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Development of a Polyclonal Antibody Against Synthetic Human Immunoglobulin A1 Hinge Glycopeptide

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Abstract

**Background:** Undergalactosylated IgA1 has been found to be increased in IgA nephropathy (IgAN) by an ELISA assay using Helix aspersa agglutinin (HAA) that recognizes N-acetylgalactosamine (GalNAc). In this study, we developed a polyclonal antibody (anti-sHGP antibody) against a synthetic IgA1 hinge peptide with five GalNAc residues.

**Methods:** The specificity of the anti-sHGP antibody was evaluated through the incremental treatment of IgA with corresponding glycosidases. Then, the susceptibility of the IgA to anti-sHGP antibody was compared among IgAN patients (n=39), patients with other forms of kidney diseases (OKD, n=36) and healthy controls (n=37), using ELISA assay. The association of the binding abilities between anti-sHGP antibody and HAA were evaluated blindly using same 85 sera.

**Results:** The binding ability of the anti-sHGP antibody was increased relative to the incremental treatments of neuraminidase (desialo-IgA), galactosidase (desialo/regalacto IgA). The binding levels of anti-sHGP antibody against serum IgA were significantly higher in IgAN patients compared to both healthy controls (P=0.008) and those with OKD (P=0.049). The binding levels of anti-sHGP antibody were closely related to those of HAA ELISA in the same patient sera (RR=0.5964).

**Conclusions:** It was certified that the anti-sHGP antibody recognized GalNAc residues in the hinge peptide of human IgA1 as well as HAA. The increased antigenicity of IgA against the antibody in IgAN suggested that a serum IgA1 exposing GalNAc residue was increased in IgAN. It would be necessary to identify the precise structure of O-glycans specific to IgAN for developing a more specific antibody.

**Keywords:** IgA nephropathy; IgA1 hinge region; N-acetylgalactosamine; Galactose; Anti-IgA1 hinge antibody

**Abbreviations:** IgA: Immunoglobulin A; GalNAc: N-acetylgalactosamine; sHGP: synthetic Hinge Glycopeptide; OKD: Other forms of Kidney Diseases; HAA: Helix Aspersa Agglutinin; MALDI-TOFMS: Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry; KLH: Keyhole Limpet Hemocyanin

Introduction

There is increasing evidence for the involvement of aberrantly glycosylated IgA1 in the pathogenesis of IgAN [1-8]. A variant with terminal GalNAc or sialylated GalNAc is rare in normal serum IgA1, but it has been suggested that the presence of the truncated O-glycan with an exposed N-acetylgalactosamine (GalNAc) residue is more common in the IgA1 of IgAN patients. In previous studies, O-glycans in the hinge region of IgA1 were evaluated by ELISA assay using Helix aspersa agglutinin (HAA), a lectin specifically binding to GalNAc residues [9,10]. The assay suggested the increased binding of HAA to IgA1 in IgAN with high specificity and sensitivity. However, it is known that the affinity of lectin is much less than that of antibody. Therefore, in this study, we tried to develop a polyclonal antibody specifically binding to undergalactosylated IgA1, expecting that the antibody would be available as a clinical tool for the diagnosis of IgAN.

**Methods**

**Patients and test sera**

Thirty-nine patients with biopsy-proven IgAN were examined. The serum samples were obtained at the time of renal biopsy. An additional 36 patients with other forms of kidney diseases (OKD) were also examined (Table 1). The absence of glomerular IgA deposits were confirmed in all OKD patients by renal biopsy. The itemization of OKD and patient number of each disease were described in Table 1. Thirty-seven healthy control subjects were selected from healthy individuals matched for gender and age to the IgAN patients. A 19 mer synthetic peptide with five GalNAc residues at 4, 7, 9, 11 and 13 [VPST(GalNAc) PPT (GalNAc) PS (GalNAc) TPPT (GalNAc) PSPS-NH2] was purchased from Peptide Institute, Inc. (Osaka, Japan). The binding sites were determined according to the report of Mattu et al. [11] in that GalNAc residues frequently bind to these sites in human IgA1 hinge region. The purity and molecular weight were confirmed by HPLC and MALDI-TOFMS. For immunization, sHGP was conjugated with Keyhole Limpet Hemocyanin (KLH, Sigma). The sHGP–KLH conjugate was subcutaneously injected with Freund’s complete adjuvant to domestic mongrel white rabbits (n=2). The immunization was performed four times in every two weeks. Then, the antisera were isolated following exsanguination. The antisera were certified to bind to sHGP at the dose – response manner. IgG fraction was then obtained by ion exchange chromatography.

The serum IgA1 was isolated from the sera of healthy individuals using jacalin affinity chromatography according to our previous study

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The binding ability of the anti-sHGP antibody was increased relative to the incremental treatment of neuraminidase (desialo-IgA), galactosidase (desialo/degalacto IgA). The anti-sHGP antibody also bound significantly to the naked IgA (Figure 1). However, the binding was slightly decreased compared to that of desialo/degalacto IgA. The levels of antibody–IgA binding were significantly higher in the IgAN group compared to OKD (P = 0.049) and to HC (P = 0.008) groups (Figure 2). There was no significant difference between HC and OKD (p=1.839). The binding levels of the anti-sHGP antibody were closely correlated to those of HAA ELISA in the same patient sera (RR²=0.5964, Figure 3).

Discussion

Human IgA1 molecule has a unique hinge structure due to its mucin-like O-linked oligosaccharides [11]. The core peptide consists of a proline-, serine, and threonine-rich amino acid sequence in which serine and threonine residues are able to bind to O-glycans consisting of neuraminic acid, galactose and N-acetylgalactosamine with microheterogeneity. In the past two decades, it has been demonstrated that there are some abnormalities in the O-glycan structure of the IgA1 hinge region in IgAN. Although the abnormalities were suggested to be underglycosylated (especially undergalactosylated), the precise structure is not yet clear. Further, although the precise analyses were performed by mass spectrometry, the analyses limited the number of samples in the comparable study. The HAA-assay seemed to overcome this problem, clarifying the significant increase of exposed GalNac residues in IgAN. In other words, IgAN patients had less Gal residues in IgA1 hinge region. However, the relatively weak binding ability

![Figure 1: Reactivity of the deglycosylated IgA1 with anti-sHGP antibody.](image-url)
Antibody produced by the immunization of the artificially synthesized hinge of human IgA1. It was certified that the polyclonal antibody against the hinge peptide included five GalNAc residues frequently found at these sites [11]. The increased antigenicity of IgA against the antibody in IgA nephropathy suggested that IgAN patients have serum underglycosylated IgA1 expressing GalNAc residues and/or hinge core peptide. However, the antibody did not have sufficient specificity for clinical application in the diagnosis of IgAN. It would be necessary to clarify the precise structure of O-glycans specific in IgAN to develop a more specific antibody.

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