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Does the Renal Expression of Toll-like Receptors Play a Role in Patients with IgA Nephropathy?

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Abstract

The onset of IgA nephropathy (IgAN), characterized by glomerular deposition of IgA-containing immune complexes, is often associated with synpharyngitic hematuria. Innate immune responses mediated by Toll-like receptors (TLR) may play a role in IgAN onset and/or progression. Here, we assessed the expression of TLR 4, 7, 8, and 9 in renal-biopsy specimens from patients with IgAN, with different degree of proteinuria and eGFR, compared with normal-kidney and disease-control tissues (ANCA-associated vasculitis). Renal-biopsy specimens from 34 patients with IgAN and 7 patients with ANCA-associated vasculitis were used. In addition, we used 15 healthy portions of renal-tissue specimens from kidneys after nephrectomy for cancer as control specimens. Expression of TLR 4, 7, 8, and 9 was assessed using immunohistochemical staining of paraffin-

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Authors contribution statement

HC, DM, ZH, JV and VT conceived and planned the experiments and worked on the manuscript. HC and AL carried out the experiments. EH and MS processed the experimental data, performed the analysis and the evaluation of the results. TZ, MN, RC and JN aided in interpreting the results and drafted and edited the manuscript. All authors discussed the results and commented on the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest concerning their work in this study. In the interest of full disclosure, we report that JN is a co-founder and a co-owner of Reliant Glycosciences, LLC.

embedded renal-biopsy tissue specimens with specific antibodies and evaluated semiquantitatively by light microscopy. Linear discriminant analysis (LDA) was used to test whether intrarenal staining of TLR 4, 7, 8, and 9 distinguished patients with IgAN from controls or correlated with eGFR and/or proteinuria. eGFR was calculated using the creatinine-based formula. Moreover, the biopsies from patients with IgAN were scored according to the Oxford Classification. LDA showed that staining for TLR 4, 7, 8, and 9 was more intense in specimens from IgAN patients compared to normal kidney tissues. The intensity of intrarenal staining of TLRs discriminated four groups of IgAN patients with different eGFR and proteinuria and MEST scoring.

Keywords

Toll-like receptors; renal biopsy; renal insufficiency; IgA nephropathy; ANCA-associated vasculitis

Introduction

A multi-hit pathophysiological process has been proposed for IgA nephropathy (IgAN), starting with the production of galactose-deficient IgA1, a process co-determined by genetic and environmental factors (1,2,3,4,5). Mucosal immune system and its capacity to manage mucosal pathogens and their products are implicated in IgAN, as upper-respiratory tract infections are often associated with IgAN onset or disease activity. Toll-like receptors (TLRs), a multifaceted system for pathogen- and damage-associated molecular patterns (PAMP, DAMPS), participate in and mediate immune and inflammatory responses upon recognition of PAMP and/or DAMP (6). TLR activation by PAMP and/or DAMP may include exacerbation of experimental glomerulonephritis by activating neutrophils, macrophages, and/or other cells of the innate immune system, leading to glomerular inflammation and renal damage. TLR activation in tubular epithelial cell has been reported to play a role in tubulo-interstitial damage and CKD progression (7). TLR activation may enhance the synthesis of IgA and alter IgA glycosylation. Upregulation of TLRs has been reported in peripheral-blood mononuclear cells (PBMCs) of patients with IgAN (8, 9, 10). However, TLR renal expression was not systematically studied in IgAN (11).

In this study, we assessed intrarenal expression of TLR 4 (ligand: lipopolysaccharides; LPS), TLR 7 and 8 (ligand: single-stranded RNA), and TLR 9 (ligand: unmethylated cytosine guanine dinucleotide motifs in microbial DNA) by immunohistochemical staining with specific antibodies and tested whether the level of TLR expression in patients with IgAN differs from that in healthy controls without any renal injury. Patients with ANCA-associated vasculitis served as disease controls. Moreover, we compared data on TLR expression with clinical and histologic prognostic factors in IgAN to assess potential association of TLRs expression with disease severity. Our data indicated that renal expression of TLR 4 and TLR 9 was associated with severity of IgAN.

Materials and Methods

Renal tissue specimens

After obtaining the approval from the local ethical board of the General Faculty Hospital and First Medical Faculty and informed consents from all patients and controls, remnant archived paraffin-embedded kidney biopsy tissue samples of thirty-four patients with IgA nephropathy, 7 patients with ANCA-associated vasculitis, and healthy portions of kidney tissues from 15 individuals obtained after nephrectomy for cancer were used. All of these biopsies were scored according to the Oxford Classification (12), the most widely accepted system for assessing histologic findings in IgAN. System was based on the scoring of four histological parameters, each of which was independently associated with clinical outcome: mesangial proliferation (M), endocapillary proliferation (E), segmental sclerosis (S), and tubular atrophy with interstitial fibrosis (T).

Renal-biopsy specimens from 34 patients with IgAN, 7 patients with ANCA-associated vasculitis, and healthy portions of kidney tissues from 15 individuals obtained after nephrectomy for cancer were used for staining with anti-TLR antibodies. All individuals included in the study were white Caucasian from the Czech Republic. The control groups were used with stabilized renal function and eGFR >60 mL/min (CKD-EPI). CKD controls with matched renal function were not included. Patients with IgAN were divided into four subgroups according their renal function (Tables 1, 2 and 3). The diagnoses in all diseases were assessed at the time of renal biopsies. The interval between disease onset and the actual diagnosis might not be precisely evaluated. All the patients included in the study have not been treated at the moment of the renal biopsy.

Immunohistochemistry and light microscopy

Paraffin-embedded renal tissue sections were deparaffinized in xylene, rehydrated through a descending ethanol series, and washed in distilled water. The slides were pre-treated using heat-induced antigen retrieval performed by immersing the slides in citrate buffer (10 mM, pH 6) or EDTA buffer (pH 8) followed by a period of heating in water bath for 45 minutes with enough buffer to prevent evaporation and drying of slides during heating period. The slides were then washed in distilled water 2 times for 3 minutes.

The endogenous peroxidase activity was blocked by immersing the section in 0.3% H₂O₂ in 70% methanol for 30 minutes. Then the slides were washed by distilled H₂O for 3 times for 5 minutes. The nonspecific binding sides were then blocked with 1% BSA for 20 minutes (Sigma-Aldrich, Darmstadt, Germany). The primary antibodies were diluted in Dako REAL™ Antibody Diluent. Separate sets of sections were incubated with polyclonal TLR 7 rabbit IgG (LifeSpan Biosciences, Inc, Seattle, WA, USA), TLR 8 rabbit IgG (Sigma Aldrich, Darmstadt, Germany), TLR 9 rabbit IgG (Sigma Aldrich, Darmstadt, German) and monoclonal TLR 4 mouse IgG (Novus Biologicals, LLC, Littleton, CO,USA). All sections were incubated overnight in a humidified chamber at 4°C.

Next day, the sections were washed 3 times for 5 minutes in PBS. The secondary antibody Histofine® - Universal Immuno-enzyme Polymer system (Nichirei Biosciences Inc., Japan) was applied for 30 minutes at 37°C and washed 3 times in PBS and incubated in peroxidase

substrate solution (DAB – DAKO, Glostrup, Denmark) for 2 minutes. Slides were washed in distilled water and counterstained in Harris hematoxylin for 10 s and washed in running water for 5 minutes, dehydrated in graded ethanol series, cleared in xylene and mounted. Stained specimens were analyzed and photographed. For the evaluation, the semiquantitative methods were used by two experienced nephropathologists. Proximal and distal tubuli, glomeruli, visceral layers of the Bowman's capsule and interstitial inflammatory cells were scored from – to +++ according to following scheme: – negative; + mild positive ($\leq 25\%$); ++ moderate positive ($\leq 50\%$); +++ strongly positive ($> 50\%$) staining (Figure 1).

Statistical methods

The main goal of this study was to classify the observations into one of the various diagnoses according to different criteria. The classification of data with their high dimensionality of qualitative and quantitative parameters results in the use of one classical statistical technique of pattern recognition methods – **linear discriminant analysis (LDA)**. Statistical-analysis approach was chosen with respect to the limited number of samples and statistical significance of specific individual parameters was assessed by linear discriminant analysis. LDA predicts a membership in a group or category on observed values of several continuous or discontinuous variables (parameters) (13). Specifically, discriminant analysis predicts a classification X variable (in this case six groups consisting of patients with IgAN, ANCA, and healthy controls) based on known responses Y (clinical parameters, various TLRs). The data for LDA consist of a sample of observations with known group membership together with their values on continuous and discontinuous variables. We used LDA with transformed variables, so-called principal components (Figure 2, Figure 3 in this article), to reduce the dimensionality of the problem and provide a better graphical view of the output. First principal component, Figure 2, explains the largest possible variation in the data and therefore that Figure 2 accounts for most information (% at the specification of Figure 2). The second principal component, Figure 3, explains the second largest variation and is orthogonal to the first one. To verify the correct discrimination function, confusion matrix has been used, which resulted in classifying each of the objects in those categories. In the case of high accuracy of discrimination (classification) there is a possibility to predict classification of new patients on the same set of observations.

Hypothesis testing was made on significance level 90%, *i.e.*, cut off value is $P = 0.1$. **Mood's median test** was used for the test of significance of parameter medians for various groups of IgAN. Mood's median test is nonparametric test that tests the null hypothesis that the medians of two or more samples are identical. This test is based on chi square distribution. All calculations were performed using XLSTAT software (14).

Results

Biopsy-tissue specimens from kidneys of 34 patients with IgAN, 7 patients with ANCA-associated vasculitis, and 15 control-tissue specimens were used. These specimens were divided in the following six groups: Group 1: Patients with IgAN with proteinuria < 1 g/day, normal renal function; group 2: Patients with IgAN with proteinuria > 1 g/day, normal renal function; group 3: Patients with IgAN with proteinuria > 1 g/day, serum creatinine > 150

μmol/l; group 4: Patients with IgAN with proteinuria <1 g/day, serum creatinine >150 μmol/l; group 5: Patients with ANCA-associated vasculitis, and group 6: Control tissues from nephrectomy specimens (Tables 1, 2, 3). Renal tissue specimens were stained with antibodies specific for TLR 4, 7, 8, and 9. Clear differences in staining were found among all groups. The groups with IgAN showed differences among subgroups divided based on serum creatinine levels and proteinuria. The tubuli showed positive staining for TLR 4, TLR 7, TLR 8, but only mild staining for TLR 9 for IgAN with the differences between the groups, however in glomeruli were positive for TLR 4. There was only mild positive staining in interstitium and glomeruli for IgAN patients with severely impaired renal function and proteinuria, as described below (Table 4).

TLR 4 staining showed diffuse positive staining in tubules and in glomeruli, including mesangial and parietal epithelial cells of Bowman's capsules in samples from IgAN patients with the level of serum creatinine >150 μmol/l (groups 3, 4) and from patients with ANCA-associated vasculitis. Samples from IgAN patients with normal renal function and from healthy controls (groups 1, 2, 6) showed weak positive staining of TLR 4 of distal tubules and in glomeruli (Figure 1). TLR 7 in cases with ANCA-associated vasculitis (group 5) showed diffuse positive staining in tubuli, and positive cells in interstitium and glomeruli. All other samples (groups 1, 2, 3, 4, 6) showed mild scattered positive cells in tubuli, interstitium and glomeruli (Figure 1). TLR 8 in biopsy samples with normal kidney function including controls (groups 1, 2, 6) showed mild diffuse staining of tubular epithelial cells. Samples from IgAN patients with the level of serum creatinine > 150 μmol/l and from patients with ANCA-associated vasculitis (groups 3, 4, 5) exhibited diffuse positive staining in the tubular epithelial and in the interstitium that was more pronounced in tissues from patients with ANCA-associated vasculitis (group 5) (Figure 1). For TLR 9, biopsy samples from individuals with normal kidney function, including controls, (groups 1, 2, 6) showed similar mild focal staining of tubular epithelial cells. Cases with IgAN with the level of serum creatinine >150 μmol/l and proteinuria >1 g/day and ANCA-associated vasculitis (groups 3, 5) demonstrate diffuse positive detection in tubular epithelial cells. IgAN with the level of serum creatinine >150 μmol/l and proteinuria >1 g/day (group 4) showed positive staining for these two patterns (Figure 1).

Linear discriminant analysis (LDA) showed that TLR 4, 7, 8, and 9 staining was more intense in specimens from IgAN patients compared to patients with ANCA-associated vasculitis and healthy-control kidney tissues (Figure 2) and was able to distinguish four subgroups of patients with IgAN (Figure 3).

Next, we evaluated the correlation between MEST score and clinical data at renal biopsy such as proteinuria and eGFR. LDA with Oxford (MEST) histological evaluation showed the distribution of subgroups of IgAN patients with the accuracy of the discrimination 65.6% (Figure 4). Addition of information of the intensity of intrarenal staining of TLR 7, 8, and 9 with the Oxford (MEST) scoring improved the accuracy of the differentiation of four subgroups of IgAN to 100% according to confusion matrix (Supplemental Figure 1). Using MEST scoring together with intensity of intrarenal staining of TLR 8 and 9 or only TLR 8 was sufficient to discriminate four groups of IgAN with 100% and 96% accuracy, respectively, according to confusion matrix. Furthermore, use of MEST scoring with TLR 7

alone was sufficient to discriminate four groups of IgAN with 100% accuracy according to confusion matrix (Supplemental Figure 1).

Next, we evaluated the impact of TLR staining on differentiation of IgAN patients with different disease severity without considering MEST scores. LDA revealed that the intrarenal staining of TLR 7, 8, and 9 distinguished four subgroups of IgAN with the accuracy of the discrimination 100% according to confusion matrix (Supplemental Figure 2). Similarly, a combination of intrarenal staining of TLR 8 and 9 or only TLR 8 differentiated subgroups of IgAN with the accuracy of discrimination 97% and 94%, respectively.

Furthermore, high intrarenal staining for TLR 4 was observed in renal-biopsy specimens of patients with IgAN with proteinuria >1 g/day and normal renal function and in IgAN patients with renal insufficiency (Table 4). More intense staining for TLR 9 was found in renal biopsy tissues of patients with IgAN with normal renal function and without proteinuria (Table 4).

Discussion

Our results for the first time showed expression of TLR proteins in renal tissue of patients with IgAN, thus extending earlier observations on elevated levels of TLR mRNAs in PBMCs from patients with IgAN and associations of specific TLR 9 polymorphisms with disease progression in Japanese patients (11,15–19). In IgAN patients, expression levels of TLR 2, 3, 5, or 9 in PBMC correlated with proteinuria and TLR 4 mRNA expression was associated with proteinuria and microscopic hematuria (11). Thus, the overexpression of specific TLRs mRNAs in PBMCs of IgAN patients may be related to disease severity and/or activity. In this study, we detected high intrarenal expression of TLR 4, 7, 8, and 9 in patients with IgAN.

An increased circulation of lipopolysaccharides due to increased intestinal permeability in IgAN patients resulting in activating tubular cells via TLRs would drive progression of IgAN (20). This explanation would be in agreement with more intense staining observed for TLR 4 in IgAN patients with advanced renal insufficiency. *In vitro* experiments revealed that TLR 4 pathway may be involved in the progression of IgAN through induction of transcription factors, mainly NF- κ B and proinflammatory cytokines. An experimental rat model showed that peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists (*e.g.*, pioglitazone) exert anti-inflammatory effects through suppression of the expression and activity of TLR 4 (18). In agreement with the findings from the rat model, in our study we found elevated intrarenal staining of TLR 4 in tissues of patients with IgAN with proteinuria >1g/day as well as those with renal insufficiency (Table 4).

Moreover, an association between TLR 9 polymorphisms, expression, and progression of IgAN was observed (21). A whole-genome screening of copy number variants (CNVs) in familial IgAN patients, healthy relatives, and unrelated healthy subjects revealed that IgAN patients with poor renal function carried low copy number of *TLR 9* gene; this low CNV was associated with down-regulation of TLR 9 expression (22). IgAN patients with normal

renal function had normal CNV and normal expression of TLR 9 (22). In agreement with this observation, we found high intrarenal staining of TLR 9 in patients with preserved renal function, although these results need to be validated in larger cohorts. Moreover, genetic analysis for confirmation of homogenous genetic background was not performed in this study.

TLR 9-induced aberrant expression of a proliferation-inducing ligand (APRIL) in tonsillar germinal center B cells was found in patients with IgAN (8). Aberrant APRIL expression in tonsillar germinal centers correlated with greater proteinuria and responded well to tonsillectomy with decreases in serum levels of galactose-deficient IgA1 in patients with IgAN (8). It was hypothesized that in patients with IgAN, a TLR 9-BAFF (B cell activation factor)-IgA axis may exist that can enhance IgA production (9). It was suggested that overexpression of TLR 9 mRNA and protein in PBMCs and elevated levels of serum BAFF maybe associated with overexpression of serum IgA1 and could have a role in the development of IgAN (10).

TLR 3 signaling contributes to the expression of a neutrophil chemoattractant, CXCL1 in human mesangial cells (23). These observations seem to implicate viral infection(s) and corresponding immune responses in the pathogenesis of autoimmune renal diseases, such as IgAN (23).

Intrarenal staining of TLR 7 did not show significant differences between patients with IgAN and healthy controls which could be linked to different ligand of single-stranded RNA contrary to lipopolysaccharides in activation of TLR 4 (24). More frequent intrarenal staining of TLR 8 in patients with IgAN and proteinuria might reflect podocyte injury, as it was described (25).

In our study, intrarenal staining of TLR 4, 7, 8, and 9 was more distinctive in specimens of IgAN patients compared to healthy-control kidney tissues and completely distinguished four subgroups of IgAN patients. Moreover, intrarenal staining of TLR 7, 8 and 9 or only TLR 8 without considering MEST scores, might help to stratify patients with IgAN at the time of renal biopsy and determine serious forms of disease with an unfavorable prognosis and possibility of early initiation of adequate treatment.

In conclusion, if our results are validated in a larger cohort, renal-biopsy specimen staining for TLRs together with clinical parameters (serum creatinine, eGFR – MDRD and proteinuria) and LDA may aid in the assessment of IgAN patients, further contributing to the disease assessment at the time of diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative examples of immunohistochemical staining for Toll-like receptors (TLRs) 4,7,8, and 9 in human kidney biopsy specimens from patients with IgAN (group 1, panel A - proteinuria <1 g /day, normal renal function; group 2, panel B - proteinuria >1 g/day, normal renal function; group 3, panel C - proteinuria >1 g/day, serum creatinine >150 $\mu\text{mol/l}$; group 4, panel D - proteinuria <1 g/day, serum creatinine >150 $\mu\text{mol/l}$), ANCA-associated vasculitis (group 5, panel E), and controls, panel F (healthy portions of renal tissue after nephrectomy for cancer) (group 6). Scale bar = 50 μm .

Immunohistochemical detection of TLR4 in kidney tissue. A. Positive staining in distal tubules and in glomerulus (arrow). B. Weak positive staining in distal and several proximal tubules. C, D and E (arrow). Very similar results of staining with diffuse positive detection in distal and proximal tubules, also in glomeruli including parietal epithelial cells of Bowman capsules (arrow). F. similar to B with weak positive staining in distal tubules and in glomerulus (arrow).

Immunohistochemical detection of TLR7 in kidney tissue. A, B, C, D and F showed very similar results with mild scattered positive staining in distal tubular cells, and also detection of positive cells in the interstitium and glomeruli (arrow). Panel E showed different pattern of diffuse positive staining in tubuli and positive cells in interstitium and glomeruli (arrow). Immunohistochemical detection of TLR8 in kidney tissue. Panel A, B and F showed similar mild diffuse staining of tubular epithelial cells (arrow). Panel C, D and E demonstrate diffuse positive staining in the tubular epithelial and in the interstitium mainly in ANCA GN (E) (arrow).

Immunohistochemical detection of TLR9 in kidney tissue. Panel A, B, and F showed similar mild focal staining of tubular epithelial cells (arrow). Panel C and E demonstrate diffuse positive detection in tubular epithelial cells (arrow). Panel D showed positive staining between these two patterns (arrow).

Figure 2.

Linear discriminant analysis (LDA) showed that the intensity of staining of the kidney biopsy tissue for TLR 4, 7, 8, and 9 differentiated all diagnoses (four groups of patients with IgA nephropathy, ANCA-associated vasculitis and healthy controls). The accuracy of the discrimination was 100% according to confusion matrix. Groups of patients with IgA nephropathy: 1. normal renal function and proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function and proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria >1 g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria <1 g/day at the time of renal biopsy. Control groups: 5 – patients with ANCA-associated vasculitis, 6 – healthy controls.

Figure 3.

Linear discriminant analysis (LDA) confirmed that the intensity of staining of the kidney biopsy tissue for TLR 4, 7, 8, and 9 discriminated four groups of patients with IgA nephropathy. The accuracy of the discrimination was 100 % according to confusion matrix. Groups of patients with IgA nephropathy: 1. normal renal function and proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function and proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria >1 g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria <1 g/day at the time of renal biopsy. Centroid is a vector of parameters mean values for each group in two-dimensional space of principal-component coordinates (F1 and F2).

Figure 4.

Linear discriminant analysis (LDA) with Oxford (MEST) histological evaluation showed the distribution of subgroups of IgAN with the accuracy of the discrimination only 65.6% according to confusion matrix. Groups of patients with IgA nephropathy: 1. normal renal function and proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function and proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria >1g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria <1 g/day at the time of renal biopsy.

Table 1.

Clinical parameters for the four groups of patients with IgAN and patients with ANCA-associated vasculitis (at the time of renal biopsy and at the end of follow-up)

	onset	follow-up	onset	follow-up	onset	follow-up
Group (no. pts)	S-Cr*		PU*		eGFR*	
1 (8)	88	94	0.4	0.2	94	79
2 (11)	81	83	2.4	0.6	101	91
3 (10)	180	294	2.4	1.0	30	17
4 (5)	231	456	0.6	2.0	21	11
5 (7)	199	193	1.3	0.3	30	38

* median values

Abbreviations: onset, parameters at the time of renal biopsy; S-Cr, serum creatinine ($\mu\text{mol/l}$); PU, proteinuria (g/day); eGFR (MDRD, ml/min/1.73 m^2); no. pts., number of patients; Groups of patients with IgAN: 1. normal renal function and proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function and proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria >1 g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria <1 g/day at the time of renal biopsy; 5. Patients with ANCA-associated vasculitis.

Table 2.

Mood test of clinical parameters [serum creatinine, proteinuria, eGFR (MDRD)] at the time of renal biopsy differentiated four groups of patients with IgAN

Parameters	P value
S-Cr	<0.0001
PU	<0.0001
eGFR	<0.0001
ERU	0.454

Groups of patients with IgAN: 1. normal renal function and proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function and proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria >1 g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 $\mu\text{mol/l}$) and proteinuria <1 g/day at the time of renal biopsy. Abbreviations of parameters assessed at the time of renal biopsy: S-Cr, serum creatinine ($\mu\text{mol/l}$); PU, proteinuria (g/day); eGFR (MDRD, ml/min/1.73 m^2); ERU, erythrocyturia ($10^6/\text{l}$).

Table 3.

The distribution of the groups of patients with IgAN, ANCA-associated vasculitis, and healthy controls

Groups 1 – 4: IgAN	Number of subjects
Group 1: PU <1 g/d, normal renal function	8
Group 2: PU >1 g/d, normal renal function	11
Group 3: renal insufficiency: S-Cr > 150, PU >1 g/d	10
Group 4: renal insufficiency: S-Cr > 150, PU <1 g/d	5
Group 5: ANCA-associated vasculitis	7
Group 6: Controls	15

Abbreviations: IgAN, IgA nephropathy; Four groups of patients with IgAN: 1. normal renal function with proteinuria <1 g/day at the time of renal biopsy, 2. normal renal function with proteinuria >1 g/day at the time of renal biopsy, 3. renal insufficiency (serum creatinine >150 µmol/l) and proteinuria >1 g/day at the time of renal biopsy, 4. renal insufficiency (serum creatinine >150 µmol/l) and proteinuria <1 g/day at the time of renal biopsy; PU, proteinuria (g/day); S-Cr, serum creatinine (µmol/l).

Table 4.

Intrarenal staining of TLRs (expressed as percentage of positive cases for each group)

TLR 4						
parameter	group-1	group-2	group-3	group-4	group-5	group-6
Mesang-0	25%	36%	10%	0%	0%	93%
Mesang-I	75%	45%	70%	100%	100%	7%
Mesang-II	0%	9%	20%	0%	0%	0%
Mesang-III	0%	9%	0%	0%	0%	0%
Podocytes/crescents-0	75%	64%	30%	20%	14%	100%
Podocytes/crescents-I	25%	36%	30%	60%	14%	0%
Podocytes/crescents-II	0%	0%	40%	20%	29%	0%
Podocytes/crescents-III	0%	0%	0%	0%	43%	0%
Prox. tubuli-0	38%	55%	40%	40%	14%	73%
Prox. tubuli-I	63%	45%	50%	60%	29%	27%
Prox. tubuli-II	0%	0%	10%	0%	57%	0%
Dist. tubuli-0	25%	27%	0%	0%	0%	67%
Dist. tubuli-I	13%	36%	10%	0%	0%	27%
Dist. tubuli-II	63%	9%	60%	20%	29%	7%
Dist. tubuli-III	0%	27%	30%	80%	71%	0%
TLR 7						
parameter	group-1	group-2	group-3	group-4	group-5	group-6
Mesang-0	38%	64%	100%	80%	43%	53%
Mesang-I	63%	36%	0%	20%	57%	47%
Podocytes/crescents-0	38%	18%	10%	0%	14%	40%
Podocytes/crescents-I	50%	45%	80%	100%	14%	60%
Podocytes/crescents-II	13%	27%	10%	0%	14%	0%
Podocytes/crescents-III	0%	9%	0%	0%	57%	0%
Prox. tubuli-0	63%	91%	80%	40%	43%	93%
Prox. tubuli-I	38%	9%	10%	60%	29%	7%
Prox. tubuli-II	0%	0%	10%	0%	29%	0%
Dist. tubuli-0	25%	9%	30%	60%	0%	73%
Dist. tubuli-I	13%	64%	60%	40%	43%	27%
Dist. tubuli-II	38%	27%	10%	0%	43%	0%
Dist. tubuli-III	25%	0%	0%	0%	14%	0%
TLR 8						
parameter	group-1	group-2	group-3	group-4	group-5	group-6
Mesang-0	25%	45%	10%	20%	14%	100%
Mesang-I	75%	55%	90%	80%	86%	0%
Podocytes/crescents-0	50%	73%	50%	60%	0%	100%
Podocytes/crescents-I	50%	27%	50%	40%	29%	0%

TLR 8

parameter	group-1	group-2	group-3	group-4	group-5	group-6
Podocytes/crescents-II	0%	0%	0%	0%	29%	0%
Podocytes/crescents-III	0%	0%	0%	0%	43%	0%
Prox. tubuli-0	0%	0%	0%	0%	0%	53%
Prox. tubuli-I	0%	100%	50%	0%	14%	33%
Prox. tubuli-II	100%	0%	40%	80%	43%	13%
Prox. tubuli-III	0%	0%	10%	20%	43%	0%
Dist. tubuli-0	0%	0%	0%	0%	0%	60%
Dist. tubuli-I	38%	100%	10%	0%	14%	33%
Dist. tubuli-II	63%	0%	60%	80%	43%	7%
Dist. tubuli-III	0%	0%	30%	20%	43%	0%

TLR 9

parameter	group-1	group-2	group-3	group-4	group-5	group-6
Mesang-0	50%	100%	100%	100%	86%	100%
Mesang-I	50%	0%	0%	0%	14%	0%
Podocytes/crescents-0	63%	100%	100%	100%	29%	100%
Podocytes/crescents-I	38%	0%	0%	0%	43%	0%
Podocytes/crescents-II	0%	0%	0%	0%	29%	0%
Prox. tubuli-0	13%	36%	40%	0%	0%	53%
Prox. tubuli-I	75%	45%	60%	80%	14%	27%
Prox. tubuli-II	0%	9%	0%	20%	43%	20%
Prox. tubuli-III	13%	9%	0%	0%	43%	0%
Dist. tubuli-0	0%	0%	0%	0%	0%	13%
Dist. tubuli-I	0%	18%	0%	0%	0%	33%
Dist. tubuli-II	88%	82%	70%	40%	29%	53%
Dist. tubuli-III	13%	0%	30%	60%	71%	0%

Mesangium [podocytes/crescents, proximal (prox.) and distal (dist.) tubuli] 0 = negative staining; mesangium (podocytes/crescents, prox. and dist. tubuli) I = mild positive staining (<25 %); mesangium (podocytes/crescents, prox. and dist. tubuli) II = moderate positive staining (<50%); mesangium (podocytes/crescents, prox. and dist. tubuli) III = strongly positive staining (>75 %). Groups 1–4: patients with IgA nephropathy, 1. with normal renal function and without proteinuria, 2. with normal renal function and proteinuria >1 g/day, 3. with renal insufficiency (serum creatinine >150 µmol/l and proteinuria >1 g/day), 4. with renal insufficiency (serum creatinine > 150 µmol/l and proteinuria <1 g/day); 5 – patients with ANCA-associated vasculitis; 6 – healthy-tissue control.