RESEARCH LETTER

IL-17A as a potential biomarker of IgA nephropathy

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Introduction Since IgA nephropathy (IgAN) was first described in 1968, it has been recognized as the most common primary glomerulonephritis in the world, affecting mainly adolescents and young adults. Clinically, IgAN manifests either as macroscopic hematuria coinciding with mucosal infections or persistent hematuria with or without proteinuria. Nephrotic syndrome and rapidly progressive glomerulonephritis with crescent formation are uncommon. End-stage renal disease develops in 20% to 30% of the patients within 20 years. Clinical markers of unfavorable outcome are hypertension, degree of proteinuria, persistence of erythocyturia, and renal impairment.[1,2] IgAN is an autoimmune disorder, characterized by the mesangial deposition of IgA1–IgG complexes. IgA1 galactosylation defect is responsible for the immunogenicity of IgA1. Galactose deficient O-glycans are recognized by IgG and form complexes deposited in the mesangium activating an inflammatory cascade.[3]

Currently, the diagnosis of IgAN is based on the confirmation of IgA deposits in kidney biopsy, which is an invasive procedure. The course of the disease, including the development of end-stage renal disease, is heterogenous; therefore, noninvasive diagnostic and prognostic biomarkers are needed. Serum levels of galactose-deficient IgA1 and glycan-specific IgG are potential diagnostic and prognostic biomarkers of IgA nephropathy.[4] Recently, a quantitative assessment of IgA galactosylation status has been developed. It has been reported that IgA O-glycosylation status improves with immunosuppressive therapy; therefore, it may serve as a marker of therapeutic response.[5] Some potential biomarkers were correlated with biopsy findings. Urinary mannose-binding lectin correlated with clinical and histopathological parameters conventionally predicting progression of IgAN.[6] Serum soluble interleukin 2 receptor alpha chain was shown to predict progression of IgAN and correlated with fibrotic lesions in biopsies.[7] Serum vascular cell adhesion molecule 1 appeared as a marker of acute kidney injury in IgAN, correlating with crescentic lesions and daily proteinuria.[8] Urine proteomics also seems to be a promising tool in the diagnosis and monitoring of IgAN.[9]

Recently, the role of Th17 cells producing proinflammatory interleukin 17A (IL-17A) was established in the pathogenesis of various glomerulonephritides.[10] Therefore, the aim of this study was to investigate the utility of IL-17A as a potential biomarker in IgAN.

Materials and methods During the years 2011 and 2012, plasma and urine samples were collected from 23 patients with biopsy-confirmed IgAN hospitalized at the Department of Nephrology and Transplantation Medicine of the Wroclaw Medical University. The pathological diagnosis was based on immunofluorescence. The secondary cases of IgAN were excluded. Twenty healthy volunteers served as a control group. Plasma and urine were also sampled from the following groups with other primary chronic nephropathies: 36 patients with focal segmental glomerulosclerosis (FSGS), 16 patients with minimal change disease (MCD), 21 patients with membranous nephropathy, and 19 patients with membrano-proliferative glomerulonephritis. The characteristics of the groups are presented in the TABLE. Patients with IgAN were divided into 2 groups according to kidney biopsy findings: FSGS-like pattern and proliferative pattern.

Samples were stored at a temperature of –80°C until assayed. Biomarkers were assayed by a commercially available enzyme-linked immunosorbent assay kit (Human Il-17 A ELISA MAX Deluxe, BioLegend, San Diego, California, United States).

Concentrations of urinary biomarkers were normalized by the urine creatinine concentration. Active urinary sediment was defined by the presence of more than 2 erythrocytes and more than 5 leukocytes in high-power field urinalysis after exclusion of urinary tract infection. Daily

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TABLE Characteristics of the study groups

Nephropathy	FSGS	MCD	MN	IgAN	MPGN	Controls	*P* value
No. of patients	36	16	21	23	19	20	–
age, y	48.3 ±18.4	31.2 ±12.8	55.2 ±15.9	37.5 ±12.3	44.5 ±18.6	34.9 ±34.8	0.0001[a]
female/male	15/21	5/11	5/16	10/13	9/10	–	–
serum creatinine, mg/dl	1.4 ±0.7	0.96 ±0.2	1.7 ±1.0	1.5 ±1.2	1.5 ±0.6	0.9 ±0.2	0.0001[b]
GFR, ml/min	62.0 ±29.0	92.4 ±19.8	56.4 ±24.8	66.2 ±27.6	58.4 ±31.2	90.5 ±17.3	0.0001[b]
IL-17 plasma, pg/ml	2.0 ±0.0	2.14 ±0.6	2.028 ±0.1	7.27 ±3.3	2.29 ±0.5	2.0 ±0.0	<0.0001[c]
IL-17 urine, ng/g creatinine	1.82 ±1.5	1.47 ±1.5	1.35 ±0.8	3.199 ±1.8	1.626 ±0.8	1.756 ±1.3	<0.0001[c]
daily proteinuria, g/24 h	2.8 ±2.7	0.8 ±1.5	3.5 ±3.1	1.2 ±1.6	2.4 ±1.6	0	0.0001[d]
diabetes, %	13.9	12.5	20	4.3	5.3	0	0.29
hypertension, %	58.3	25	76	43.5	68.4	0	<0.05[e]
ACEIs, %	66.7	50	76	65	58	0	>0.05
prednisone, mg/24 h	17.3 ±36.0	13.3 ±11.5	3.7 ±8.8	15.1 ±43.3	7.3 ±11.0	0	0.0001[f]

a no difference between IgAN and control; IgAN patients were younger from FSGS and MN patients
b no difference in serum creatinine among nephropathy patients except MCD and control groups
c plasma and urine IL-17 concentration was higher in IgAN patients compared with control and other nephropathy groups
d MN and FSGS are different from IgAN and MCD; IgAN and MCD are different from MN, FSGS, and MPGN; MPGN is different from MN and MCD
e only the MCD group had a lower hypertension rate
f mean daily prednisone dose was lower in MN and MPGN groups

Conversion factor to SI units for serum creatinine is 88.4 µmol/l.

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; GFR, glomerular filtration rate; IgAN, IgA nephropathy; IL-17, interleukin 17; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; MPGN, membranoproliferative nephropathy

proteinuria was calculated using the protein-to-creatinine ratio from a single morning urine sample.

IgAN patients were treated with oral steroids, cyclosporine, or a combination of both. A mean serum cyclosporine concentration was 9.03 ng/ml. A mean oral daily prednisone dose was 15.12 mg. Fifteen patients (65.2%) took also angiotensin-converting enzyme inhibitors (ACEIs). Seven patients received only steroids (5 with an FSGS lesion pattern, 2 with a proliferative pattern). Only 1 patient (with an FSGS pattern) received monotheraphy with cyclosporine. In addition, only 1 patient (with a proliferative pattern) was treated with adrenocorticotropic hormone with cyclosporine. Three patients were treated with the combination of steroids and cyclosporine (1 with an FSGS pattern, 2 with a proliferative pattern). Of the remaining 11 patients without immunosuppression, 4 did not receive any drugs (2 with an FSGS pattern and 2 with a proliferative pattern) and 7 received a monotherapy with ACEIs (4 with a proliferative pattern and 3 with an FSGS pattern).

Normal distribution of the data was assessed by the D'Agostino–Pearson test. A correlation between biomarker concentrations and the type of nephropathy was tested by means of the analysis of variance with a post hoc analysis using the Student–Newman–Keuls method for data with normal distribution or the Kruskall–Wallis test with a post hoc analysis according to the Conover method for data without normal distribution. Correlations between biomarkers and quantitative laboratory data were assessed using the Spearman correlation coefficient, while correlations between biomarkers and qualitative data—either by the *t* test or Mann–Whitney test. For all tests, A *P* value of less than 0.05 was considered significant.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee. A written informed consent was obtained from all subjects included in the study.

Results Patients with IgAN are characterized by increased plasma and urinary concentrations of IL-17A (TABLE). Plasma and urinary IL-17A concentrations in all other chronic nephropathies were not different from those in the control group. Plasma IL-17A was increased in patients with MCD with active urinary sediment ($P = 0.037$).

In patients with IgAN, urinary IL-17A correlated negatively with glomerular filtration rate (GFR; $r = -0.4$; $P = 0.03$) and hemoglobin ($r = -0.5$; $P = 0.016$). Neither plasma nor urinary IL-17A concentrations correlated with daily proteinuria in any of the chronic nephropathies.

Urinary IL-17A concentrations were higher in patients with membranous nephropathy and FSGS with diabetes ($P = 0.007$ and $P = 0.047$, respectively) and with hypertension ($P = 0.011$ and $P = 0.012$, respectively).

Steroid (prednisone dose), ACEI, or cyclosporine therapy was not correlated either with plasma or urinary IL-17A concentrations. In IgAN patients, plasma IL-17A concentrations were higher in patients treated with statins ($P = 0.04$).

IgAN patients were divided into 2 groups depending on renal biopsy findings: patients with an FSGS pattern and those with a proliferative

pattern. No differences in plasma and urinary IL-17A concentrations were observed between both groups. Steroid therapy (prednisone dose) negatively correlated with plasma IL-17A concentrations only in IgAN patients with the FSGS pattern ($r = -0.4$; $P = 0.02$). Plasma and urinary IL-17A concentrations were not affected by hypertension, presence of the active urinary sediment, or ACEI use in any of the groups.

Discussion The current study is the first to reveal a significant increase in plasma and urinary concentrations of IL-17A in IgAN patients. In patients with the remaining chronic nephropathies, plasma and urinary IL-17A concentrations were not different from those in the control group. Moreover, in the IgAN group, urinary IL-17A concentrations negatively correlated with GFR. No other correlations between IL-17A concentrations and other conventional clinical markers such as daily proteinuria or active urinary sediment were observed. There were no associations between the two different morphological patterns of IgAN (FSGS or proliferative) and IL-17A concentrations.

Th17 cells were shown to mediate neutrophil--dependent glomerular injury in animal models. IL-17A produced by Th17 cells stimulated tubular and mesangial cells to secrete chemoatractants for neutrophils and macrophages.[11] The role of IL-17A in the murine crescentic glomerulonephritis model has been confirmed.[12] Th17 cells, releasing IL-17A, have been shown to participate in the development of various types of glomerulonephritis such as lupus nephritis, antiglomerular basement membrane nephritis and pauci-immune glomerulonephritis associated with antineutrophil cytoplasmic antibodies.[10] Th17 cells are increased in the plasma of patients with IgAN compared with those with non-IgA mesangial proliferative nephropathy and the control group.[13] In patients with IgAN, an imbalance between circulating regulatory T cells and proinflammatory Th17 cells was observed. The ratio of regulatory cells to Th17 cells was shown to be decreased compared with controls, which was postulated to participate in the development of glomerular damage in IgAN. Also in this study, serum IL-17A levels were increased and correlated with daily proteinuria. In IgAN patients with prominent tubular IL-17A expression, lower renal function, greater proteinuria, and more tubular damage were noted.[14]

IL-17A has been implicated in various types of secondary nephropathies. The current study tested the usefulness of IL-17A for diagnostic and prognostic purposes in patients with primary nephropathies. IL-17A appeared specific for IgAN. The study limitations include a small study group and the lack of prospective data that could provide information as to whether IL-17A correlates with exacerbations of the disease. Further studies are needed to support the role and specificity of IL-17A for this particular type of chronic nephropathy.

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