH 8.5, with Tris/HCl.

For double-labeling studies with antibodies, specimens were incubated with the respective antibody at 37°C for 60 minutes (anti-I/anti-i: 4°C overnight for 12 hours). Specimens were washed thoroughly with PBS and incubated with an appropriate FITC-labeled or rhodamine-labeled second antibody for 60 minutes at 37°C. Subsequently, lectin staining was performed under the conditions previously described.

The following lectins were examined: *Arachis hypogaea* agglutinin ([aggl] peanut lectin); *Artocarpus integrifolia* aggl (Jacalin); *Canavalia ensiformis* aggl; *Datura stramonium* aggl; *Dolichos biflorus* aggl (DBA); elderberry bark aggl; *Erythrina cristagalli* aggl (ECA); *Glycine maximum* aggl (soybean lectin); *Griffonia simplicifolia* I aggl; *Griffonia simplicifolia* II aggl (GS II); *Lens culinaris* aggl; *Lotus tetragonolobus* aggl (LTA); *Lycopersicon esculentum* aggl; *Phaseolus vulgaris*, agglutinating erythrocytes; *Phaseolus vulgaris*, agglutinating leukocytes; *Phytolacca americana* aggl; *Pisum sativum* aggl; *Ricinus communis* I aggl; *Sophora japonica* aggl (SJA); *Solanum tuberosum* aggl; *Tritium vulgaris* aggl (wheat germ [WGA]); *Tritium vulgaris* aggl, succinylated (succinylated WGA); *Ulex europaeus* aggl; *Vicia villosa* lectin; and *Wisteria floribunda* aggl (WFA). All lectins were obtained from Vector Laboratories (Burlingame, CA), with the exception of LTA and WFA, which were obtained from Sigma.

The antibodies used in this study and their binding characteristics are listed in Table 1.

Evaluation of Urine Cytologic Specimens

For each specimen, the number of cells in 10 high-power fields (HPF) were counted at original magnification ×500 using a fluorescence microscope (Orthoplan; Zeiss Microscope, Oberkochen, Germany) equipped with filters for UV, FITC, and rhodamine. First, the number of DAPI-stained nuclei in the field of vision was identified by means of the UV filter. By switching to the FITC and rhodamine filters, the lectin-binding characteristics of the nucleated cells were determined, and cells were classified according to their binding pattern. Morphological evaluation of urinary cell types was performed according to criteria described by

Table 1. Antibodies Used to Characterize the Binding Pattern of Lectins in Double-Labeling Studies

Antibody (Working Dilution, Source)	Marked Structure
CD 13[12] (1:20; Dakopatts, Glostrup, Denmark)	Proximal tubules
Tamm-Horsefall[12] (1:20; Vector Laboratories)	Thick ascending limbs and distal tubules
Human cold isoagglutinin anti-I[13] (1:10*)	Thin limbs of Henle
Human cold isoagglutinin anti-i[13] (1:10*)	Collecting ducts
Factor VIII–related antigen (1:20; Behringwerke, Marburg, Germany)	Endothelial cells
CD 3 (1:20; Dakopatts)	T lymphocytes
CD 15 (1:20; Dakopatts)	Granulocytes
CD 64 (1:20; Dakopatts)	Monocytes

*Gift of D. Roelcke, Institute of Immunology, Heidelberg, Germany.

Schumann and Weiss[2] and Piccoli et al.[3] Sizes of cells were estimated by reference to the size of leukocytes, assumed to be 11 to 14 μm in diameter. Numbers are given as cells/10 HPF.

Statistics

Because of the skewed distribution of the data, values are given as median and 25% and 75% quantiles. The nonoutlier maxima and minima are shown in some experiments. Data were statistically analyzed by Mann-Whitney *U* test. Correlations were calculated using Pearson's correlation coefficient and Spearman's rank correlation coefficient. The statistical software, Statistica, version 5.1 (Statsoft, Hamburg, Germany), was used.

RESULTS

Lectin-Binding Pattern to Structures of the Kidney, Urinary Tract, and Nucleated Blood Cells

Twenty-five lectins, listed in Patients and Methods, were examined with respect to their binding pattern on cryosections of the human kidney and urinary tract, as well as to blood cells (blood groups A, B, and O). Structures were identified by morphological criteria and double immunolabeling with known markers, listed in Table 1.

Only lectin GS II exclusively marked one of the examined structures, namely the transitional cells throughout all layers of the mucous tunic of both the ureter and bladder, hereafter called urothelium. It permitted the identification of this cell type in urine using this lectin. Such lectins as Jacalin, succinylated WGA, *Phytolacca americana* aggl, or *Phaseolus vulgaris*, agglutinating erythrocytes, bound to almost all structures of the kidney, excluding them as markers. Conversely, other lectins selectively stained some of the examined structures.

Lectins LTA, SJA, and ECA proved to be useful for the differentiation of various cell types. ECA bound in the cortex to proximal and distal tubules and in the inner medulla to the loops of Henle. It also showed binding sites on urothelium, but not on collecting ducts. SJA bound in the cortex to proximal tubules and in the inner medulla to collecting ducts (Fig 1). Therefore, only the cells of the proximal tubule bound to

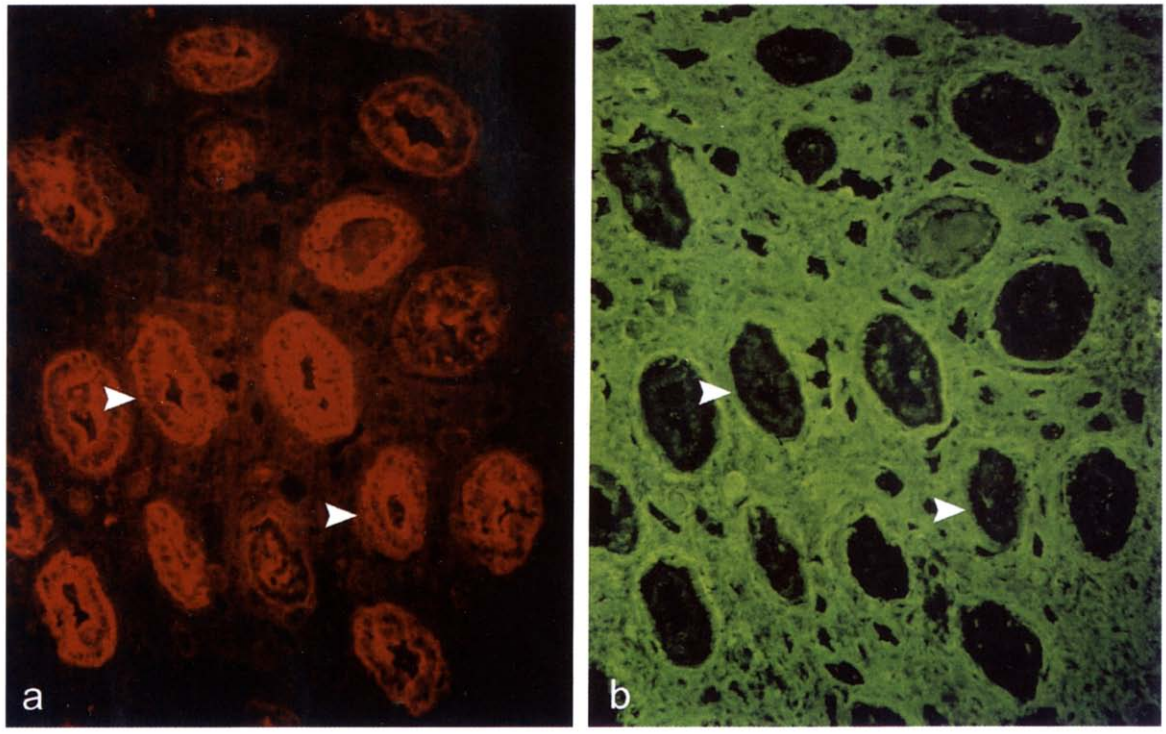

Fig 1. Lectin binding to renal structures. Binding pattern of lectin SJA (rhodamine coupled) and ECA (FITC coupled) to cryosections of the human inner medulla. (a) Rhodamine filter: only the collecting ducts, some marked by arrowheads, bound SJA. (b) FITC filter: lectin ECA showed a complementary binding pattern to SJA. The collecting ducts did not bind this lectin, whereas the other structures were all positive. (Original magnification ×380.)

both lectins (SJA positive [SJA+], ECA+). This combination permitted the differentiation of this cell type from distal tubules (SJA negative [SJA–], ECA+), collecting ducts (SJA+, ECA–), and lymphocytes (SJA–, ECA–). In addition, the distal tubules, granulocytes, cells of the glomerulus, loop of Henle, and urothelium were also negative for SJA and positive for ECA. Granulocytes could be clearly differentiated from other cell types with this binding pattern by their segmented nuclei in DAPI staining with the UV filter. Cells of the distal tubule, which are irregularly polygonal, cuboid, or faceted, differed from transitional cells of the urothelium, which are rounded, raquet-shaped, flat, or umbrella-shaped. The lymphocytes were very faintly positive for ECA, especially in patients with blood group O. This binding was substantially weaker than in the other cell types positive for ECA. The combination of SJA/LTA had a binding pattern similar to that of SJA/ECA. Only the proximal tubules were positive for both lectins. Collecting duct cells were SJA+ and LTA–. The cells of the thin loop of Henle and granulocytes were SJA– and LTA+, but the latter could easily be identified by the shape of their nuclei. Glomeruli, distal tubules, transitional cells of the urothelium, and lymphocytes were negative for both these lectins. These cell types were differentiated by morphological criteria. For example, lymphocytes were characterized by their small cytoplasms. With respect to these lectins, monocytes showed a similar binding pattern to the granulocytes. The binding patterns of these lectins are listed in Table 2.

Our studies of lectin-binding patterns suggested additional lectin combinations for the specific identification of particular structures, listed in Table 3. To evaluate the applicability of lectin staining for the facilitated differentiation of nucleated cells in the urine, we focused on the lectin combinations of SJA/LTA and, in particular, SJA/ECA.

Examination of Lectin Binding in Urinary Sediment

To assess whether the lectin-binding patterns of cells voided into the urine corresponded to those in situ, conformity between the morphological characteristics of the cell type and its lectin-binding pattern was examined in acetone-fixed urinary sediments. Examining urine with lectin combinations SJA-FITC and LTA-rhodamine, the lectin-binding pattern was in agreement with the respective morphological characteristics of the cell type. Double staining of urine specimens with one of the previously mentioned marker antibodies and a lectin was also performed. The lectin-binding pattern of cell type identified by the antibody always agreed with the binding characteristics predicted from the studies of the cryosections and isolated blood cells. Double labeling with the antibody against Tamm-Horsefall protein was not possible in urinary sediment because this urine secretory protein coated all urinary cells, allowing no differentiation. Furthermore, parallel staining of specimens from the same urinary sediment with both the lectin combination SJA/ECA and antibodies anti-CD 13 or anti-CD 3 was performed.

As shown in Fig 2, the number of proximal tubular cells and T lymphocytes identified by lectin or antibody staining in the sediment of the same urine yielded a good correlation between both methods. Thus, preservation of lectin binding on cells voided into the urine

Table 2. Binding Pattern of Lectins SJA, ECA, and LTA

Lectin	Specificity	Gl	PT	tlH	TalH	DT	CD	Uro	Ly	Gr/Mo
SJA	βD-galNAc	–	+	–	–	–	+	–	–	–
ECA	βD-gal(14)DglcNAc	+	+	+	+	+	–	+	(–)	+
LTA	αL-fuc	–	+	+	–	–	–	–	–	+

NOTE. Lectins SJA, ECA, and LTA were the most useful to differentiate between the cell types occurring in urinary sediment. Granulocytes and monocytes showed a similar binding pattern with respect to these lectins.

Abbreviations: Gl, glomerulus; PT, proximal tubule; tlH, thin limb of Henle; TalH, thick ascending limb of Henle; DT, distal tubule; CD, collecting duct; Uro, urothelium, including the transitional cells of the ureter and bladder; Ly, T lymphocytes; Gr, granulocytes; Mo, monocytes; +, lectin binds to the indicated structure; –, no binding of the lectin, (–), very faint binding of the lectin.

Table 3. Identification of Urinary Cell Types by the Unique Binding Pattern of a Lectin Combination

Cell Type	Lectin Binding
Glomerulus	ECA– and WFA+ or LCA+
Proximal tubule	SJA+ and ECA+ or EBA–
Thin limb of Henle's loop	WGA– and LTA+
Thick ascending limb and distal tubule	DBA+ and Con A+*
Collecting duct	ECA– and GS I+
Urothelium	GS II+
Granulocytes	LTA+ and VVL–†
Lymphocytes	LTA– and VVL–

Abbreviations: +, binding; –, no binding of the lectin to the cell type; LCA, *Lens culinans* agglutinin; EBA, elderberry bark agglutinin; Con A, *Canavalia ensi formis* agglutinin; GS I, *Griffonia simplicifolia* I agglutinin; VVL, *Vicia villosa* lectin.

*Similar binding pattern to granulocytes, but different morphological characteristics of the nuclei.

†Granulocytes of patients with blood group O were only weakly positive for LTA and faintly positive for VVL.

was confirmed by all experimental approaches. An example for the identification of various cell types in urinary sediment by lectin combinations SJA-FITC and LTA-tetramethylrhodamineisothiocyanate (TRITC) is shown in Fig 3, which permitted the differentiation between proximal tubular cells, collecting duct cells, granulocytes, and lymphocytes.

Applicability of Lectin Staining to Urine Examination in Nephropathies

To evaluate the clinical applicability of lectin staining, we examined the urinary sediment of healthy volunteers and patients with various chronic nephropathies by the lectin combination of SJA/ECA in preliminary studies. The cellular excretion pattern of healthy male and female volunteers is listed in Table 4. In healthy individuals (n = 20), the number of cells excreted into the urine was very low for all cell types, with the exception of the superficial squamous epithelial cells observed in greater numbers in urine from women.

Examining urinary sediment in different nephropathies, we first studied the applicability of the method. Of 528 specimens, a clear assessment could be made in 439 specimens (83%), whereas in the remainder, the evaluation was limited for the following reasons: mucous (5.4%), massive granulocyturia (4.3%), overlaying by squamous epithelial cells (in women; 4.3%), massive hematuria (1.2%), and complete degeneration of the cells (1.8%). Differences in the affinity of lectins to urinary mucous was observed. Lectins SJA, ECA, and LTA showed only weak binding. Preliminary experiments had shown that, for example, lectin DBA had a strong affinity to mucous, limiting its use for urine cytologic diagnostic.

Compared with the urine of healthy controls (median, 0.5 cells/10 HPF; 25% to 75% quantiles, 0 to 1.5 cells/10 HPF; n = 20), we observed

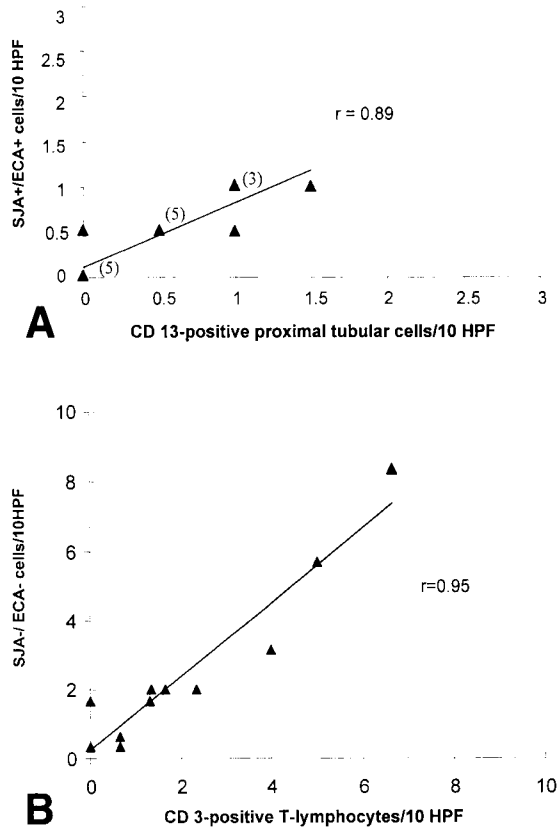

Fig 2. Conservation of the lectin-binding pattern in cells of urinary sediment. Identification of a particular cell type in the urinary sediment by lectin combination SJA/ECA or an antibody that reacts specifically with the respective cell type in specimens derived from the same urine showed a very high correlation between the two methods. (A) Staining of specimens (n = 16) with anti-CD 13 or lectin combination SJA/ECA. Cells of the proximal tubule were positive for both lectins. If one symbol represents more than one specimen, the total number of specimens is given in parentheses close to this symbol. (B) Staining of specimens (n = 11) with anti-CD 3 or lectin combination SJA/ECA. Lymphocytes were negative for both lectins. Pearson's correlation coefficient is given.

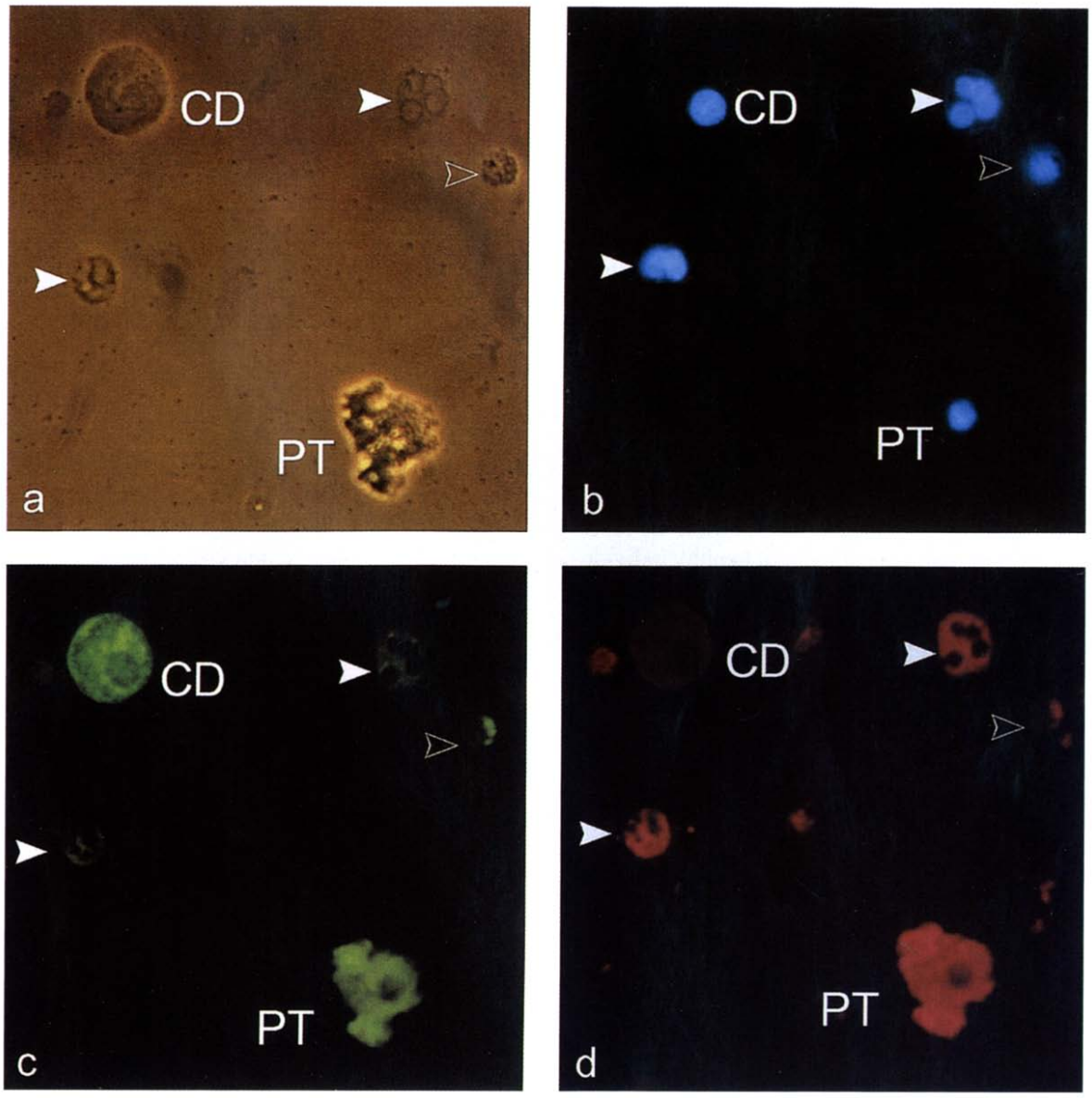

Fig 3. Identification of nucleated cells in urinary sediment by lectins. Urine cytologic specimen stained with a lectin combination of SJA (FITC coupled) and LTA (rhodamine coupled). (a) Phase contrast: a differentiation between respective cell types by morphological criteria alone was very difficult. (b) DAPI staining (UV filter) provided an overview of the number of nucleated cells in the field of vision. (c and d) Identification of cell types by double staining with lectins (c) SJA (FITC filter) and (d) LTA (rhodamine filter) allowed a differentiation between the respective cell types. Proximal tubular cells (PT) were positive for both lectins, cells of the collecting duct (CD) were positive for SJA and negative for LTA, and lymphocytes (open arrow heads) were negative for both lectins. Granulocytes (closed arrow heads) were negative for SJA and positive for LTA. They showed the same binding pattern as the cells of the thin loop of Henle, from which they could be differentiated by their characteristic segmented nuclei, assessed by DAPI staining. (Original magnification ×625.)

a significantly increased excretion of distal tubular cells in glomerulonephritis (median, 2.0 cells/10 HPF; 25% to 75% quantiles, 1.0 to 4.0 cells/10 HPF; n = 13; $P < 0.05$), diabetic nephropathy (median, 3.75 cells/10 HPF; 25% to 75% quantiles, 2.25 to 5 cells/10 HPF; n = 12; $P < 0.01$), hypertensive nephropathy (median, 3.0 cells/10 HPF; 25% to 75% quantiles, 0.5 to 4.0 cells/10 HPF; n = 10; $P < 0.05$), and lupus nephritis (median, 3.5 cells/10 HPF; 25% to 75%

Table 4. Number of Cells Excreted in Urine From Healthy Male and Female Volunteers

	Men (n = 7)			Women (n = 13)		
	25% Quantile	Median	75% Quantile	25% Quantile	Median	75% Quantile
Proximal tubular cells (/10 HPF)	0	0	0	0	0	0
Distal tubular cells (/10 HPF)	0	0.5	1.8	0	0.5	1.0
Collecting duct cells (/10 HPF)	0	0	0.3	0	0	0
Urothelial cells (/10 HPF)	0.5	1.5	3.5	0.5	1.0	1.5
Squamous epithelial cells (/10 HPF)	0	0.5	0.5	13	30	40
Lymphocytes (/10 HPF)	0	0	0	0	0	0
Granulocytes (/10 HPF)	1.5	2.0	5.8	1.5	2.5	12

quantiles, 3.0 to 3.5 cells/10 HPF; n = 5; $P < 0.01$). Conversely, the excretion of proximal tubule cells, as well as transitional cells of the urothelium, was not increased, and glomerular cells were not found in these chronic diseases. Compared with the sediment of healthy individuals (median, 0 cells/10 HPF; 25% to 75% quantiles, 0 to 0 cells/10 HPF), lymphocyturia, although not significant, tended to be increased in glomerulonephritis (median, 0 cells/10 HPF; 25% to 75% quantiles, 0 to 1 cells/10 HPF), lupus nephritis (median, 0.5 cells/10 HPF; 25% to 75% quantiles, 0 to 1 cells/10 HPF), and diabetic nephropathy (median, 0.25 cells/10 HPF; 25% to 75% quantiles, 0 to 1 cells/10 HPF), but not in hypertensive nephropathy (median, 0 cells/10 HPF; 25% to 75% quantiles, 0 to 0 cells/10 HPF). The excretion pattern of the cell types in the respective nephropathies is shown in Fig 4.

DISCUSSION

The aim of the present study is to evaluate lectin staining of urinary sediment as a tool to improve the identification and differentiation of nucleated cells in the urine, thus facilitating urine cytologic diagnostics. The following questions had to be clarified: Can a combination of lectins facilitate the differentiation between the major cell types in urine? Is the lectin-binding pattern preserved in cells voided in the urine? Is this technique applicable for routine diagnostics?

Although lectin binding has been characterized in the kidney, principally in the cortex[4-9] and urinary tract,[14] and in nucleated blood cells,[15,16] no systematic evaluation has been performed with respect to their binding properties to the cell types potentially occurring in urine. On screening 25 lectins for their binding characteristics to these structures in situ, lectins ECA, SJA, and LTA in particular proved useful for urine cytologic diagnostics. In agreement with others,[6-8,17] we observed that lectin LTA binds to proximal tubules and the loop of Henle, but not to glomeruli, distal tubules, and collecting ducts. The binding of this lectin to granulocytes, but not lymphocytes, has been reported.[18,19] In agreement with Laitinen et al,[7] we found that SJA bound to the proximal tubules and collecting ducts. Other binding sites of these lectins, especially ECA, have not been reported to date.

As a second step, the preservation of lectin binding on cells voided in the urine was studied. We examined whether a particular cell type characterized by both antibody staining and morphological criteria as reported by Schumann and Weiss[2] and Piccoli et al[3] showed the lectin-binding pattern predicted by in situ studies. This was the case.

Third, we examined the clinical applicability of lectin staining of urinary sediment to enhance the recognizability of cellular elements. Their differentiation by morphological criteria using phase contrast or bright field microscopy, even after staining with such techniques as Papanicolaou, Giemsa, methylene blue, or New-Sternheimer, is difficult even for a skilled examiner.[1,2] The differentiation between various tubuloepithelial cells and mononucleated cells, such as lymphocytes or monocytes, by morphological criteria alone is particularly difficult[20] because the size of the cells may vary with the tonicity of the urine because of swelling or shrinking.[21] Selecting the previously mentioned lectins, the differentiation of cell groups potentially occurring in urine was significantly facilitated. Antibody staining is an alternative technique for the specific

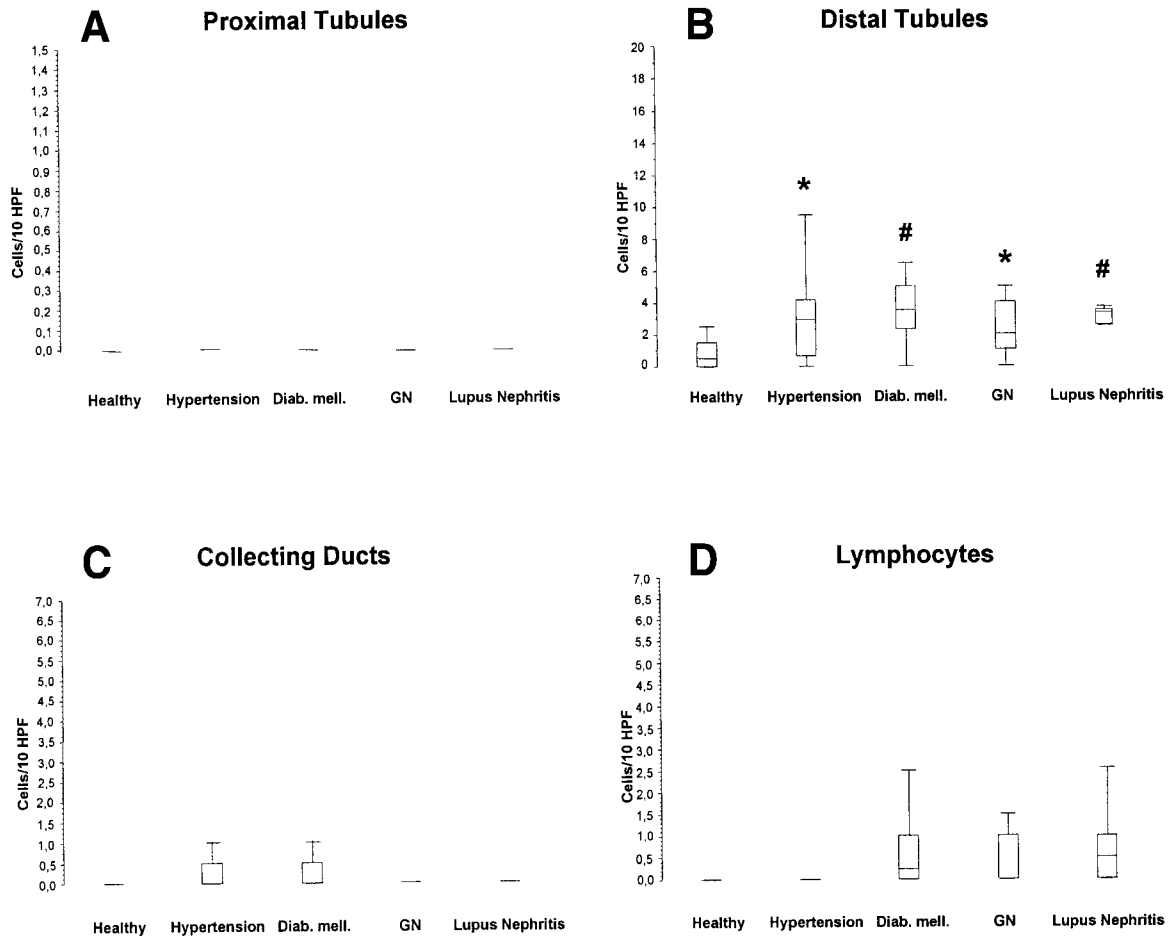

Fig 4. Distribution of different cell types in urinary sediment in various chronic nephropathies. All specimens were examined with lectin combination SJA/ECA. (A) Proximal tubule and (C) collecting duct cells occurred very rarely in healthy volunteers (n = 20) and patients with various kidney diseases (hypertensive nephropathy, n = 10; diabetic nephropathy [Diab mell], n = 12; glomerulonephritis [GN], n = 13; and lupus nephritis, n = 5), whereas (B) distal tubule cells were observed in all groups. Compared with healthy controls, excretion of distal tubular cells was significantly increased in patients with kidney diseases. (D) Lymphocyte cells were rarely observed in healthy volunteers and hypertensive patients in contrast to those with diabetic nephropathy. Median, 25% and 75% quantiles, and the nonoutlier maxima and minima are given. *$P < 0.05$. #$P < 0.01$.

identification of cells. However, compared with lectin staining, it is more time consuming and expensive and allows the identification of only one or two cell types in the same specimen.[22,23] Other approaches, such as examining cells in urinary sediment by transmission electron microscopy,[24] are even more time consuming and not applicable to routine diagnosis.

The difficulty differentiating urinary cell types by means of the available techniques with certainty and at reasonable efforts might be one reason that only a few reports exist of urine cytologic findings in nephropathies. Most of these reports refer to examinations in patients with acute renal failure or rapidly deteriorating renal function,[25] and especially acute transplant rejection,[26-31] in which relative high urinary cell numbers are expected. Only limited data exist with respect to chronic nephropathies.[32,33]

We evaluated the clinical applicability of lectin staining in the urine of patients with chronic nephropathies. Urine from healthy individuals contained, in agreement with the findings of Piccoli et al,[3] very few nucleated cells. In various chronic nephropathies, characteristic changes in the cellular excretion profile were noted. Although no increase in glomerular, proximal tubular, and urothelial cells was observed, excretion

of distal tubular cells was significantly elevated in all examined nephropathies. Because of the relatively high oxygen consumption of the distal tubular cells,[34] these are regarded as especially sensitive for disturbances in energy metabolism. Noxious agents affecting the kidney might preferentially damage this cell type, leading to shedding into the urine. Although not significant, increased excretion of lymphocytes was observed in glomerulonephritis, lupus nephritis, and diabetic nephropathy, but not in hypertensive nephropathy. This suggests that in the first cited diseases, primary or secondary activation of immune mechanisms might be involved in their pathogenesis. A close correlation between a urine cytologic score comprising both renal tubular cells and lymphocytes and the extent of renal involvement has been reported by Roberti et al[33] in patients with systemic lupus erythemathosus.

In summary, our studies show that lectin staining facilitates the differentiation of cellular elements in urine. This technique should provide a basis to reevaluate the importance of urine cytologic evaluation to assess not only chronic nephropathies, but also acute deterioration of renal function, and to monitor renal graft function. Although not a substitute for kidney biopsy, urine cytologic examination may provide information about the localization of the structures mainly affected and contribute to a better understanding of the pathomechanisms underlying a renal disease. It also could permit a more specific classification of a renal disease, allowing the monitoring of its course and providing prognostic information. Urine cytologic evaluation could be useful to plan therapeutic approaches and evaluate their effects. The elucidation of these items was beyond the scope of this study. Their evaluation in larger collectives will help clarify the diagnostic power of urine cytologic examination. Lectin staining of urinary sediment as an easy and rapidly applicable tool to differentiate urinary cell types should provide a basis to address these questions.

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