Immune complex formation in IgA nephropathy:
A case of the ‘right’ antibodies in the ‘wrong’ place at the ‘wrong’ time?


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Running Head: IgA immune complex formation in IgA nephropathy

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Summary

One of the fundamental features of IgA nephropathy (IgAN) is the presence in the circulation of IgA1-containing immune complexes (IgA-IC) with a propensity for mesangial deposition and triggering of glomerular injury [1]. Immune complex formation in IgAN remains a poorly understood process, however Novak and colleagues have over the past 5 years steadily unravelled some of the intricacies, in particular the potential contribution of IgG anti-IgA1 autoantibodies to IgA-IC formation [2]. In contrast to other diseases associated with immune complex formation, immune complexes in IgAN appear to develop as a result of a shift in the distribution of IgA1 O-glycoforms in the serum. In the present paper, Suzuki and colleagues demonstrate IgA1 O-glycoform specific IgG antibodies in the serum of IgAN patients from both USA and Japan. They have gone on to generate B-cell lines from these patients and used the IgG secreted by these cell lines to characterise its specificity. They show that IgG from cells derived from IgAN patients binds poorly galactosylated IgA1 O-glycoforms and in vitro triggers the formation of IgA1-IgG immune complexes. They also present preliminary data suggesting that measurement of IgG with specificity for poorly galactosylated IgA1 may in the future form the basis of a non-invasive diagnostic test for IgAN.

Review of the field

One of the most striking findings in IgAN is an increase in the circulating levels of poorly galactosylated IgA1 O-glycoforms (Figure 1B). This has been observed in patient populations from North America, Europe and Asia using a variety of techniques [3-5]. Importantly, two studies of IgA1 eluted from isolated glomeruli have shown that mesangial IgA is enriched with poorly galactosylated IgA1 O-glycoforms, strongly implicating the composition of IgA1 hinge region glycans in the
mechanism of IgA1 deposition [6, 7]. Novak and colleagues have also reported that these poorly galactosylated IgA1 O-glycoforms are predominantly found in circulating high molecular weight IgA-IC in IgAN [2].

IgA1 is one of the very few serum proteins to have O-linked sugars. The 18-amino acid hinge region of IgA1 can carry from zero to 6 O-glycan moieties, each of which is a relatively short and simple sugar chain (Figure 1A). An IgA1 monomer, consisting of two \( \alpha_1 \) heavy chains therefore carries multiple closely adjacent O-linked sugars in the hinge region, providing a tight clustering of sialic acid (NeuNAc), galactose (Gal) and \( N \)-acetylgalactosamine (GalNAc) residues, variations in which may exert significant effects on the overall physicochemical properties of the IgA1 molecule [1]. A variety of IgA1 O-glycoforms exist due to the heterogeneity at each of the O-glycosylation sites.

While formation of IgA-IC in IgAN is poorly understood, Suzuki and colleagues present new evidence that poorly galactosylated IgA1 O-glycoforms may act as autoantigens, potentially triggering the production of glycan-specific IgG autoantibodies (Figure 1). Data from Japan also suggests that poorly galactosylated IgA1 O-glycoforms are liable to self-aggregation and immune complex formation, although it is unclear whether this is through antigen-independent hinge region attraction or the presence of O-glycan-specific IgA1 autoantibodies [8]. Once formed there is strong in vitro evidence that IgA-IC display a high affinity for the extracellular matrix components fibronectin and type IV collagen [9], preferentially bind and activate mesangial cells [10] and activate complement through the alternate and lectin binding pathways [11]. Together these in vitro data support a pathogenic role for circulating IgA-IC in glomerular IgA deposition and mesangial cell activation in IgAN. This is supported by clinical studies which have shown that the distribution
of IgA1 O-glycoforms in the serum correlates with the degree of glomerular injury at the time of renal biopsy [12].

**Discussion**

The mechanism underlying this increase in poorly galactosylated IgA1 O-glycoforms in IgAN remains controversial; a fundamental abnormality of O-glycosylation machinery, with at least in part a genetic basis, has been hypothesised but remains unproven [13]. However, it must be appreciated that even normal individuals have some poorly galactosylated IgA1 O-glycoforms in their circulation as part of the immune response to mucosal antigens e.g. *Helicobacter pylori* and mucosal vaccines. In contrast, systemic antigen challenge is associated with the predominant production of IgA1 O-glycoforms with greater numbers of terminal galactose residues [14, 15]. Why mucosal and systemic O-glycosylation of IgA1 differs so much remains unexplained. However, this raises the intriguing possibility that in IgAN there is no defect in IgA O-glycosylation (i.e IgA1 is not “undergalactosylated”) but rather there is an increase in “mucosal-type” IgA1 in the serum. We and others reported some 15 years ago that IgAN is indeed associated with a maldistribution of IgA1 secreting plasma cells from mucosal to systemic sites [16, 17]. More recently, changes in the homing of lymphocytes between mucosal and systemic sites has also been reported in IgAN [18, 19]. One possibility therefore is that mucosal IgA1-committed plasma cells are misdirected to systemic sites in IgAN and then secrete “mucosal-type” IgA1 into the circulation - the “right” IgA1 molecules ending up in the “wrong” place (Figure 2).

Immune complex formation in IgAN may similarly be a case of the “right” IgG antibodies being in the “wrong” place at the “wrong” time. Suzuki and colleagues propose that the IgG anti-IgA1 autoantibodies could in fact be antibodies specific for
bacterial and viral surface glycoproteins and that recognition of poorly galactosylated IgA1 O-glycoforms is an unfortunate coincidence, a phenomenon known as molecular mimicry [2] (Figure 2). Such a hypothesis may partly explain the well described association of macroscopic haematuria with upper respiratory tract infections, often thought of as pathognomonic of IgAN. During a normal response to infection serum levels of microbial-specific IgG (and IgA) rise. In patients with IgAN it is possible that a proportion of the glycan-specific anti-microbial antibodies bind in error to poorly galactosylated IgA1 O-glycoforms present in the serum at high levels, resulting in rapid IgA-IC formation. A sudden increase in IgA-IC levels may then lead to rapid glomerular IgA-IC deposition, mesangial cell activation and glomerular haematuria. An increase in circulating IgA-IC levels during episodes of macroscopic haematuria has been reported, however, any involvement of molecular mimicry in IgA-IC formation is at present pure speculation [10, 20].

While these new observations strongly support a role for IgG anti-IgA1 autoantibodies in IgAN there is a note of caution. IgG deposition is not universally seen in renal biopsies in IgAN, and in some series IgA1-IgG co-deposition is reported in only a minority of cases. The authors have argued (unpublished observations) that this is due to the use of immunohistopathological techniques with low sensitivity. However, until we have unequivocal evidence for generalised co-deposition of IgA1-IgG it is difficult to truly judge the importance of IgG anti-IgA1 autoantibodies in IgAN and the potential clinical utility of measuring them in IgAN.

**Take home message**

A previously unsuspected link between IgG specific for microbial glycoproteins and mucosal IgA inadvertently entering the circulation may underlie IgA immune complex formation in IgAN.
References


Legends to Figures

Figure 1: O-glycosylation of IgA1

1A. The O-linked sugars of IgA1 are attached to serine or threonine residues in the IgA1 hinge region which lies between the CH1 and CH2 domains of the α1 heavy chain. The O-linked sugar chains are core 1 structures based on N-acetylgalactosamine (GalNAc) in O-linkage with serine (usually) or threonine. This core GalNAc may be further extended with galactose (Gal) in the β1,3 configuration or sialic acid (N-acetylneuraminic acid, NeuNAc) in an α2,6 configuration. GalNAc-Gal may be further extended with sialic acid in α2,3-linkage with Gal.

1B. In IgAN there is an increase in the serum levels of poorly galactosylated IgA1 O-glycoforms. Suzuki and colleagues describe IgG anti-IgA1 autoantibodies with specificity for IgA1 O-glycoforms displaying exposed GalNAc residues.

Figure 2: Pathogenic components involved in the generation of IgA immune complexes in IgA nephropathy

It has been proposed by Novak and colleagues that the development of IgAN requires two “hits”. The first is an increase in the circulation of poorly galactosylated IgA1 O-glycoforms. The reasons for this increase are at present unknown. The second hit is the generation of IgG (and probably IgA1) antibodies specific for these poorly galactosylated IgA1 O-glycoforms. IgA-IC formation may then occur either within the circulation or in situ within the glomerulus and results in mesangial cell activation and glomerular injury.
1A
IgA1 hinge region O-glycans

1B
IgA1 hinge region in IgA nephropathy

O-glycan specific IgG anti-IgA1 in IgA nephropathy
Presence of increased amounts of poorly galactosylated IgA1 \( O \)-glycoforms in the circulation

Generation of IgG antibodies specific for poorly galactosylated IgA1 \( O \)-glycoforms

Mesangial deposition and/or \textit{in situ} formation of IgG-IgA1 immune complexes

Mesangial cell proliferation, extracellular matrix overproduction