Multi-hit Pathogenesis of IgA Nephropathy

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IgA nephropathy (IgAN) is characterised by the deposition of IgA in the glomerular mesangium and is the most commonly reported primary glomerulonephritis worldwide. Thirty to forty percent of patients with IgAN develop progressive renal function decline, requiring renal replacement therapy within two decades of diagnosis. Development of a curative treatment and strategies for early diagnosis and treatment are necessary. Renal biopsy is the gold standard for the diagnosis and assessment of disease activity. Therefore, reliable biomarkers are needed for the non-invasive diagnosis of this disease and to more fully delineate the risk for progression. For the pathogenesis of IgAN, it is now widely accepted that multi-hit hypothesis, including production of galactose-deficient IgA1 (Gd-IgA1) (1st hit), and IgG autoantibodies that recognize Gd-IgA1 (2nd hit) and their subsequent immune complex formation (3rd hit) and glomerular deposition (4th hit). Although the prognostic values of several biomarkers have been discussed, we recently developed a highly sensitive and specific diagnostic method by measuring serum levels of Gd-IgA1 and Gd-IgA1 contained immune complexes. In addition, urinary Gd-IgA1 may represent a disease-specific biomarker of IgAN. We also confirmed a significant correlation between serum levels of these effector molecules and disease activity, suggesting that each can be considered as a practical surrogate marker of therapeutic response. A better understanding of the molecular basis of the pathogenesis of IgAN will likely result in disease-specific serum and urinary biomarkers. Thus, these disease-oriented specific biomarkers may be useful for screening of potential IgAN with isolated hematuria, earlier diagnosis, disease activity, and eventually, response to treatment.

Key words: IgA nephropathy (IgAN), galactose-deficient IgA1 (Gd-IgA1), immune complexes, biomarker

Introduction

IgA nephropathy (IgAN; nephropathy with mesangial IgA and IgG deposits, so-called Berger disease in France) is the most common type of primary chronic glomerulonephritis in the world and was first described by Berger and Hinglais in 1968. IgAN is the most common in Asian populations, moderately prevalent in European and US, and rare in Africans. The difference in the prevalence of IgAN between world populations, together with the familial aggregation of disease, suggests a genetic mechanism. IgAN has a signifi-

cant morbidity, culminating in end-stage of kidney disease (ESKD) in about 30–40% of patients within 20 years of the diagnosis¹⁾.

Histopathologically, IgAN is characterized by the expansion of glomerular mesangial matrices with mesangial cell proliferation and/or mononuclear cell infiltration. Glomeruli typically contain generalized diffuse granular mesangial deposits of IgA (mainly polymeric IgA1), IgG, and C3. Electron microscopy revealed electron-dense deposits in the glomerular mesangial areas. Therefore, this disease is generally considered to be an immune complex-mediated glomerulonephritis.

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Renal biopsy is required for the definite diagnosis of IgAN. However, the findings may differ according to the timing of renal biopsy during the 20-years clinical course of IgAN. Moreover, renal biopsy is not frequently performed because of procedural risks, and renal biopsy provides only a snap-shot of the disease status in the assessment of disease activity. Poor prognosis of IgAN is partly a result of delayed diagnosis. The strategies for early diagnosis leading to early and effective medical intervention are urgently needed. Taken together, useful methods for activity assessment other than renal biopsy and urinalysis are needed. In this review we explore the evidence base current approaches to management and explore new strategies and future directions in the diagnosis and management of IgAN.

Current clinical approaches for diagnosis and assessment of disease activity

The poor prognosis of IgAN is partly due to the delayed diagnosis. The renal biopsy is the gold standard for diagnosis as well as assessment of disease activity and prognosis of IgAN. However, the pathological findings differ according to the time point of renal biopsy during the long chronic course of IgAN^{1) 2)}. Different timing of intervention of renal biopsy may yield variable pathological severity and chronicity on renal biopsy due to the different indication of renal biopsy in each country. These observations emphasize that renal biopsy is just a snap-shot, and thus has a limitation in the assessment of disease activity.

The degree of proteinuria is one of most important prognostic factors not only in IgAN but also in all renal diseases at present^{3) 4)}, and there have been a substantial number of clinical research studies on renal disease^{5) 6)} in which both a decrease in kidney function and proteinuria have been considered as an endpoint. Therefore, many clinical guidelines recommend therapeutic indication based on the degree of proteinuria^{7) 8)}. However, it is difficult to distinguish proteinuria from acute glomerular inflammatory lesions such as cellular crescents or burned-out sclerotic glomerulus in IgAN. In general, IgAN may have a long chronic course with mixture of such acute inflammatory lesions and the common pathway based on chronic

lesions¹⁾. Proteinuria must be derived from both lesions. Therefore, it is hard to qualitatively discriminate proteinuria at acute phase and chronic phase, suggesting the limitation of proteinuria-based assessment of the disease activity.

Taken together, activity assessment methods other than renal biopsy and urinalysis are needed. In addition to assess disease activity, a simple and safe method of early diagnosis using valid biomarkers based on the pathogenesis of IgAN should be established.

Mucosal immune response for IgA production

The main production site of IgA is the mucosa of the respiratory tract and intestine, and the onset and exacerbation of IgAN are often related with infections of the upper respiratory tract and gastrointestinal tract. IgA secreted from the mucosa is mainly polymeric form, the IgA in the glomerular deposits is exclusively of the IgA1 subclass and levels of the polymeric form of IgA1 are elevated in the serum of IgAN^{9)–11)}. Thus, mucosal immunity may play a central role in the pathogenesis of this disease. It is postulated that many antigenic substances, *i. e.*, viruses, bacteria, fungi, or food, may stimulate the IgA-containing immune complexes formation in patients with IgAN.

Aberrantly glycosylated IgA1

Galactose-deficient IgA1 (Gd-IgA1) consist of terminal *N*-acetylgalactosamine (GalNAc) or sialylated GalNAc in the hinge legion of IgA1 (Figure-1)^{12)–14)}. Normal serum IgA1 was thought to contain little galactose-deficient *O*-glycans¹⁵⁾. An increase of serum levels of Gd-IgA1 in patients with IgAN was quantitatively confirmed for the first time by Moldoveanu, *et al.* using *Helix aspersa agglutinin* lectin, which recognizes GalNAc residues¹⁶⁾. The immortalized IgA1-secreting cells derived from the circulation of patients with IgAN and healthy controls have provided a new insight that the Gd-IgA1 is related to decreased activity of core 1 β 1,3-galactosyltransferase (C1GalT1) and elevated activity of α -2,6-sialyltransferase 2 (ST6GalNAc-II)¹⁷⁾. Recent quantitative genome-wide association study for serum levels of Gd-IgA1 in 2,633 subjects

Figure-1 Structure of IgA1 and synthesis of IgA1 O-Glycans

IgA1 has O-glycans located in the hinge region between constant-region domains 1 and 2 of the heavy chain. Serum levels of IgA1 with galactose-deficient glycans (Gd-IgA1) are elevated in patients with IgAN.

of European and East Asian ancestry discovered significant loci in C1GALT1¹⁸⁾.

A fraction of Gd-IgA1 from the glomerular deposits is excreted into the urine and thus represents a disease-specific marker of IgAN. Urinary excretion of Gd-IgA1 discriminated patients with IgAN from patients with other proteinuric renal diseases. Furthermore, the level of urinary Gd-IgA1 correlated with proteinuria in patients with IgAN. Urinary Gd-IgA1 thus may represent a disease-specific marker of IgAN¹⁹⁾. We recently established novel lectin-independent method with monoclonal antibody (KM55) for measuring serum levels of Gd-IgA1²⁰⁾. It could be the worldwide standard measurement system of serum Gd-IgA1.

Essential role of Gd-IgA1 specific autoantibodies

Serum Gd-IgA1 levels in the relatives were still elevated compared with those in normal individuals who were not blood relatives, regardless of the absence of nephropathy²¹⁾. *In vitro*, Gd-IgA1

containing immune complexes, but not Gd-IgA1 alone induced the proliferation of mesangial cells²²⁾. Furthermore, the concentration of immune complexes containing Gd-IgA1 is increased in the blood and urine of IgAN patients²³⁾⁻²⁵⁾. These facts implicate additional pathogenic hits are necessary in the pathogenesis of IgAN. Gd-IgA1 in the serum of patients with IgAN is found exclusively within immune complexes bound to IgG. The IgG autoantibodies recognize glycan-containing epitopes on Gd-IgA1 and exhibit unique features in the complementarity-determining region 3 of the variable region of their heavy chains²⁶⁾. Furthermore, serum levels of Gd-IgA1 specific IgG autoantibodies correlated with disease severity, as assessed by magnitude of proteinuria²⁶⁾. The onset and progression of IgAN is believed to require Gd-IgA1 (1st hit), as well as endogenous anti-glycan antibodies (2nd hit) and subsequent immune complexes formation (3rd hit) and glomerular depositions (4th hit) (Figure-2)²⁷⁾.

Figure-2 Hypothesis on the pathogenesis of IgAN

Mucosal immunity may play a central role in the pathogenesis of IgAN. Synthesis of IgA1 with some *O*-glycans deficient in galactose is elevated. Gd-IgA1 is present in the circulation at increased levels (1st hit). This immunoglobulin is recognized by unique anti-glycan autoantibodies (2nd hit). This process results in the formation of pathogenic IgA1-containing circulating immune complexes (3rd hit), some of which deposit in the glomeruli and induce renal injury (4th hit).

Gd-IgA1 and its related immune complexes as biomarkers for disease activity of IgAN

The above-described clinical findings suggest that Gd-IgA1 and its related immune complex containing with anti-glycan autoantibodies are essential effector molecules in the pathogenesis of IgAN. Recent studies demonstrated that increased Gd-IgA1 levels associated with progression of proteinuria and a greater risk of deterioration of renal function in IgAN²⁸⁾. In addition, the combination of high serum Gd-IgA1 levels and circulating levels of advanced oxidation protein products were correlated with progression of renal injuries in IgAN²⁹⁾. Berthoux *et al.* reported that serum levels

of IgG autoantibodies specific for Gd-IgA1 at the time of renal biopsy were significantly associated with clinical progression of IgAN towards end stage of renal failure³⁰⁾. Moreover, our recent study revealed that improvement of urinary abnormalities was well correlated with decreased serum levels of Gd-IgA1 and Gd-IgA1 containing immune complexes³¹⁾. Those findings confirmed the multi-hit hypothesis for the disease mechanism of IgAN²⁷⁾, and indicate the possibility that evaluation of not only serum levels of the autoantigen (Gd-IgA1) but also those of autoantibodies against Gd-IgA1 or immune complexes should be required as disease markers for IgAN²⁷⁾.

Biomarkers for diagnosis of IgAN

Serum level of Gd-IgA1 that provided a 0.77 sensitivity and a specificity of 0.90 to distinguish IgAN patients from healthy controls by receiver operating characteristic (ROC) curve analysis¹⁶⁾. We recently reported that serum levels of IgA, Gd-IgA1, Gd-IgA1 specific IgG and Gd-IgA1 specific IgA were elevated in patients with IgAN compared with those of healthy and other renal disease controls³²⁾. Gd-IgA1 specific IgG showed the best performance for diagnosis of IgAN, with a sensitivity of 89% and specificity of 92%. Although those biomarkers may be useful for the diagnosis of IgAN, there are substantial overlap in serum levels of individual biomarkers between patients with IgAN, other renal diseases, and healthy controls³²⁾. Consequently, no single biomarker was sufficiently specific for IgAN. It is suggested that a panel of such serum biomarkers may be helpful to differentiate IgAN from other glomerular diseases. Those serum biomarkers have potential for diagnosis of IgAN, even though the renal biopsy is a gold standard for diagnosis and prediction of renal prognosis in patients with IgAN.

Biomarkers for early screening of IgAN

Annual checking system for urine analysis is well developed from childhood in Japan. About 75 % of Japanese IgAN patients are initially discovered through chance hematuria. The renal biopsy is not recommended for patients presenting with isolated hematuria. Thus, there are still many patients who show delayed intervention of therapy, resulted in deterioration of renal function. Therefore, these biomarkers may be applicable to second screening to the examinees with hematuria in a general checkup. Hematuria generally precede to proteinuria in IgAN, such new screening system with these biomarkers may change the importance of hematuria screening.

Conclusion

IgAN is an autoimmune disease caused by the glomerular deposition of nephritogenic circulating immune complexes consisting of Gd-IgA1 bound by anti-glycan autoantibodies. A better understanding

of the multi-step process of the pathogenesis of IgAN (Figure-2) will lead to the development of specific biomarkers. Although accurate individual risk stratification at diagnosis and predicting treatment response remains a challenge, noninvasive and real-time examination with such biomarkers on the basis of pathogenesis, besides renal biopsy, is critical to elucidate the efficacy of treatment and disease activity. Moreover, for the early screening of potential IgAN with isolated hematuria, those biomarkers may be applicable for early intervention of therapy resulted in reduce the risk of deterioration of renal function.

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Conflict of interest statement

The author declares that there are no competing interests.

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