Incidence of latent mesangial IgA deposition in renal allograft donors in Japan

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Incidence of latent mesangial IgA deposition in renal allograft donors in Japan.

Background. Mesangial immunoglobulin A (IgA) deposition is incidentally encountered in asymptomatic individuals, but its precise frequency and significance had not been clarified. The background of the latent IgA deposition is related to the epidemiology and pathogenesis of IgA nephropathy.

Methods. Zero-hour allograft biopsies were performed in 510 renal transplantations (446 living donors, and 64 cadaveric donors) at the Kidney Center of Tokyo Women’s Medical University. Mesangial IgA and C3 deposition were analyzed immunohistochemically, and the frequency and clinicopathologic features of mesangial IgA deposition were investigated.

Results. Mesangial IgA deposition was present in 82 (16.1%) of the total 510 allografts with no statistical difference between living donors (72/446, 16.1%) and cadaveric donors (10/64, 15.6%) or between blood-related donors (66/392, 16.8%) and nonblood-related donors (16/110, 14.5%). Mesangial C3 deposition was present in 16 (19.5%) of the 82 allografts with mesangial IgA deposition. The grade of hematuria in IgA(+) donors was significantly higher than IgA(-) donors (1.30 ± 1.17 vs. 0.86 ± 0.89, P = 0.025). Histologic investigation of IgA(+) allografts revealed the frequency of mesangioproliferative glomerulonephritis (PGN) was significantly higher in IgA(+)/C3(+) allografts (8/16, 50%) than in IgA(+)/C3(-) allografts (11/66, 16.7%) (P = 0.0084). Moreover, the number of infiltrated macrophages to glomerulus (cells/glomerular cross section) was significantly higher in the IgA(+)C3(+) allografts than in IgA(+)C3(-), IgA(-)C3(+) and IgA(-)C3(-) allografts (1.10 ± 0.62 vs. 0.61 ± 0.42, P = 0.0008; 0.47 ± 0.34, P = 0.023; and 0.37 ± 0.23, P = 0.002, respectively).

Conclusion. The latent mesangial IgA deposition was a relatively common phenomenon in the healthy Japanese donors. This phenomenon was associated with mild degree of microhematuria, mesangial proliferation and glomerular macrophage infiltration in some of the affected individuals, especially with combined IgA and C3 deposition.

Key words: IgA nephropathy, renal transplantation, donor kidney, zero-hour biopsy, C3, human leukocyte antigen (HLA)

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Immunoglobulin A (IgA) nephropathy (IgAN) was originally described by Berger and Hinglais in 1968 [1] and is now considered the most common form of primary glomerulonephritis worldwide [2]. It is characterized by predominantly mesangial IgA and C3 deposition, which is related to mesangial proliferative changes. At first, IgAN was considered to be a mild clinical manifestation with a relatively good prognosis [1], but, recent reports suggest that 20% to 30% of all patients develop end-stage renal disease (ESRD) 10 to 30 years after the disease onset [2, 3]. Consequently, the prognosis of IgAN is now regarded as more serious than previously thought, and IgAN is one of the most important causes of ESRD worldwide [2].

The prevalence of mesangial IgA deposition in normal populations has been investigated previously. Sinniah [4] demonstrated mesangial IgA deposition in 8/200 (4%) necropsy cases in Singapore, while another necropsy study revealed that 10% to 30% of renal specimens from patients without any manifestation of renal disease had mesangial IgA deposition [5, 6]. Mesangial IgA deposition has been observed in some donor kidneys at transplantation, but this incidental IgA deposition has not always been related to glomerular inflammatory changes [7, 8]. The exact frequency and clinicopathologic significance of the latent mesangial IgA deposition remain unclear.

In this study, we examined the latent mesangial IgA and C3 deposition in Japanese donor kidneys (zero-hour biopsy). Because the donors did not usually have any symptoms or signs of renal disease, the deposition rate was thought to represent an actual frequency of mesangial IgA deposition in the so-called general population. We also investigated the clinical features of the individuals with latent mesangial IgA deposition and the histologic findings in their kidneys. The results should provide insights into the epidemiology and pathogenesis of IgAN, the most common form of glomerulonephritis in many countries.
METHODS

Subjects

Between 1992 and 1999, 561 renal transplantations (477 living kidney and 84 cadaveric kidney transplantations) were performed at the Kidney Center of Tokyo Women’s Medical University. Zero-hour renal biopsy before transplantation was performed in 510 allografts (446 from living donors and 64 from cadaveric donors). The donors consisted of 184 males and 287 females, and their mean age was 52.3 ± 13.4 years old (range, 1 to 76 years old). Among the 510 donors, the age was unknown in 45 donors and the gender was unknown in 39 donors.

Evaluation of light microscopic and immunohistochemical findings

Wedge renal biopsy tissue was obtained at transplantation and fixed with 10% phosphate-buffered formation (pH 7.2), embedded in paraffin, and cut into 2 μm sections. The sections were then stained with hematoxylin and cosin, periodic acid-Schiff (PAS) stain, Masson trichrome stain, and periodic acid methenamine silver (PAM) stain for light microscopy. For immunohistochemistry, paraffin sections on glass slides coated with saline were stained by using a peroxidase-labeled streptavidin-biotin (SAB) staining kit (DAKO Co.). The primary antibodies were rabbit polyclonal antibodies against IgG, IgA, IgM, and C3 (Hoechst, Behringwerke AG, Marburg, Germany) and monoclonal antibody against macrophage PG-M1 (CD68) (DAKO Co.).

The histologic findings were evaluated based on the following parameters: percentage of glomeruli exhibiting glomerular obsolescence, crescent formation, glomerular tuft adhesion to Bowman’s capsule, grades of mesangial cell proliferation, mesangial matrix increase, inflammatory cell infiltration and fibrosis in the interstitium, tubular atrophy, arteriosclerosis and arteriolosclerosis. The severity of each histologic parameter was semiquantitatively evaluated and categorized into following four grades: grade 0, none; grade 1, mild; grade 2, moderate; and grade 3, severe. Glomerular lesion was classified into three categories: minor glomerular abnormality (MGA), focal proliferative glomerulonephritis (FPGN), and diffuse proliferative glomerulonephritis (DPGN), according to the severity and distribution of mesangial proliferative changes. All histologic evaluations were performed by two independent pathologists (K.H. and Y.Y.) without any clinical information about the donors.

Concerning the immunohistochemical deposition for IgA and C3 in glomerular mesangium, “positive” was defined as its presence in more than 30% of all glomeruli examined. Some of the cases with mesangial IgA and C3 deposition in paraffin-embedded sections were reconfirmed by immunofluorescence method using frozen sections. In the present study, the frozen sections were not available in all biopsy specimens, so the analysis was performed by immunohistochemistry using formalin-fixed, paraffin-embedded sections. In general, sensitivity for immunohistologic detection is superior in nonfixed frozen section than in formalin-fixed section. However, as far as IgG, IgA, IgM, and C3, the detection sensitivity by SAB method is almost same as by immunofluorescent method using frozen section, although the sensitivity for C3 using formalin-fixed section is slightly lower than using frozen sections. In some cases, we failed to detect C3 in formalin-fixed sections but false positive was not detected. So, the positive case in formalin-fixed sections can be regarded as a more definitive case in C3 deposition.

Immunohistochemical staining of a macrophage marker, PG-M1 (CD68) (DAKO Co.), was performed to evaluate glomerular macrophage infiltration in all 82 IgA(+) allografts, seven IgA(-)/C3(+) allografts, and randomly selected 10 IgA(-)/C3(-) allografts as a control. All glomeruli in each specimen (range, 10 to 50 glomeruli; mean, 12 glomeruli) were examined, and the number of PG-M1(CD68)-positive cells in each glomerular cross section was counted. The mean number of PG-M1(CD68)-positive cells per glomerular cross-section was calculated in each group.

Evaluation of clinical data

The urine of the donors was examined before transplantation, usually using the morning urine sample at their hospitalization. The evaluation could be done in 67 IgA(+) donors and randomly selected 84 IgA(-) donors. Microhematuria was evaluated by the number of urinary red blood cell (RBC) sediment. Briefly, 10 mL urine sample was centrifuged (1500 rpm, 5 minutes) and the sediment was resuspended in the residual 200 μL urine (×50 concentration), and total volume of the suspension was tipped on the slide glass and covered by cover glass (18 × 18 mm). The cell number is counted under ×400 magnification by light microscopy (high-power field). The grade of hematuria was divided into six grades according to the number of RBC sediments per high-power field (×400): grade 0, no RBC; grade 1, <1 RBC; grade 2, 1 to 5 RBC; grade 3, 6 to 10 RBC; grade 4, 11 to 20 RBC; and grade 5, >20 RBC. The urinary protein was evaluated qualitatively using test paper method. The age, gender, and serum creatinine of renal allograft donors were also evaluated. The human leukocyte antigen (HLA) type of the 493 donors was analyzed and the frequencies of HLA-DR4, Bw35, and Bw51 were evaluated and compared between IgA(+) group (N = 82) and IgA(-) group (N = 411).

Statistical analysis

The relationship between mesangial IgA deposition and donor category (living donor or cadaveric donor, blood-related or nonblood-related) was evaluated by chi-squared test. Comparisons of clinical and immuno-
histochemical data between two groups were made by using the Mann-Whitney U test or unpaired Student t test. The relationship between HLA frequency and mesangial IgA deposition was evaluated by chi-squared test. P < 0.05 was considered significant in all analyses.

RESULTS
Mesangial IgA and/or C3 deposition rate of renal allograft donors

Figure 1 shows a histologic and immunohistochemical finding of the renal allograft at transplantation (zero-hour

Fig. 1. Histologic and immunohistochemical finding of zero-hour renal allograft biopsy with mesangial immunoglobulin A (IgA) and C3 deposition. (A) Biopsy specimen showed mild mesangial proliferative change and histologically diagnosed with focal and segmental proliferative glomerulonephritis (FPGN) [periodic acid-Schiff (PAS)]. (B) IgA staining by peroxidase-labeled streptavidin-biotin (SAB) method revealed mesangial IgA deposition with mild degree. (C) C3 staining by the same SAB method also showed mesangial C3 deposition in combination with IgA. (D) Immunofluorescence staining method using a frozen section confirmed the similar mesangial IgA deposition. (E) Mesangial C3 deposition was also confirmed by the immunofluorescence staining method using the frozen section.
biopsy) with mesangial IgA and C3 deposition. Light microscopy revealed a focal and segmental mesangial proliferation with mild degree (Fig. 1A). By immunohistochemistry (SAB method) using formalin-fixed, paraffin-embedded section, granular IgA and C3 deposition were demonstrated in the glomerular mesangium (Fig. 1B and C). These findings were confirmed by immunofluorescence staining method using frozen section (Fig. 1D and E).

Mesangial IgA deposition was detected in 82 (16.1%) of all 510 donor allografts; in 72 (16.1%) of the 446 allografts from living donors, and in 10 (15.6%) of the 64 allografts from cadaveric donors. The difference in IgA deposition rates between living donor and cadaveric donor group was not statistically significant (Fig. 2). Comparison of the IgA deposition rate according to blood relationship revealed mesangial IgA deposition in 66 (16.8%) of the 392 allografts from blood-related donors, as opposed to 16 (14.5%) of the 110 allografts from nonblood-related donors. The difference in IgA deposition rate between the blood-related donors and nonblood-related donor group was not significant, although the rate tended to be higher in the blood-related donor group than in the nonblood-related donor group (Fig. 3). On the other hand, mesangial C3 deposition was detected in 31 (6.1%) of the total of 510 allografts. The combined presence of IgA and C3 was detected in only 16 allografts (3.1%). In 15 (2.9%) cases, C3 deposition was observed without IgA deposition (Fig. 4).

Mesangial IgG deposition was detected in 14/510 cases (2.7%), in which 5/510 cases (1.0%) showed concomitant IgA and IgG deposition, while mesangial IgM deposition was detected in 160/510 cases (31.4%), in which 35/510 cases (6.9%) showed concomitant IgA and IgM deposition. (The significance of mesangial IgG and IgM deposition was not referred in this study.)

**Correlation between age and mesangial IgA deposition rate**

The distribution of IgA deposition rate in each generation of the 465 donors is shown in Figure 5. The IgA deposition rate was lower in donors under 30 years old (6.9%) and increased in the 30s (19.6%). A higher IgA deposition rate was also observed in the aged donors, 60 years old and older.

**Urinary findings in renal allograft donors with and without mesangial IgA deposition**

The urinary red blood cell count and hematuria grade of IgA(+) and IgA(−) donors are demonstrated in Figure 6. In both groups, the urinary RBC count was less...
than 20/high-power field. However, the hematuria grade in IgA(+) donors was shifted to relatively high grade comparing to IgA(−) donors. The mean grade of hematuria in IgA(+) donors was significantly higher than IgA(−) donors (1.30 ± 1.17 vs. 0.86 ± 0.89, P = 0.025) (Fig. 6). Concerning C3 deposition, there was no significant difference of hematuria grade between IgA(+) /C3(+) and IgA(+) /C3(−) donors, nor IgA(−)/C3(+) and IgA(−)/C3(−) donors (data not shown). Proteinuria was not detected in any of the 67 donors with mesangial IgA deposition.

**Histologic characteristics of the renal allografts with mesangial IgA deposition: Comparison between groups with and without C3 deposition**

Histologic examination defined three categories of the glomerular change: MGA, FPGN, and DPGN, according to the grade and distribution of mesangial proliferation. The typical glomerular feature of each category is shown in Figure 7. In the 82 IgA(+) allografts, the histologic classification was composed of 63 (76.8%) MGA, 15 (18.3%) FPGN, and four (4.9%) DPGN. The frequencies of the each histologic category in two groups IgA(+) /C3(+) and IgA(+) /C3(−) allografts are demonstrated in Figure 8. In IgA(+) /C3(+) group (N = 16), eight allografts (50%) were diagnosed with mesangioproliferative glomerulonephritis (five cases of FPGN and three cases of DPGN) and the other eight (50%) were diagnosed with MGA. By contrast, in the IgA(+) /C3(−) group (N = 66), 55 allografts (83.3%) were diagnosed with MGA, and 11 (16.7%) were diagnosed with mesangioproliferative glomerulonephritis (10 cases of FPGN and one case of DPGN). The frequency of mesangioproliferative glomerulonephritis was significantly higher in IgA(+) /C3(+) allografts (8/16, 50%) than in IgA(+) /C3(−) allografts (11/66, 17.2%) (P = 0.0084).

Table 1 demonstrated the comparison of histologic findings of the 82 IgA(+) donors with and without C3 deposition. The glomerular obsolescence rate in both two groups was approximately 10%, and not significantly different. Glomerular crescent and tuft adhesion to Bowman’s capsule were rarely observed in each group. The grade of mesangial cell proliferation was significantly different in the C3(+) group than in the C3(−) group (0.6 ± 0.1 vs. 0.2 ± 0.4, P = 0.0063), although the grade of mesangial matrix increase was not different between two groups. Tubulointerstitial lesions were absent or very mild in both groups. Arterial and arteriolar sclerosis were observed in both groups, without any statistical differences between them.

**Glomerular macrophage infiltration of renal allografts with mesangial IgA deposition**

Figure 9A showed PG-M1 (CD68)–positive cells infiltrated in the glomerulus of IgA(+) /C3(+) allograft (same case as Fig. 1). In the cadaveric allografts, glomerular macrophage infiltration (4.95 ± 3.52 cells/glomerular cross-section, N = 10) was greater than that of the living allografts (0.70 ± 0.50 cells/glomerular cross-section, N = 72) (P < 0.0001), probably because of their mortal and postmortem condition (Fig. 9 B and C). Therefore, we excluded the cadaveric allografts from the present analysis. Figure 10 shows the comparison of glomerular macrophage infiltration in four groups classified by IgA and C3 deposition. The IgA(+) /C3(+) allografts had significantly higher macrophage infiltration.
Fig. 7. Histologic classification of glomerular lesion [periodic acid-Schiff (PAS)]. Histologic examination defined three categories of glomerular lesion according to the grade and distribution of mesangial proliferation. (A) Minor glomerular abnormality (MGA) showed no significant mesangial proliferation. (B) Focal proliferative glomerulonephritis (FPGN) showed mild mesangial proliferation with focal and segmental accentuation. (C) Diffuse proliferative glomerulonephritis (DPGN) showed mild to moderate mesangial proliferative change with relatively diffuse distribution.

Table 2 shows the frequency of each HLA type in IgA(+) group (N = 82) and IgA(−) group (N = 411). The frequency of HLA-DR4 and Bw35 was not statistically different between the groups. HLA-Bw51 was more frequent in IgA(−) group than in the IgA(+) group: 20.7% (17/82 cases) vs. 11.9% (49/411 cases) (P = 0.032).
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Fig. 9. Glomerular macrophage infiltration of the renal allograft with mesangial immunoglobulin A (IgA) deposition [PG-M1 (CD68) immunostaining, streptavidin-biotin (SAB) method]. A few CD68-positive macrophage was infiltrated in the glomerulus of IgA(+)/C3(+) allograft diagnosed with focal proliferative glomerulonephritis (FPGN) (A) (the same case as Fig. 1). In cadaveric allograft, CD68-positive macrophage infiltration was frequently observed in the renal interstitium (B) and glomerulus (C). The number of macrophage infiltration in glomerulus (cells/glomerular cross-section) was 4.95 ± 3.52 in the cadaveric allografts (N = 10) and 0.70 ± 0.50 in the living allografts (N = 72) with significant difference (P < 0.0001).

Table 2. Human leukocyte antigen (HLA) frequency of renal allograft donors with and without immunoglobulin A (IgA) deposition

<table>
<thead>
<tr>
<th></th>
<th>(+) N = 82</th>
<th>(−) N = 411</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>DR4</td>
<td>35 (42.7%)</td>
<td>163 (39.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bw35</td>
<td>10 (12.2%)</td>
<td>71 (17.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>DR4 and Bw35</td>
<td>6 (7.3%)</td>
<td>42 (10.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bw51</td>
<td>17 (20.7%)</td>
<td>49 (11.9%)</td>
<td>0.032†</td>
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† Chi-squared test = 4.6

DISCUSSION

The present study revealed that mesangial IgA deposition was a relatively frequent phenomenon in the Japanese renal allograft donors, who could be regarded as the so-called “general population.” Previous studies had reported that mesangial IgA deposition was found in 4% to 10% of consecutive necropsies without clinical evidence of renal disease [4–6], while, another study found IgA deposition in 10% to 30% of renal allografts at transplantation [7, 8]. The reason why IgA deposition is so frequent among Japanese allograft donors had been regarded due to high frequency of blood-related renal transplantation instead of cadaveric transplantation. Because the blood-related donors have a genetic background similar to the recipient, who had the renal disease that progressed to ESRD, they are considered to be prone to renal diseases, including IgAN, the most common renal disease in Japan. To confirm the significance of blood relationship, we compared the IgA deposition...
rate of blood-related and nonblood-related donors; however, no significant difference of IgA deposition rate was found in both groups. Therefore, we are considering that the mesangial IgA deposition is a relatively common phenomenon in general population in Japan and the blood relationship between the donor and recipient is not so significant a factor in this phenomenon.

The correlation between donor age and IgA deposition rate was interesting. The IgA deposition rate was relatively higher in the donors in their 30s (19.6%), 60s (22.2%), and the 70s (26.1%). Because IgAN is frequently encountered in individuals aged in their teens to their 30s, relatively high incidence of IgA deposition in the 30s may represent a similar epidemiologic background of IgAN. The reason why IgA deposition rate increased with age in the elderly donors is uncertain. Abnormal immunologic regulation, including IgA immune system in the elderly individuals, may be concerned with the increased mesangial IgA deposition. Further knowledge should be accumulated to elucidate the relationship between aging and glomerular immunoglobulin deposition.

To evaluate a clinical significance of the latent mesangial IgA deposition, we first analyzed the urinary abnormality of the donors with IgA deposition. Although the grade of microhematuria of renal allograft donors was usually very low, it was significantly higher in IgA(+) donors than in IgA(−) donors (Fig. 6). This result suggested that the latent mesangial IgA deposition was associated with a mild degree of hematuria, which was a common symptom of IgAN.

Next, the histologic analysis revealed that some of the cases (19/82, 23.2%) with mesangial IgA deposition showed mild mesangial proliferative changes and the degree of mesangial proliferation was associated with the presence of combined C3 deposition. A previous study [9] demonstrated that a greater degree of C3 deposition was associated with higher histologic activity in IgA nephropathy and that C3 deposition was a useful indicator of histologic activity and clinical severity. Our results also support the relationship between the grade of mesangial cell proliferation and mesangial C3 deposition.

Furthermore, the combined IgA and C3 deposition was associated with the increased number of glomerular macrophage infiltration. Several recent studies have shown that intraglomerular infiltrating macrophages play an important role in both experimental [10, 11] and human [12–14] glomerulonephritis, and the macrophage infiltration has been found to be induced by several cytokines [monocyte chemoattractant protein-1 (MCP-1) [15], interleukin-6 (IL-6) [16], macrophage migration inhibitory factor (MIF) [17]] secreted by mesangial cells or infiltrating inflammatory cells. Since mesangial cells have receptors for IgA (FcεR) [18, 19] and complement [20], the IgA and complement deposited presumably stimulate the mesangial cells via these receptors and induce cytokine production, which promotes macrophage migration. Although the subjects of the present study were restricted to early or silent IgAN with low histologic activity, the combined deposition of IgA and C3 in the glomerular mesangium was shown to be related to mesangial hypercellularity and glomerular macrophage infiltration in renal allografts with latent IgA deposition.

Finally, our results suggest that HLA antigen may be associated with the latent mesangial IgA deposition. Certain genetic backgrounds have been reported in IgAN, indicating that HLA-Bw35 [21–23], HLA-DR4 [23, 24], and HLA-DQw4 [25] are related to IgAN patients. In particular, many reports have shown higher frequency of HLA-Bw35 [21–23] and HLA-DR4 [23, 24] in patients with IgAN than in controls. Although our results showed no difference in HLA-Bw35 or HLA-DR4 frequency between IgA(+) and IgA(−) donors, the frequency of HLA-Bw51 was higher in the IgA(+) donors than in the IgA(−) donors. These results suggest that the patients with latent IgA deposition may have a different genetic background from that of patients with clinically apparent IgAN. Further investigation is required to clarify the association between HLA antigen and latent mesangial IgA deposition.

In conclusion, mesangial IgA deposition was observed in approximately 16% of the Japanese renal allografts, regardless of blood relationship. The mesangial IgA deposition was associated with mild degree of microhematuria clinically and mesangial proliferation and glomerular macrophage infiltration histologically in some of the affected individuals, especially with combined IgA and C3 deposition. It should be kept in mind that latent mesangial IgA deposition is a relatively common phenomenon, and it might represent the epidemiological background of IgAN, the most common glomerulonephritis in the world.

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