Profile of Autoantibodies in IgA Nephropathy, an Integrative Antiobiomics Approach

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Summary

Background and objectives IgG commonly co-exists with IgA in the glomerular mesangium of patients with IgA nephropathy (IgAN) with unclear clinical relevance. Autoantibody (autoAb) biomarkers to detect and track progression of IgAN are an unmet clinical need. The objective of the study was to identify IgA-specific autoAbs specific to IgAN.

Design, setting, participants, & measurements High-density protein microarrays were evaluated IgG autoAbs in the serum of IgAN patients (n = 22) and controls (n = 10). Clinical parameters, including annual GFR and urine protein measurements, were collected on all patients over 5 years. Bioinformatic data analysis was performed to select targets for further validation by immunohistochemistry (IHC).

Results One hundred seventeen (1.4%) specific antibodies were increased in IgAN. Among the most significant were the autoAb to the Ig family of proteins. IgAN-specific autoAbs (approximately 50%) were mounted against proteins predominantly expressed in glomeruli and tubules, and selected candidates were verified by IHC. Receiver operating characteristic analysis of our study demonstrated that IgG autoAb levels (matrilin 2, ubiquitin-conjugating enzyme E2W, DEAD box protein, and protein kinase D1) might be used in combination with 24-hour proteinuria to improve prediction of the progression of IgAN (area under the curve = 0.86, P = 0.02).

Conclusions IgAN is associated with elevated IgG autoAbs to multiple proteins in the kidney. This first analysis of the repertoire of autoAbs in IgAN identifies novel, immunogenic protein targets that are highly expressed in the kidney glomerulus and tubules that may bear relevance in the pathogenesis and progression of IgAN.

Introduction

IgA nephropathy (IgAN) is diagnosed by evidence of mesangial IgA deposits along with proliferation of mesangial cells on renal biopsy. Although named for the deposition of IgA in the kidney, other types of immunoglobulins may also be involved (1). In fact, Berger described IgAN as “Les depots intercapillaires d’IgA-IgG” (2). IgG and IgM deposits accompany IgA in most cases, with IgA deposits alone seen in approximately 15% of biopsies (3). Suzuki et al. have highlighted the potential importance of IgG in IgAN when they found that specific IgG antibodies recognize aberrantly glycosylated IgA, and these antibody levels correlated with disease activity in terms of proteinuria (1). The specificities of other IgG antibodies are currently unknown in this disease.

High-density protein microarrays have been successfully used to identify surrogate biomarkers for kidney and other diseases (4–6). For this report, we used protein arrays to characterize the profile of IgG autoantibodies (autoAbs) in patients with IgAN. We used an integrative genomics approach to map the significant antibodies to protein targets. The overall approach is summarized in Figure 1.

Materials and Methods

Patients

Thirty-two subjects participated in this study, including 22 patients with biopsy-confirmed IgAN and 10 age- and gender-matched healthy controls (HCs). Subjects were divided into two groups on the basis of their rate of decline of measured GFR over the 5-year follow-up. Patients were labeled as progressors (IgANp; n = 7) if their rate of measured GFR decline was ≥5 ml/min per 1.73 m² per year. IgAN patients with a ΔGFR of <5 ml/min per 1.73 m² per year were labeled nonprogressors (IgANnp). The demographics of all 22 IgAN patients are provided in Tables 1 and 2. The IgAN patients underwent annual clearance studies over 5 years, with the exception of those who had progressed to end-stage renal failure. GFR was examined using the urinary clearance of inulin, as described previously (7). Serum and urine samples were...
collected annually over 4 to 5 years. Seventeen patients with non-IgAN glomerular disease (nine focal segmental glomerulosclerosis [FSGS] and eight membranous nephropathy) were chosen for a comparison with IgAN autoAb profiling. Demographics of patients with non-IgA glomerular diseases include age (49 ± 13 years), gender (male = 10, female = 7), and serum creatinine (1.6 ± 0.83 mg/dl). Two patients with membranous glomerulonephritis were on immunosuppressant agents (one on prednisone and cyclosporine, one on cyclosporine and mycophenolate), and one patient with FSGS was on prednisone. AutoAbs elevated in non-IgAN glomerular disease were based on the comparison with autoAb levels from 12 separate HCs.

Measurement of IgG and IgA Levels in the Serum
To control for differences in levels of immunoglobulins in IgAN patients, human IgG ELISA (catalog no. E-80G) and human IgA ELISA (catalog no. E-80A) kits (Immunology Consultants Laboratory, Inc., Newberg, OR) were used to measure total IgG and IgA in the sera. After 1:80,000 dilution, ELISA analysis was done following standard protocols (8). Protein concentration was determined from the generated standard curve.

Immune Response Profiling Using Protein Microarrays
ProtoArray Human Protein Microarray version 4.0 (Invitrogen, Carlsbad, CA) was used to characterize the IgG-specific autoAb responses in IgAN. The arrays contain approximately 8000 recombinant human proteins (Figure 1B). The established protocol (http://www.invitrogen.com) (5) was followed for autoAb data acquisition. The slides were scanned using an Axon GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA). Raw signal intensity data were acquired using GenePix pro 6.0 software (Mo-
Table 2. Patient demographics: Progressors versus nonprogressors

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Progressor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nonprogressor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.7 ± 7.3</td>
<td>37.6 ± 11.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>5/2</td>
<td>7/8</td>
<td>0.30</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.5 ± 8.9</td>
<td>168 ± 12.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean systolic BP (mmHg)</td>
<td>131.5 ± 9.8</td>
<td>130.3 ± 12.4</td>
<td>0.82</td>
</tr>
<tr>
<td>Mean diastolic BP (mmHg)</td>
<td>82.2 ± 9.1</td>
<td>77.9 ± 14.8</td>
<td>0.49</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.38 ± 0.22</td>
<td>1.05 ± 0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>57 ± 13.7</td>
<td>79 ± 21.4</td>
<td>0.02</td>
</tr>
<tr>
<td>ΔGFR (ml/min per 1.73 m² per year)</td>
<td>−16.4 ± 15.3</td>
<td>0.19 ± 4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>3.87 ± 2.63</td>
<td>1.28 ± 0.93</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean values are presented as mean ± SD. Other values are presented as the number of patients.

<sup>b</sup>P values are calculated to evaluate whether there is any significant difference between the two groups.

**Results**

**IgG and IgA Levels in IgAN Patients**

Commercially available ELISA was used to measure total IgA level in the sera. As expected, the IgA levels were found to be significantly higher in IgAN patients (6.8 ± 3.8 mg/ml in IgAN versus 2.9 ± 1.5 mg/ml in HC; P < 0.004). Despite greater variability in the level of IgG in subjects with IgAN, there was no statistically significant difference in IgG levels (8.8 ± 5.1 mg/ml in IgAN versus 9.4 ± 4.1 mg/ml in HCs; P > 0.75). There was also no significant difference between the IgG levels of IgANp and IgANnp (P > 0.25).

**Immunohistochemistry**

Tissue-specific expression by immunohistochemical staining was performed on formalin-fixed paraffin-embedded kidney tissue to evaluate the presence of protein kinase D1 (PKD1), ubiquitin-conjugating enzyme E2W (UBE2W), and immunoglobulin kappa constant (IGKC) using corresponding antibodies. Immunohistochemistry (IHC) of these antigens was tested on a new set of five IgAN kidney biopsy samples and five new normal kidney control samples obtained from the normal kidney region obtained from renal tumor nephrectomy samples. Protein-G-purified rabbit anti-human PKD1 polyclonal antibody (Lifespan Biosciences, Seattle, WA; catalog no. LS-C98928), protein-G-purified mouse anti-human UBE2W monoclonal antibody, and goat anti-IGKC polyclonal antibody (Abcam Inc., Cambridge, MA; catalog no. ab55034) were used for this purpose.

**Immunofluorescence Microscopy**

Tissue-specific expression by immunofluorescence microscopy was performed in normal and IgAN kidney biopsies. As expected, IgA levels were found to be significantly higher in IgAN patients (6.8 ± 3.8 mg/ml in IgAN versus 2.9 ± 1.5 mg/ml in HCs; P < 0.004). Despite greater variability in the level of IgG in subjects with IgAN, there was no statistically significant difference in IgG levels (8.8 ± 5.1 mg/ml in IgAN versus 9.4 ± 4.1 mg/ml in HCs; P > 0.75). There was also no significant difference between the IgG levels of IgANp and IgANnp (P > 0.25).
the detection of these different Ig reactivities in IgAN patients. Of the 117 autoAbs in IgAN, 99 were mounted against non-Ig protein targets (Table 3).

For a functional analysis of the non-Ig protein targets in IgAN, we used pathway analysis. This revealed that increased autoAb responses against cyclin-dependent kinase inhibitor 1B and superoxide dismutase 2 proteins could alter their functions, known to be involved in mesangial cell apoptosis (11,12). Furthermore, an analysis of the main functional classes by pathway analysis revealed that the most significant autoAbs were reactive against proteins involved in apoptosis (P ≤ 0.05; 21 proteins), cellular assembly and organization (P < 0.04; 20 proteins), and cellular development (P ≤ 0.05; 19 proteins). These autoAb targets are also associated with dysregulation of proteins in immunologic disease (P ≤ 0.045), connective tissue disorders (P ≤ 0.045), and dermatological conditions (P ≤ 0.045), reflecting some systems affected in IgA disease (Table 4). Other antibody targets (cAMP responsive element modulator 1, Fc fragment of IgG, low-affinity IIIa receptor for CD16, and CD79a molecule Ig-associated alpha) were reported to be dysregulated in systemic lupus erythematosus (13–15). Four of the most significantly elevated antibodies were against proteins found in or highly specific to the kidney. These were matrilin2 (MATN2), UBE2W, DEAD box protein (DDX17), and PRKD1. Signal

![Figure 3](image)

Figure 3. | An increased reactivity against several immunoglobulins was observed among IgA nephropathy (IgAN) patients. (A) The list of immunoglobulins significantly increased in the sera of IgAN patients. (B) A heat map that demonstrates separation of healthy controls from IgAN patients purely on the basis of the intensity of the different Ig responses in IgAN.
differences for these autoAbs are shown for each individual patient and HCs in Figure 4. PRKD1 antibody with IgAN remained significant after the patient with the highest signal was removed from the analysis.

**Immune Response Profiling of IgANp and IgANnp**

AutoAbs against five targets, primarily protein kinases, were significantly increased in the IgANp group compared with the IgANnp group ($P < 0.05$). The list includes (1) the mitogen-activated protein kinase-interacting and spindle-stabilizing protein, SMITH antigen; (2) RIO kinase 3; (3) protein tyrosine phosphatase type IVA, member 1; (4) leucine-rich repeat containing 8 family, member D; and (5) and the death-associated protein kinase 3. Leucine-rich repeat containing 8 family, member D is present in glomeruli and renal tubules and is reported to be involved in cell-cycle exit in T lymphoblasts by IL-2 withdrawal (16,17). RIO kinase 3, reported to interact with caspase-10 and inhibit the nuclear factor-κB signaling pathway, is expressed in renal tubules (18).

**Integrative Bioinformatics and IHC**

We used publicly available tissue-specific gene expression data (19) to perform integrative genomic and

### Table 4. Associated pathways and molecular functions for increased autoantibodies specific to IgA nephropathy

<table>
<thead>
<tr>
<th>Molecular Pathway/Function</th>
<th>Associated Antigens</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>IGHG1, IGHG3, NEK2, TNS1, EDIL3, CDKN1B, ABCD1, KIFC3, HSPA8, RAB38, SOD2, TOM1, RASA1, AGAPI, CD7, IGHM, CKM, JAK3, SEPT5, BLZF1, and CDKN1B</td>
<td>$\leq0.05$</td>
</tr>
<tr>
<td>Cellular assembly and organization</td>
<td>ABCD1, AGAPI, BLZF1, CD7, CDKN1B, CKM, EDIL3, HSPA8, IGHG1, IGHM, JAK3, KIFC3, NEK2, SEPT5, SOD2, TNS1, RASA1, IGHG3, TOM1, and RAB38</td>
<td>$\leq0.04$</td>
</tr>
<tr>
<td>Cellular development</td>
<td>CD79A, IGHG1, SART1, RASA1, EFNA3, GNAT2, MED6, CDKN1B, MEF2D, SOX5, CIAPIN1, IGHM, JAK3, NIF3L1, PRKD1, RBPJ, SOD2, TRPV4, and TGM1</td>
<td>$\leq0.05$</td>
</tr>
<tr>
<td>Immunologic disease</td>
<td>IGHM, IGKC, JAK3, CDKN1B, ALCAM, FCGR3A, and IGHG1</td>
<td>$\leq0.05$</td>
</tr>
<tr>
<td>Connective tissue disorders</td>
<td>TPRA1</td>
<td>$\leq0.05$</td>
</tr>
<tr>
<td>Dermatologic conditions</td>
<td>CD79A, IGHG3, IGHM, IGK@, IGL@, PLXNA1, HN1, IGHG1, IGKC, POLH, and TGM1</td>
<td>$\leq0.03$</td>
</tr>
</tbody>
</table>

Figure 4. | Increased matriline 2 (MATN2), ubiquitin-conjugating enzyme E2W (UBE2W), DEAD box protein (DDX17), and protein kinase D1 (PRKD1) antibodies in IgA nephropathy (IgAN). Several autoAbs were identified with a significant increase in reactivities against their targets (Table 2). Scatterplots for antibodies of (A) MATN2, (B) UBE2W, (C) DDX17, and (D) PRKD1 are shown in terms of their relative fluorescence intensities in healthy controls and IgAN.
antibiotic analyses to predict which, if any, corresponding proteins to the 99 autoAbs were highly expressed in the kidney (5). We next examined the kidney-specific expression of all 99 proteins by examining the publicly available IHC data from the Human Protein Atlas (http://www.proteinatlas.org) (17). There was significant enrichment for antigens expressed in the glomeruli and tubules of the kidney (n/H11021 32; P/H11003 106) (17), and 15 additional proteins were only expressed in the renal tubules (P/H11003 106). A list of 20 biologically relevant antigenic targets and the information regarding their presence and location in the kidney by IHC are also provided in Table 2.

PRKD1, IGKC, and UBE2W were chosen for IHC (1) because a previously published integrative genomics study showed high expression of these proteins in the kidney and (2) on the basis of our data (5). The IHC of PRKD1 showed weak, patchy cytoplasmic staining within podocytes in a few biopsies of IgAN tissue. Mildly increased immunostaining was observed within proximal and distal tubules, especially along the apical aspects (Figure 5B); however, there was no significant staining in normal kidney tissue (Figure 5A).

In controls, no significant staining for IGKC was observed in glomeruli and tubules, and UBE2W staining revealed a moderate granular cytoplasmic pattern in proximal tubules but no staining in glomeruli (Figure 5, C and E). However, there was increased staining for IGKC in the glomerular endothelium and proximal tubules (Figure 5D) and increased staining for UBE2W in the proximal tubular cells with accentuation of the brush border in kidney tissue from IgAN patients (Figure 5F).

**Prediction of Renal Function Decline**

The positive predictive value (PPV) and negative predictive value of 24-hour proteinuria for the risk of ending up in the IgANp group was 42.8% and 85.7%, respectively (a cutoff of 24-hour proteinuria/H11022 1 g per day). We investigated IgG autoAbs to see if they could predict the progression of IgAN or could be used in combination with 24-hour proteinuria to improve its PPV.

The signal intensity of IgG against PRKD1 in IgAN significantly correlated with the observed decline of kidney function during the study (inulin GFR; R/H11005/H11002 0.58; P/H11005 0.004). The results of linear regression analyses between the decline of kidney function and clinical vari-

### Table 3. List of the most significant and biologically relevant proteins with increased IgG antibodies in IgAN

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Present in Glomerulusa</th>
<th>Present in Tubulesb</th>
<th>Highly Kidney Specific from Gene Expression Datab</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PRKD1</td>
<td>Serine/threonine-protein kinase D1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>MATN2</td>
<td>Matrilin 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>DDX17</td>
<td>DEAD (Asp-Glu-Ala-Asp) box polypeptide 17 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>UBE2W</td>
<td>Ubiquitin-conjugating enzyme E2W</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>CDKN1B</td>
<td>Cyclin-dependent kinase inhibitor 1B</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>SOD2</td>
<td>Superoxide dismutase 2, mitochondrial</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>IQCK</td>
<td>IQ motif containing K</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>8</td>
<td>BLZF1</td>
<td>Basic leucine zipper nuclear factor 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.02</td>
</tr>
<tr>
<td>9</td>
<td>EFNA3</td>
<td>Ephrin A3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.01</td>
</tr>
<tr>
<td>10</td>
<td>EIF4A2</td>
<td>Eukaryotic translation initiation factor 4A</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>11</td>
<td>FLII</td>
<td>Flightless 1 homolog</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>12</td>
<td>LIMCH1</td>
<td>LIM and calponin homology domains 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>13</td>
<td>MAGEA4</td>
<td>Melanoma antigen family A, 4</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>14</td>
<td>MEF2D</td>
<td>Myocyte enhancer factor 2D</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.03</td>
</tr>
<tr>
<td>15</td>
<td>MLLT6</td>
<td>Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Dro sophila); translocated to, 6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.02</td>
</tr>
<tr>
<td>16</td>
<td>CIAPIN1</td>
<td>Cytokine induced apoptosis inhibitor 1</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>0.05</td>
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<td>17</td>
<td>GD12</td>
<td>GDP dissociation inhibitor 2</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>0.02</td>
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<tr>
<td>18</td>
<td>HSPA8</td>
<td>Heat shock 70-kD protein 8, transcript variant 1</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>19</td>
<td>SERPINA5</td>
<td>Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
<td>0.05</td>
</tr>
<tr>
<td>20</td>
<td>TGM1</td>
<td>Transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.05</td>
</tr>
</tbody>
</table>

All of the autoantibodies for the corresponding proteins were significantly increased in IgA nephropathy (IgAN) compared with healthy controls (P ≤ 0.05). X, yes. NA, not available.

aNo immunohistochemistry data available.

bWith a false discovery rate < 5%.

In controls, no significant staining for IGKC was observed in glomeruli and tubules, and UBE2W staining revealed a moderate granular cytoplasmic pattern in proximal tubules but no staining in glomeruli (Figure 5, C and E). However, there was increased staining for IGKC in the glomerular endothelium and proximal tubules (Figure 5D) and increased staining for UBE2W in the proximal tubular cells with accentuation of the brush border in kidney tissue from IgAN patients (Figure 5F).
ables are shown in Figure 6. By univariate analysis, there was no significant correlation of age at presentation, initial systolic and diastolic BP, or initial GFR with the subsequent decline in kidney function. The amount of 24-hour proteinuria at the study start correlated, as expected, with a decline in GFR ($R = -0.60; P = 0.004$). In multivariate analysis, after adjusting for other clinical variables including age, systolic BP, diastolic BP, hematocrit, and initial GFR, the intensity of the autoAb signal against PRKD1 correlated with a decline in kidney function better than the extent of proteinuria over 24 hours (regression coefficient: $-4.73$ versus $-2.72$). Figure 6B shows the receiver operating characteristic curve analysis when the IgG antibodies against MATN2, UBE2W, DDX17, and PRKD1 (which were selected because of the significant correlation among each other as well as significant increase in IgAN) were used in combination. The area under the curve (AUC) was $0.86 (P = 0.02)$ on the basis of the logistic regression model by combining the four antibodies’ signal intensities when the arbitrary cutoff of $-5 \text{ ml/yr}$ of renal function decline was used to diagnose disease progression. Figure 6C shows receiver operating characteristic curve analysis when the four antibodies (MATN2, UBE2W, DDX17, and PRKD1) and 24-hour proteinuria were used in combination. The AUC was $0.98 (P = 0.0008)$ by performing logistic regression. The AUC of 24-hour proteinuria alone was $0.82 (P = 0.02)$. This finding suggests that using IgG autoAbs in addition to 24-hour proteinuria could improve the ability to predict the progression of disease.
Specificity of AutoAbs to IgAN
Protoarray analysis was done in a cohort of 15 patients with other glomerular diseases (membranous and FSGS) with a similar range of GFR and urine protein as our IgAN subjects. Their autoAb profile (data not shown) was distinct with only six of the autoAbs overlapping the IgAN group as significantly increased, none of which included the autoAbs of greatest significance. This suggests that the autoAb pattern is relatively specific to IgAN, at least as compared with other proteinuric glomerular diseases.

Discussion
IgG has been identified as an important participant in the pathogenesis of IgAN. This is the first study using high-density protein protoarrays to characterize the specificity of IgG autoAb responses in patients with IgAN. In this disease, it has been demonstrated that IgG antibodies can recognize aberrant IgA and form immune complexes leading to deposition in the glomerular mesangium (20). The purpose of this study was to more fully identify specific IgG autoAbs in this disease with a view to understanding pathogenesis, to track disease by noninvasive biomarkers, and to follow disease progression. We analyzed a carefully collected and serially followed cohort of patients with biopsy-proven IgAN who had serial GFR measurements by inulin clearance on a yearly basis, such that over a 5-year period, patients could be identified as patients with or without disease progression.

We found a nonsignificant trend toward more overall IgG and a greater variability in IgG levels in IgAN patients when compared with normal controls. Mimicking the biopsy findings of different classes of Ig deposits in IgA, we found a significant and broad increase in IgG targeting multiple immunoglobulins (IgA, IgG, and IgM) in patients with IgAN. IgG targeting against total IgA was increased, but not statistically significant (\(P = 0.08\)), possibly because the assay measured the IgG against all IgA including non-aberrant IgA. This finding likely supports the prior hypotheses that increased concentrations of polymeric IgA in IgAN (21,22) activates immune complexes between IgA and IgG and could be a critical mechanism involved in immune complex formation and deposition in the mesangium in IgAN.

In addition, this study identifies a specific repertoire of autoAbs in IgAN, including antibodies to targets that may have important regulatory properties in the kidney. For example, MATN2 is expressed in various extracellular matrices that can form collagen-dependent and collagen-independent filamentous networks (23). DDX17 are putative...
RNA helicases involved in alteration of RNA secondary structure, leading to cellular growth and division (24). UBE2W is involved in the ubiquitination reaction that targets proteins for proteasomal degradation (25). These findings suggest that IgAN involves the development of IgG antibodies specific to the kidney and that these antibodies may play an important role in determining the manifestations and clinical course of the disease.

If these findings are further validated in other cohorts of patients, then it would suggest that the role of IgG in IgAN is not limited to forming immune complexes with IgA. It has been known that IgAN is often associated with other diseases. Examples include dermatologic diseases such as erythema nodosum, cancers such as lymphoma, infectious diseases such as HIV, and rheumatologic diseases such as systemic lupus erythematosus and inflammatory bowel disease (26–32). Our study results could help to understand common pathophysiology between IgAN and these diseases. The protein targets of the IgGs identified herein are involved in various cellular and molecular functions in cancer, cell death, cellular assembly/orGANization, and molecular biochemistry. The molecular networks of protein targets are associated with dermatologic diseases, infectious disease, lipid metabolism, and hair and skin development and function. There may be common pathologic pathways causing production of IgGs in these diseases that lead to forming immune complex with IgAs and developing IgAN.

On the basis of our integrative genomic analysis and an IHC database, antigen targets corresponding to approximately 50% of the increased antibodies are expressed in glomeruli and tubules of the kidney. We believe these findings support that IgG targeting of these antigens in the kidney is an important pathophysiological process in IgAN that may cause renal injury and loss of function. By IHC, separate markers were seen in glomerular endothelial cells, but we have no present explanation why the endothelium of the kidney should be disproportionately affected in this disease. A larger clinical study may be necessary to investigate these antigens and antibodies in IgAN.

We also analyzed the data to find potential biomarkers that can complement existing prognostic indicators. Traditional prognostic indicators are not able to accurately predict the progression as shown in this study by the low PPV (42%) of 24-hour proteinuria.

Analysis of our data (including MATN2, UBE2W, DDX17, and PRKD1) demonstrated that IgG autoAb levels might be useful in combination with 24-hour proteinuria to improve prediction of the progression of IgAN. Broader clinical validation will be necessary to determine if IgG autoAbs may be used as biomarkers in this clinical setting.

Potential limitations of the study include the limited size of the study, but the study needed a population that was well characterized for kidney function, structure, and outcome with adequate follow-up to classify patients into IgANp or IgANnp. Another potential drawback is the use of fusion proteins that are expressed in insect cells as antigens. The insect-expressed human proteins may not possess disease-specific characteristics associated with post-translational modification and thus might not present epitopes necessary for the identification of immune reactivity. However, the differences we see in IHC suggest that at least some of these antigens are acting differently in the kidneys of patients with IgAN. The comparisons to other glomerular diseases suggest that the response is relatively specific to IgAN. Finally, further analysis for the pathogenicity of these antibodies and validation of these autoAbs for accuracy, consistency, sensitivity, and specificity for IgAN will need to be pursued by larger, prospective studies.

In summary, this report provides new insight into the potential roles of IgGs in IgAN. These IgGs appear to be broadly involved in many molecular networks and functions not limited to forming immune complexes with IgA. The further investigation of IgG autoAbs may lead to the discovery of new biomarkers to predict the progression of IgAN and a better understanding of the pathophysiology and progression of this disease.

Acknowledgments

The work was supported by the National Institutes of Health (Grant R01-AI-061739; to M.S.); the Deans Fellowship Child Health Research Program (to T.S. and M.S.); and the Sobrato Research Fund (S.H.W. and R.L.). We also thank Neeraja Kambham at Stanford Pathology for IHC and its interpretation, the Stanford Functional Genomics Center at Stanford University for the Axon scanner, and Van Dinh and the Sarwal Laboratory members for their help.

Disclosures

None.

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Received: May 13, 2011 Accepted: August 17, 2011

Published online ahead of print. Publication date available at www.cjasn.org.