The Pathophysiology of IgA Nephropathy

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IgA nephropathy (IgAN) was described histologically for the first time in 1968 by Berger and Hinglais as les dépôts intercapillaires d’IgA-IgG (intercapillary deposits of IgA-IgG). Over the ensuing decades, this renal disease has been recognized as the most common primary glomerulonephritis and has been shown to arise from a systemic process wherein the kidneys are damaged as innocent bystanders. The latter point is best illustrated by the experience with renal transplantation. IgAN frequently recurs in allografts; in contrast, kidneys from donors with subclinical IgAN are clear of IgA deposits shortly after transplantation into recipients with non-IgAN renal diseases. The glomerular IgA eluted from tissue specimens from patients with IgAN is exclusively of the IgA1 subclass, predominantly in the polymeric form, and, most importantly, glycosylated aberrantly. Specifically, this aberrant IgA1 exhibits galactose deficiency in the O-linked glycans in the hinge region of the heavy chain. Blood levels of a similarly aberrantly glycosylated IgA1 are higher in patients with IgAN than in healthy controls or patients with other kidney diseases. However, as we discuss here, a high circulating load of galactose-deficient IgA1 alone does not induce the renal injury. Rather, several sequential processes or hits are necessary for the clinical expression of IgAN.

PATHOGENESIS OF IgAN

Four processes come together to induce renal injury that culminates in IgAN: aberrant glycosylation of IgA1, synthesis of antibodies directed against galactose-deficient IgA1, binding of the galactose-deficient IgA1 by the anti-glycan/glycopeptide antibodies to form immune complexes, and accumulation of these complexes in the glomerular mesangium to initiate renal injury. We recently performed a genome-wide association study and identified five distinct susceptibility loci—in the MHC on chromosome 6p21, the complement factor H locus on chromosome 1q32, and in a cluster of genes on chromosome 22q22—that potentially influence these processes and contain candidate mediators of disease. The significant variation in prevalence of risk alleles among different populations may also explain some of the sizable geographic variation in disease prevalence. Elucidation of the pathogenesis of IgA nephropathy provides an opportunity to develop disease-specific therapies.

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wide association study (GWAS) that identified five susceptibility loci for IgAN and provided molecular candidates for these processes.³

**Hit 1: Hereditary Increase in Galactose-Deficient Circulating IgA1**

As is the case for other immunoglobulins, IgA1 is glycosylated. An altered pattern of its glycosylation has been recognized as a potentially pathogenic abnormality in IgAN for nearly 20 years.⁴ The key feature is the deficiency of galactose in the hinge region of the IgA1 heavy chains. The hinge region of IgA1 extends by 13 amino acids longer than the hinge region of IgA2 and is found only in humans and higher primates (Figure 1A).³ It can carry up to six relatively short and simple sugar chains, called O-linked glycans, each attached by glycosidic linkage to an oxygen atom of a serine or threonine. These glycans are synthesized in stepwise fashion and include up to six different forms (Figure 1B). Patients with IgAN have increased circulating levels of IgA1 with abbreviated glycans composed of N-acetylgalactosamine (GalNAc), with or without sialic acid, that are devoid of a galactose moiety, as in Figure 1B structures I and II. A GalNAc-specific lectin, *Helix aspersa* agglutinin, is frequently used in an ELISA to measure the amount of galactose-deficient IgA1 (IgA1 with hinge-region O-glycan structures I and II in Figure 1B) after treatment of the IgA1 with neuraminidase to remove terminal sialic acid.⁶ Recent high-resolution mass spectrometry studies demonstrate significant heterogeneity in the composition and attachment sites of these glycans.⁷—¹² Determination of the precise structural abnormality in IgAN is complicated by the multiplicity of O-glycosylation sites and combinatorial possibilities of glycan variants. However, examination of IgA1-producing cells shows that aberrant O-glycosylation in IgAN is driven by combined abnormalities in expression or activity of the glycosyltransferases involved in the sequential post-translational modification of IgA1 (Figure 1), implicating complex regulatory defects.¹³

In patients with IgAN, the predominant sites where the cells secreting galactose-deficient IgA1 originate from and reside remain uncertain. Circulatory IgA1 is produced mainly in the bone marrow, whereas aberrantly glycosylated IgA1 may be syn-
the level of galactose-deficient IgA1 increased in patients with IgAN compared with those of healthy controls. This process may include dysregulated innate immune responses through Toll-like receptors.

Serum levels of galactose-deficient IgA1 are above the 90th percentile for healthy controls in as many as 70 to 80% of IgAN patients. Furthermore, 40 to 50% of first-degree relatives of IgAN patients have elevated levels comparable to that of patients, demonstrating significant heritability of this trait. The heritability of galactose-deficient IgA1 is observed in diverse racial groups and is not explained by variation in serum IgA levels, suggesting that distinct genetic mechanisms influence IgA1 glycosylation and production. These data also indicate that aberrant IgA1 glycosylation precedes clinically overt disease and constitutes an inherited risk factor for the development of IgAN. Moreover, because most persons with elevated levels of galactose-deficient IgA1 do not exhibit clinical signs of renal injury, this hereditary defect is insufficient to cause IgAN, implicating additional pathogenic hits as described in this article.

GWAS data have identified a major locus on chromosome 22q12.2 influencing susceptibility to IgAN. This locus is also associated with variation in serum IgA levels and has been previously associated with risk of inflammatory bowel disease, further implicating this interval in the regulation of mucosal inflammation. Two cytokine genes within the associated region, LIF and OSM, are excellent positional candidates, as both are expressed in B cells and may participate in the regulation of mucosal immunity. It is not yet known if this locus also influences aberrant IgA glycosylation. Further studies, including resequencing of this locus and evaluation of its effect on serum levels of galactose-deficient IgA1, will clarify causal variants and their role in the synthesis of IgA1.

**Hit 2: Circulating Antibodies Directed against Galactose-Deficient IgA1**

Aberrantly glycosylated IgA1 in the blood of patients with IgAN is found nearly exclusively within immune complexes bound to IgG or IgA1 antibodies. We have recently shown that these IgG antibodies recognize GalNAc-containing epitopes on the galactose-deficient hinge region O-glycans of IgA1, defining an autoimmune component to IgAN. Furthermore, these IgG autoantibodies exhibit unique features in the complementarity-determining region 3 (CDR3) of the variable region of their heavy chains. Specifically, the third position in CDR3 is typically serine in patients with IgAN, a feature necessary for efficient binding of the IgG to galactose-deficient IgA1. Importantly, serum levels of IgG antibodies specific for galactose-deficient IgA1 correlated with disease severity, as assessed by the magnitude of proteinuria. It is not known whether the CDR3 serine substitution originates from somatic mutations that arise during maturation of the antibody-producing cells or from inherited germline mutations. These antibodies are also present in sera of healthy individuals, albeit at lower levels. One can postulate that these antibodies are produced in response to bacterial or viral cell-surface GalNAc-containing glycoconjugates on commensal or infectious microorganisms and then cross-react with galactose-deficient O-linked glycans on IgA1. A predominant IgA1 autoantibody response directed against galactose-deficient IgA1 may explain why some patients have IgA1 as the sole Ig isotype in the glomeruli. It is to be noted, however, that the presence of IgG in the renal biopsy specimens also correlates with mesangial and endocapillary cellularity.

The strongest signals in the recent GWAS for IgAN were localized within the MHC complex (encoding polypeptides that present antigens to T cells), a region highly associated with risk for many autoimmune disorders. Based on careful conditional analyses, we identified three independent susceptibility loci within the MHC complex. The strongest genetic effect was observed for the MHC-II locus containing the HLA-DQB1, DQA1, and DRB1 genes. This effect appeared to be conveyed by a highly protective haplotype DRB1*1501-DQA1*0102-DQB1*0602. Specifically, the DQB1*0602 allele reduced the odds of disease by over 50% per copy. This is a relatively common classical HLA allele, present in 10 to 20% of Europeans and 2 to 10% of Asians. The second independent genetic effect is from a region encompassing two genes encoding transporters associated with antigen processing (TAP1 and TAP2) and two genes encoding components of the immunoproteasome (PSMB8 and PSMB9). These molecules process antigens in the cytosol and transport them into the endoplasmic reticulum for delivery to the cell surface in association with MHC-I molecules. It is not yet clear which of these four genes is involved in the susceptibility to IgAN. Finally, the third locus of association is on chromosome 6p21 and encodes MHC-II molecules DP1, DPB1, and DPB2, and thus also relates to the process of antigen presentation. Taken together, these new genetic findings strongly implicate adaptive immunity in the pathogenesis of IgAN, and define the genetic context required for the recognition of galactose-deficient IgA1 as an antigen and for generation of pathogenic anti-glycan antibodies.

**Hit 3: Formation of Pathogenic IgA1-Containing Immune Complexes**

It is generally agreed that, in IgAN, the mesangial cells represent the primary target of pathogenic deposits formed by circulating immune complexes (Figure 2, solid lines) or by lanthanic deposits of aberrantly glycosylated IgA1, followed by binding of newly generated anti-glycan antibodies to form immune complexes in situ (Figure 2, broken lines). The presence of circulating IgA1-containing immune complexes is not unique to patients with IgAN. Such complexes can be detected in persons without apparent renal disease, includ-
healthy individuals and patients with Henoch-Schoenlein purpura without nephritis. The complexes in patients with Henoch-Schoenlein purpura without nephritis consist of IgA, but not IgG, and are of smaller mass than the complexes found in patients with IgAN. As these persons do not develop overt renal disease, it can be assumed that these IgA complexes are not nephritogenic. In contrast, patients with Henoch-Schoenlein purpura with nephritis have larger circulating immune complexes containing IgA and IgG. By analogy with other human diseases caused by immune complexes, it is likely that, in IgAN, the molecular proportion of antigens (galactose-deficient IgA1) and antibodies (IgG or IgA1) determines the size of the formed immune complexes and, consequently, their rate of removal from the circulation, as well as biologic activity. The pathogenic circulating IgA1-IgG immune complexes in patients with IgAN are relatively large (>800 kD) and thus may be excluded from entry into the hepatic space of Disse to reach the asialoglycoprotein receptor (ASGP-R) on hepatocytes, the normal catabolic pathway for circulating IgA1. As a result, these immune complexes enter the renal circulation. Due to the unique location of the mesangium between the fenestrated endothelial lining of the capillaries and the glomerular basement membrane, the mesangium is prone to deposition of immune complexes. While it is not completely understood what determines the entry of circulating immune complexes into the mesangium, the factors involved likely include the size of immune complexes, their amount, and local hemodynamic factors. The biologic activity of large circulating immune complexes with galactose-deficient IgA1 increases in IgAN patients during episodes of macroscopic hematuria. However, it is not known whether this increase in activity is due to greater production of galactose-deficient IgA1, anti-glycan antibodies, or other undefined factors influencing the formation of these complexes and/or their composi-
The pathogenetic importance of immune complexes has been shown by in vitro studies. The glomerular injury of IgAN histologically manifests as proliferation of mesangial cells and expansion of extracellular-matrix components. The detailed mechanisms of activation of mesangial cells remain to be elucidated. Nonetheless, cultured human mesangial cells provide a convenient model for evaluating the biologic activities of IgA1-containing complexes. Immune complexes from patients with IgAN containing galactose-deficient IgA1 bind to the cells more efficiently than do uncomplexed IgA1 or immune complexes from healthy controls. Complexes with galactose-deficient IgA1 induce cultured human mesangial cells to proliferate, secrete extracellular matrix components, and release humoral factors such as TNFα, IL-6, and TGFβ. These factors can, in turn, alter podocyte gene expression and glomerular permeability. In contrast, uncomplexed galactose-deficient IgA1 or relatively small immune complexes (<800 kD) have no stimulatory effect on cellular proliferation.

The cellular receptors on mesangial cells involved in the binding of IgA1 are not well characterized. IgA1-containing immune complexes display a high affinity for the extracellular-matrix components fibronectin and type IV collagen in the mesangium, and preferentially bind and activate mesangial cells. None of the well-known IgA receptors (CD89, polymeric Ig receptor, ASGP-R) and complement receptors (CR 1–3) have been confirmed on human mesangial cells. However, transferrin receptor (CD71), which is expressed on the surface of proliferating human mesangial cells, can bind polymeric IgA1. Moreover, CD71 on human mesangial cells effectively binds immune complexes containing galactose-deficient IgA1, leading to enhanced expression of CD71. This binding creates a positive feedback loop, causing overexpression of CD71 on proliferating mesangial cells. However, it is not known whether CD71 is the only receptor that binds IgA1-containing immune complexes or whether it has a direct pathogenic role in IgAN.

Activation of the complement system in glomeruli augments the inflammatory cascade and potentiates tissue injury in IgAN. The immune complexes with IgA1 can activate complement via the alternative or lectin pathway. The pattern of glycosylation of IgA1 and the molecular mass of IgA1-containing immune complexes are also important factors in the ability of IgA1 to activate the alternative complement pathway. Accordingly, renal biopsy specimens have usually detectable C3, while the components of the classical pathway, such as C1q, are typically absent. Our recent GWAS identified a major IgAN susceptibility locus within the complement factor H gene (CFH) cluster on chromosome 1q32. Products of CFH and its neighboring CFHR (CFH-related) genes participate in the modulation of the alternative pathway by binding C3a and C5a convertases. Mutations in CFH lead to uncontrolled activation of the alternative pathway and cause inherited forms of membranoproliferative glomerulonephritis type II, a disease pathologically distinct from IgAN. However, carriers of a common deletion encompassing the neighboring CFHR1 and CFHR3 genes had an approximately 30% decreased risk of developing IgAN. The risk was almost 60% lower in the rare individuals who carry two copies of this deletion. The role of CFHR1 and CFHR3 proteins in the regulation of complement cascade is currently under active investigation. Based on early experimental data, however, CFHR1 and CFHR3 compete with CFH for binding to C3b, the key activator of the terminal portion of the complement pathway. Therefore, a relative loss of CFHR1 and CFHR3 may enhance the inhibitory action of CFH and thus convey protection against local inflammation.

These mechanistic issues have important clinical and therapeutic implications because subclinical findings consistent with IgAN are common in the general population. Necropsy series found glomerular IgA deposits in 2% and 4% of individuals in Singapore and Germany, respectively. Even more striking, a study in Japan showed that 16% of 510 renal allografts at engraftment were affected, of which 19 (3.7%) had a mesangioproliferative nephritis. To date, the glycosylation pattern of this lanthanic glomerular IgA has not been examined to determine if it differs from that found in IgAN patients. Such an analysis would clarify whether the IgA deposits are clinically silent because they have a different composition that renders them relatively inert or because there is an intrinsic hypersensitiveness of the kidney in which they are deposited.

**Potential New Diagnostic and Prognostic Markers**

**In Blood**

Based on the central role of galactose-deficient IgA1 in the pathogenesis, Moldoveanu et al. investigated the value of serum levels of this protein as a diagnostic test. By receiver operating characteristic (ROC) curve analysis, the serum level of galactose-deficient IgA1 that provided a 0.77 sensitivity had a specificity of 0.90 to distinguish IgAN patients from healthy controls, while a level with a specificity of 1.00 had a sensitivity of 0.44. Other groups have replicated these findings. Importantly, the serum level of galactose-deficient IgA1 may be significantly elevated long before the diagnosis of IgAN (Olson S. et al., unpublished observation).

IgG specific for galactose-deficient IgA1 represents another potential biomarker, as serum levels of this antibody are significantly elevated in IgAN patients, and the levels correlate with proteinuria. ROC curve analysis indicates that when the specificity of the level of serum IgG antibody directed against galactose-deficient IgA1 reached 0.95, the corresponding sensitivity was 0.88.
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HAA; Helix aspersa agglutinin, a lectin specific for terminal GalNAc
In Urine
Urinary proteomics holds promise for development of noninvasive tests for IgAN. A subset of mesangial immune complexes apparently enters the urinary space. aberrantly galactosylated IgA1 within immune complexes has been found in the urine of patients with IgAN but not in patients with non-IgAN proteinuric glomerular diseases. It is also possible to develop a diagnostic test without a detailed knowledge of the pathogenesis, based on analysis of the urinary peptidome. In a preliminary study, Julian et al. found that analysis of urine samples by capillary electrophoresis, coupled online with mass spectrometry, distinguished patients with primary IgAN from patients with IgA-immune-complex renal disease due to cirrhosis, even if clinical proteinuria was absent.

Genetic
The GWAS of sporadic IgAN identified five novel genetic variants with relatively strong protective effects against IgAN. While these variants are all common, their frequencies vary significantly across different continental populations and closely parallel the prevalence rates of IgAN. For example, African populations, which have the lowest reported prevalence of IgAN, carry the most protective alleles, while Asians, who have the highest reported prevalence, have significantly fewer protective variants. The ROC analysis for a genetic risk score based on these five alleles is estimated in the range of 0.60 to 0.63, with the risk score explaining up to 7% of disease variation among Asian and Caucasian populations. Identification of additional genetic susceptibility loci in follow-up GWAS studies of Caucasian populations will likely improve the predictive power of the genetic risk score. Additionally, a composite risk score based on a combined assessment of circulating, urinary, and genetic disease biomarkers holds promise to ultimately provide a tool for the noninvasive diagnosis of IgAN.

POtential Approaches for Disease-Specific Therapy
The pathogenesis model (Figure 2) provides an opportunity to design and test rational therapies for IgAN (Table 1). This model predicts that the generation of nephritogenic immune complexes composed of galactose-deficient IgA1 and anti-glycan autoantibodies initiates disease. Therefore, interventions that can reduce generation of galactose-deficient IgA1 or anti-glycan antibodies, or block the interaction between these two components to form nephritogenic immune complexes, may prove effective. This result can be achieved by enhancing enzymatic activity of glycosyltransferases for synthesis of galactose-replete hinge-region O-glycans to reduce the availability of aberrantly galactosylated IgA1 for formation of nephritogenic immune complexes. Alternatively, generation of non-cross-linking, monovalent reagents (single-chain antibodies) with high affinity for GalNAc on galactose-deficient IgA1 could theoretically prevent binding of anti-glycan antibodies. Lastly, blocking the antibodies with a glycopeptide may be another strategy to prevent formation of immune complexes (Table 1).

Similarly, interventions aimed at reducing immune complex deposition and the downstream inflammatory signals may prove beneficial. The genetic studies identify the alternative complement pathway as a prime candidate for intervention, and predict that targeted depletion of CFHR1 and/or CFHR3 would be tolerated and prove protective. Moreover, blocking of specific signaling pathways induced in mesangial cells by pathogenic IgA1-containing complexes can be theoretically accomplished by protein-kinase inhibitors, a class of drugs that is frequently used in the treatment of some types of cancer.

Conclusions
IgAN is an autoimmune renal disease arising from consequences of increased circulating levels of IgA1 with galactose-deficient hinge-region O-glycans. However, this glycosylation aberrancy alone is not sufficient to induce nephritis. For the clinical manifestation of renal injury, several additional hits are required, including synthesis of circulating antibody directed against the aberrantly glycosylated O-linked hinge-region glycans to form immune complexes, accumulation of the complexes in the mesangium, and activation of mesangial cells. Genetic factors apparently influence the expression of these hit mechanisms. Elucidation of the pathogenesis of IgAN provides an opportunity to develop a disease-specific therapy that heretofore has been missing.

Disclosures
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