

IgA Glycosylation and IgA Immune Complexes in the Pathogenesis of IgA Nephropathy

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Summary: Circulating immune complexes containing aberrantly glycosylated IgA1 play a pivotal role in the pathogenesis of IgA nephropathy (IgAN). A portion of IgA1 secreted by IgA1-producing cells in patients with IgAN is galactose-deficient and consequently recognized by anti-glycan IgG or IgA1 antibodies. Some of the resultant immune complexes in the circulation escape normal clearance mechanisms, deposit in the renal mesangium, and induce glomerular injury. Recent studies of the origin of these aberrant molecules, their glycosylation profiles, and mechanisms of biosynthesis have provided new insight into the autoimmune nature of the pathogenesis of this common renal disease. An imbalance in the activities of the pertinent glycosyltransferases in the IgA1-producing cells favors production of molecules with galactose-deficient O-linked glycans at specific sites in the hinge region of the α heavy chains. By using sophisticated analytic methods, it may be possible to define biomarkers for diagnostic purposes and identify new therapeutic targets for a future disease-specific therapy. *Semin Nephrol* 28:78-87 © 2008 Elsevier Inc. All rights reserved.

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IgA nephropathy (IgAN) is characterized by immune deposits with dominant or co-dominant IgA in the glomerular mesangium.^{1,2} The IgA is of the IgA1 subclass³ and may be accompanied by C3, and IgG or IgM, or both.⁴ Mesangial proliferation and expansion of extracellular matrix is present in biopsy specimens from patients with even mild clinical disease. Glomerular sclerosis and interstitial fibrosis generally signify more serious disease and are associated frequently with progressive disease that leads to end-stage renal failure in 20% to 40% of patients

within 20 years of diagnosis.^{4,6} Hematuria is typical and often includes episodes of macroscopic bleeding that coincide with mucosal infections, including those of the upper respiratory tract and digestive system.^{4,7,9}

There are substantial data suggesting that the mesangial immune deposits originate from circulating IgA immune complexes. Evidence that the primary cause of IgAN is extrarenal includes recurrence of the disease in 50% to 60% of patients undergoing renal transplantation.¹⁰⁻¹⁴ Moreover, in the few instances in which a kidney from a donor with subclinical IgAN has been engrafted into a patient with end-stage renal failure due to a disorder other than IgAN, the immune deposits cleared from the allograft within several weeks.¹⁵ Many patients with IgAN have increased levels of IgA and IgA-containing immune complexes in the circulation.¹⁶⁻¹⁹ Idiotypic determinants are shared between the circulating complexes and the mesangial deposits²⁰; however, disease-specific idiotypes have not been identified.²¹ Furthermore, circulating immune complexes in pa-

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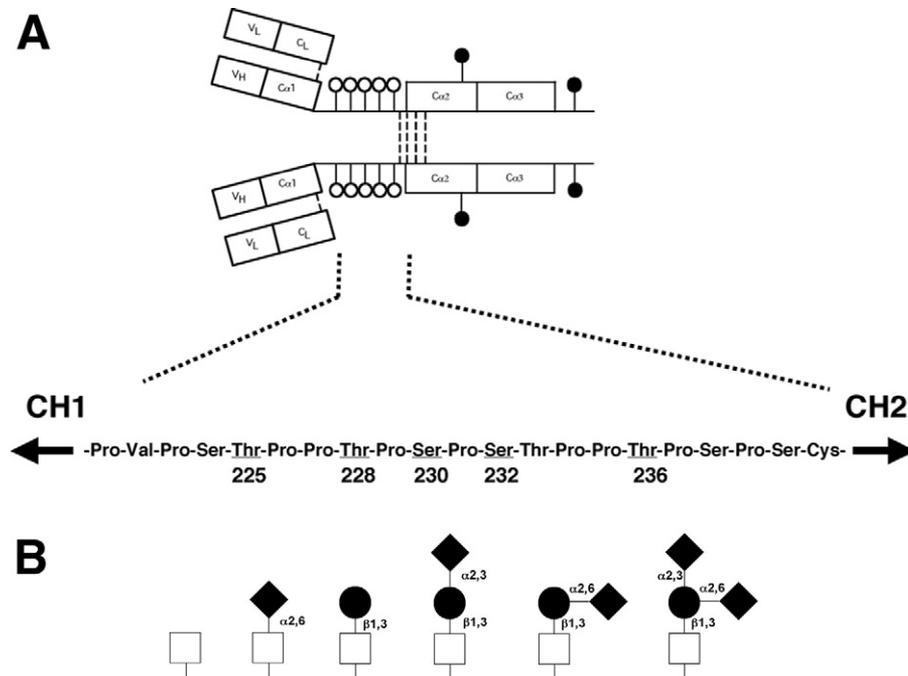


Figure 1. (A) IgA1 and its hinge region with O-linked glycans (○) and N-linked glycans (●). Underlined and numbered amino acids denote usual sites of attachment of as many as 5 O-linked glycans.^{31,34} (B) Variants of O-linked glycans in the hinge region of human circulatory IgA1.^{30,31} □, N-acetylgalactosamine; ●, galactose; ◆, sialic acid.

tients with IgAN and in the immune deposits contain IgA1, but not IgA2.^{16,22-25}

IgA1: STRUCTURE AND GLYCOSYLATION

IgA1 represents 1 of 2 structurally and functionally distinct subclasses of IgA, the other being IgA2.²⁶⁻²⁸ Unlike IgA2, the heavy chains of IgA1 molecules contain a unique insertion in its hinge-region segment between the first and second constant region domains (Fig. 1A). This hinge region, which has a high content of proline, serine, and threonine, is the site of attachment of as many as 5 O-linked glycan chains consisting of N-acetylgalactosamine with a β 1,3-linked galactose that may be sialylated.²⁹⁻³⁴ Sialic acid also may be attached to N-acetylgalactosamine by an α 2,6 linkage. The carbohydrate composition of these O-linked glycans in the hinge region of normal circulatory IgA1 is variable. The prevailing forms include N-acetylgalactosamine-galactose disaccharide, and its monosialylated and disialylated forms (Fig. 1B).^{18,31,35} Galactose-deficient variants with terminal N-acetylgalactosamine or sialylated N-acetylgalactosamine rarely are found in the O-glycans of normal

circulatory IgA1,³¹ but are much more common in patients with IgAN, predominantly in the glomerular immune deposits and in circulating immune complexes.^{18,23,36-39}

IgAN: A DISEASE OF ABERRANT GLYCOSYLATION

Analysis of the glycosylation of IgA1 in patients with IgAN has provided new insights into the mechanisms underlying formation of immune complexes and their deposition in the mesangium.^{18,23,40-44} Specifically, aberrant glycosylation of the O-linked glycans (galactose deficiency) in IgA1 hinge region appears to be a key pathogenetic factor contributing to the development of IgAN.^{18,23,39-41,45} Notably, galactose-deficient IgA1 is the predominant glycosylation variant of IgA1 in the mesangium.^{37,38} A relationship between galactose deficiency and nephritis has been observed in other diseases. Galactose-deficient IgA1⁴⁶ and IgA-IgG circulating complexes⁴⁷ are found in sera of patients with Henoch-Schönlein purpura who develop nephritis, but not in sera of patients who do not. Also, patients with IgA1 myeloma may

Table 1. Examples of Techniques for Analysis of IgA1 O-Glycans

Technology	Data Generated	References
Gas-liquid chromatography	Composition of monosaccharides	18,39
Lectin ELISA	Terminal sugar	18,36,37,39,43,44
Lectin Western blotting	Terminal sugar	43,44
Fluorophore-assisted carbohydrate electrophoresis	Profile of released O-glycans	96
HPLC	Profile of released O-glycans	97
MALDI-TOF MS of trypsin-digested hinge-region fragment	Profile of hinge-region glycopeptides	33,75,98,99
ESI MS of trypsin-digested hinge-region fragment	Profile of hinge-region glycopeptides	38
MALDI-TOF MS of hinge-region fragment generated with two bacterial proteases	Profile of hinge-region glycopeptides	35
FT-ICR MS of hinge-region proteolytic fragment	Profile of hinge-region glycopeptides	34,52,75
SELDI MS	Profile of hinge-region glycopeptides with aberrant glycans	100
Lectin Western blotting of IgA1 digested with bacterial proteases	Sites of glycan attachment	44
AI-ECD FT-ICR MS of hinge-region proteolytic fragment	Sites of glycan attachment of individual glycopeptides	34,52,75

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HPLC, high-pressure liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; MS, mass spectrometry; ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; SELDI, surface-enhanced laser desorption ionization; AI-ECD, activated-ion electron capture dissociation.

have very high levels of circulatory IgA1, but only those with aberrantly glycosylated IgA1 develop an immune-complex glomerulonephritis.^{48,49}

Analysis of O-glycans on IgA1 has been challenging.⁵⁰ The reasons include the structure of the hinge region (there are 9 serine and threonine residues, the potential sites of attachment of 5 glycan chains⁵¹) and the heterogeneity of composition of the attached glycans.⁵⁵ The techniques applied to the analysis include the use of lectins, enzyme-linked immunosorbent assay, Western blotting, mass spectrometry, gas-liquid chromatography, and specific proteases (Table 1). The greatest challenge has been the assignment of the sites of attachment of the glycans, including those that are glycosylated aberrantly. The most promising techniques are based on high-resolution mass spectrometry, such as Fourier transform ion cyclotron resonance mass spectrometry, using electron-capture or electron-transfer fragmentation techniques.^{34,52}

BIOSYNTHESIS AND CATABOLISM OF IgA1

When the daily synthesis of all isotypes of immunoglobulins is taken into account, the production of IgA far exceeds the synthesis of IgG, IgM, IgD, and IgE combined. However, more than two thirds of all IgA finishes its short life-span in the external secretions (half-life of IgA in the circulation is ~4-5 days).²⁸ Quantitative studies of IgA production and the distribution of IgA-producing cells in tissues clearly indicate that 90% to 95% of circulatory IgA is produced in the bone marrow, lymph nodes, and spleen, with a small contribution from the mucosal tissues.²⁸ In contrast, most external secretions, with the exception of those in the urine and the male and female genital tracts, contain IgA originating from local synthesis by the abundant IgA-producing plasma cells. Studies in primates⁵³ showed that IgA is catabolized by hepatocytes that recognize glycan moieties on IgA

heavy chains. In contrast to other immunoglobulin isotypes, human IgA occurs in 2 subclasses and 2 molecular forms, monomeric and polymeric, with characteristic distributions in various body fluids and in immunoglobulin-producing cells in the systemic and mucosal tissues.²⁸ Typically, most of the IgA-secreting cells in the bone marrow and lymph nodes produce monomeric IgA (>95%) of the IgA1 subclass (~85%). In mucosal tissues, IgA-secreting cells produce polymeric IgA with J chain. However, the distribution of IgA1- or IgA2-producing cells displays marked differences among mucosal tissues. For example, the upper respiratory and gastrointestinal tracts are populated by IgA1-producing cells whereas the large intestine and the female genital tract contain equal numbers or a slight excess of IgA2-producing cells. Human salivary, lacrimal, and mammary glands contain approximately equal numbers of IgA1- and IgA2-producing cells.²⁸

Whether the bone marrow or mucosal tissue is the origin of IgA1 in circulating immune complexes and the mesangial deposits of patients with IgAN has been a matter of controversy. A signature clinical manifestation of the disease, macroscopic hematuria, often is accompanied by a concurrent infection of the upper respiratory tract. Because this site is populated by cells producing polymeric IgA1, this feature favors a mucosal origin of the pathogenic IgA1.⁵⁴ However, in other studies, polymeric IgA1- and J chain-producing cells have been detected in the bone marrow of patients with IgAN.⁵⁵⁻⁵⁸ Unfortunately, samples of mucosal tissues, lymph nodes, and bone marrow from patients with IgAN are not easily available to resolve this dispute.

BIOSYNTHESIS OF O-LINKED GLYCANS ON IgA1

O-linked glycans of IgA1 are synthesized in a step-wise manner, beginning with attachment of *N*-acetylgalactosamine to serine or threonine, catalyzed by uridine-5'-diphospho-*N*-acetylgalactosaminyl-transferase 2 (GalNAcT2) (Fig. 2).^{59,60} The O-glycan chain then is extended by sequential attachment of galactose and/or sialic acid residues to the *N*-acetylgalactosamine. The addition of galactose is mediated by core 1 β 1,3-galactosyltrans-

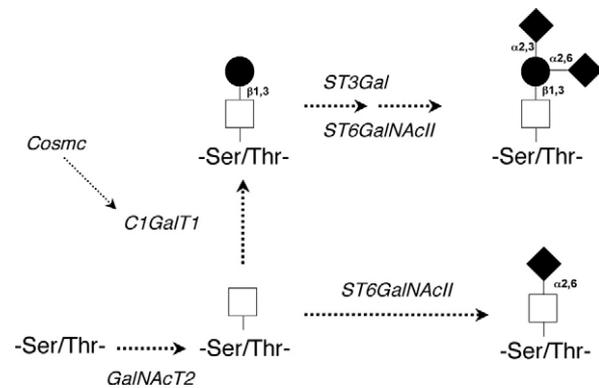


Figure 2. Enzymes involved in glycosylation of hinge region of IgA1. Galactose-deficient IgA1 may be generated by decreased activity of C1GalT1/Cosmc, by increased activity of ST6GalNAcII, or both.

ferase (C1 β GalT1) that transfers galactose from UDP-galactose to a *N*-acetylgalactosamine residue.⁶¹ The stability of this enzyme depends on its interaction with C1- β gal T1-specific molecular chaperone (Cosmc).⁶¹⁻⁶⁴ In the absence of Cosmc, the C1 β GalT1 protein is degraded rapidly, thereby resulting in undergalactosylation of *N*-acetylgalactosamine in O-linked glycans. The glycan structure is completed by the sialyltransferases that attach negatively charged sialic acid to the galactose (α 2,3-linked) or *N*-acetylgalactosamine (α 2,6-linked) residues. Sialylation of *N*-acetylgalactosamine in IgA1-secreting cells is mediated by a *N*-acetylgalactosamine-specific α 2,6-sialyltransferase (ST6GalNAcII).⁶⁵ If sialic acid is linked to *N*-acetylgalactosamine before attachment of galactose, this premature sialylation precludes subsequent attachment of a galactose residue.⁶⁵⁻⁶⁷ Thus, the relative activity of ST6GalNAcII and C1 β GalT1/Cosmc directly can influence the glycosylation of IgA1. Studies of the variants of the C1 β GalT1 gene found an association of certain polymorphisms with IgAN.⁶⁸ Other studies looking at genetic factors contributing to IgAN are ongoing.⁶⁹

Recent studies with Epstein-Barr virus-immortalized cell lines from peripheral blood lymphocytes of patients with IgAN and healthy controls confirmed the earlier-described pathways in the IgA1-secreting cells.⁷⁰ Furthermore, detailed analysis of enzymatic activities in the cell lines from patients with IgAN indicated an imbalance in the activities of the pertinent glycosyltransferases.

The β 1,3-galactosyltransferase activity was significantly lower and the *N*-acetylgalactosamine-specific α 2,6-sialyltransferase activity was significantly higher.⁷¹ Studies of the aberrant glycosylation in IgAN represent a promising field with a potentially great impact on the future care of patients.⁷²

ANTI-IgA1 ANTIBODIES AS A COMPONENT OF CIRCULATING IMMUNE COMPLEXES

Although IgA1-IgG immune complexes have been detected by many investigators, the true nature of IgA1-IgG interaction was shown only recently.²³ Dissociability of circulating immune complexes at acidic pH and inhibition of reformation by *N*-acetylgalactosamine-bearing glycoproteins implied an antigen-antibody nature of the IgA1-IgG interaction in these complexes.²³ The presence of IgG antibodies, and to a lesser degree IgA1 or IgM antibodies, to IgA1 in sera of healthy individuals and patients with IgAN has been described.^{73,74} However, the antigenic determinants expressed on the IgA1 molecules were unknown. More recently, with the application of bacterial IgA proteases, lectin Western blots, and high-resolution mass spectrometry, the antigenic determinants have been shown to consist of a *N*-acetylgalactosamine residue in the central and C-terminal portions of the hinge region of the heavy chain.⁷⁵ We have detected and isolated circulating IgG-producing cells that secrete IgG specific for aberrantly glycosylated IgA1.⁷⁰

The stimulus that leads to the formation of serum antibodies specific for *N*-acetylgalactosamine in the IgA1 hinge-region in healthy individuals and patients with IgAN is unknown. However, some viruses (eg, respiratory syncytial virus, Epstein-Barr virus) and Gram-positive bacteria (eg, streptococcus) express GalNAc-containing structures on their surfaces. It is therefore possible that these structures may mimic the glycan epitopes on galactose-deficient IgA1.

The relatively short half-life of normal serum IgA is the result of its rapid catabolism by hepatocytes.^{32,53,76,77} Hepatocytes express the asialoglycoprotein receptor^{32,77} that binds glycoproteins through a terminal galactose or

N-acetylgalactosamine residue.^{32,77-79} Because of this structural prerequisite, the absence or enzymatic removal of the otherwise terminal sialic acid is essential for effective binding of IgA1. Indeed, human IgA1 myeloma proteins and polyclonal human IgA1 are removed promptly from the circulation after enzymatic cleavage of terminal sialic acid.^{32,80,81}

Galactose-deficient IgA1 has a longer lifespan than normal IgA1.⁸² Galactose deficiency by itself should not hinder disposal of IgA1 molecules because the asialoglycoprotein receptor recognizes terminal *N*-acetylgalactosamine as well as galactose.⁷⁸ However, if the *N*-acetylgalactosamine is linked to sialic acid or is occupied by an antibody, the IgA1 cannot be recognized by the asialoglycoprotein receptor and therefore escapes hepatic catabolism.^{40,83} Because galactose-deficient circulatory IgA1 is present predominantly in the form of immune complexes, it is plausible to speculate that this IgA1 does not effectively reach the hepatic asialoglycoprotein receptor (Fig. 3). The larger size of the complexes, compared with uncomplexed IgA1, precludes binding to this receptor because the relatively small endothelial fenestrae block entry into the space of Disse. Thus, immune complexes containing aberrantly glycosylated IgA1 are not cleared efficiently by the liver and reach the glomerular capillaries where larger endothelial fenestrae permit their entry into the mesangium.^{25,45,67,84}

BIOLOGICAL ACTIVITIES OF IgA1-CONTAINING IMMUNE COMPLEXES

Cultured human mesangial cells present a convenient model to evaluate biologic activities of IgA complexes.^{40,41,85,86} Immune complexes from sera of patients with IgAN containing galactose-deficient IgA1 bind to the mesangial cells more efficiently than do uncomplexed IgA1 or immune complexes from healthy controls. Assessment of the biological activity of IgA1 complexes showed that large-molecular-weight IgA1 complexes stimulated cellular proliferation and production of some cytokines (eg, interleukin-6, transforming growth factor- β). In contrast, IgA1-depleted fractions were devoid of stimulatory activity.⁴¹ Further support for the pathogenic role of IgA1 complexes has

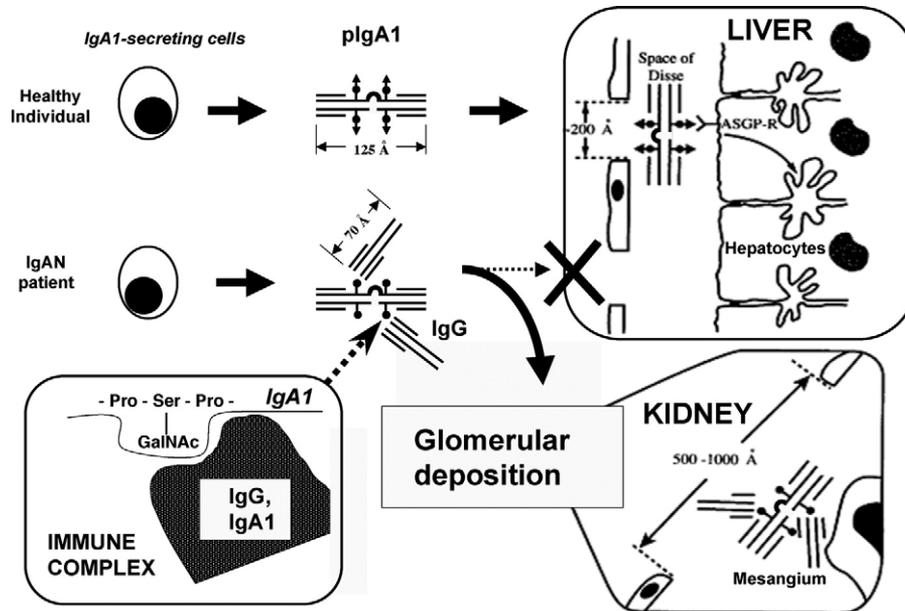


Figure 3. A model of pathogenesis of IgAN. Some polymeric IgA1 (pIgA1) produced by B cells and plasma cells in patients with IgAN is galactose deficient and is recognized by anti-glycan IgG or IgA1 antibodies. The resultant immune complexes are too bulky to enter the space of Disse to reach the asialoglycoprotein receptor (ASGP-R) on hepatocytes, but are able to pass through the larger fenestrae in glomerular capillaries overlying the mesangium. These deposited complexes induce glomerular injury.

come from supplementation experiments. Addition of small amounts of desialylated polymeric IgA1 to sera of patients with IgAN led to formation of new immune complexes: the amount of stimulatory complexes with a molecular mass of 800 to 900 kDa increased. Enzyme-linked immunosorbent assay indicated that these complexes contained IgG and IgA1.⁴¹ In contrast, uncomplexed IgA1 did not alter cellular proliferation. Complexes in the native sera of patients with IgAN enhanced cellular proliferation more than did complexes of similar mass from healthy volunteers.⁴¹ Furthermore, complexes from patients with IgAN collected during an episode of macroscopic hematuria stimulated cellular proliferation more than did complexes obtained during a later quiescent phase. IgA1 complexes with high levels of galactose-deficient IgA1 induced more proliferation than did complexes with low levels of galactose-deficient IgA1. In vivo studies in experimental animals showed that large-molecular-weight immune complexes generally induced more severe glomerular lesions than did small complexes.⁸⁷

Several findings point to activation of mesangial cells through an IgA-specific recep-

tor(s).^{40,41,88} However, none of the known IgA receptors (CD89, asialoglycoprotein receptor, and polymeric immunoglobulin receptor) is expressed on human mesangial cells.^{24,67,89-91} Among the recently identified candidate receptors that may mediate binding of IgA1 and IgA1 complexes are CD71 (transferrin receptor)^{88,91-93} and the Fc α / μ receptor.⁹⁴ CD71 appears to be the major IgA1 receptor on human mesangial cells (reviewed by Moura, pp. 88-95).^{88,92} Notably, the expression of CD71 is enhanced in the mesangium of IgAN patients and it co-localizes with IgA1 deposits.⁹⁵ Engagement of CD71 by IgA1 induces cellular proliferation and cytokine production. This accentuated cellular proliferation and cytokine production by IgA1 is inhibited completely by anti-CD71 blocking antibody, indicating that CD71 plays a major role in IgA1 binding.⁹³

HYPOTHETICAL MODEL OF THE PATHOGENESIS OF IgAN

Based on published data, a hypothetical model of the pathogenesis of IgAN is emerg-

ing. Some IgA1 molecules produced by immunoglobulin-secreting cells in patients with IgAN are galactose deficient and consequently recognized by anti-glycan IgG (or IgA1) antibodies. The resultant immune complexes are too bulky to enter the space of Disse in the liver. IgA1-containing immune complexes that escape normal clearance mechanisms reach the renal circulation and pass through the larger fenestrae in the glomerular capillaries overlying the mesangium. These complexes bind to mesangial cells and induce glomerular injury (Fig. 3). Together, these characteristics classify IgAN as an autoimmune disease, with the aberrantly glycosylated IgA1 being the autoantigen.

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