The pathogenic role of IgA1 0-linked glycosylation in the pathogenesis of IgA nephropathy

Jonathan Barratt, Alice C Smith and John Feehally

The John Walls Renal Unit, Leicester General Hospital

and

University of Leicester Department of Infection, Immunity and Inflammation

Leicester, UK

Corresponding Author:

Dr Jonathan Barratt
John Walls Renal Unit
Leicester General Hospital

Tel: (44) 116 258 8043
Fax: (44) 116 258 4764
Email: jb81@le.ac.uk

Leicester LE4 5PW, UK

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Abstract

Numerous abnormalities of the IgA immune system have been reported in IgAN but the most consistent finding remains aberrant IgA1 O-linked glycosylation of the IgA1 hinge region. The defect comprises reduced galactosylation of O-linked N-acetylgalactosamine residues with or without changes in the terminal sialylation of the O-linked sugars. Aberrant O-galactosylation has been found in serum IgA1, in IgA1 isolated from tonsillar lymphocytes, and in IgA1 eluted from mesangial deposits. There is evidence that changes in IgA1 O-galactosylation lead to IgA immune complex formation and mesangial IgA deposition. Mesangial cells exposed to these IgA immune complexes proliferate and adopt a pro-inflammatory phenotype; they secrete cytokines, chemokines, growth factors and extracellular matrix components promoting glomerular inflammation and glomerulosclerosis. Recent evidence suggests that the control of IgA1 O-glycosylation is linked to class switching from IgD to IgA1 synthesis and that the pattern of IgA1 O-glycosylation may be programmed at the time of initial antigen encounter. IgA1 glycosylation varies between systemic and mucosal sites and the association of aberrant IgA1 galactosylation with low affinity, polymeric IgA1 antibodies against mucosal antigens suggests undergalactosylated IgA1 may in fact be a mucosal glycoform of IgA1. Although suited to the mucosal compartment, when these IgA1 glycoforms enter the systemic circulation in appreciable quantities they deposit in the mesangium and trigger glomerular inflammation. This review will discuss the evidence for the role of IgA1 O-glycosylation in the pathogenesis of IgAN and propose an explanation for the presence of aberrantly O-glycosylated IgA1 in the circulation of patients with IgAN.
Introduction

IgA nephropathy (IgAN) is the commonest pattern of primary glomerulonephritis in all countries where renal biopsy is widely practiced and is an important cause of end-stage renal disease at all ages[1]. It is characterised by the predominant mesangial deposition of polymeric IgA1 molecules with an associated mesangial proliferative glomerulonephritis. The degree of histopathologic injury is extremely variable and this is reflected in the varied tempo and severity of clinical presentation seen in this disease [2]. The mechanisms involved in mesangial IgA deposition, and the initiation of inflammatory glomerular injury, remain obscure. A variety of modest abnormalities of the IgA immune system have been reported in IgAN [3]. Despite this the deposited IgA displays no apparent antigen restriction, and for this reason, physicochemical abnormalities of IgA which may promote deposition in a non-immunological fashion have been studied. We and others have found that both serum and mesangial IgA1 is aberrantly O-glycosylated in IgAN and that this defect may play an integral role in both mesangial IgA1 deposition and triggering of glomerular injury in IgAN [4].

Human IgA

There are two subclasses of human IgA, IgA1 and IgA2, and both can exist in monomeric (mIgA) or polymeric (pIgA) forms [5]. Human IgA is produced in both mucosal and systemic immune compartments. The vast majority of IgA is produced at mucosal surfaces and is secreted directly with very little of this IgA entering the circulation. Both IgA1 and IgA2 are produced, with their relative proportions varying at different mucosal sites. Mucosal IgA is almost exclusively polymeric. Systemic IgA production is based in the bone marrow, and is the source of circulating IgA.
Bone marrow IgA production is mostly mIgA1, and therefore this is also the predominant form of serum IgA [6].

**IgA glycosylation**

Glycosylation plays an important role in production, maintenance, handling, and function of all glycoproteins and occurs during and immediately after protein synthesis [7]. In common with other immunoglobulins and many other serum proteins human IgA is heavily glycosylated. There are two distinct forms of protein glycosylation, N-linked and O-linked. N-linked carbohydrates, linked to asparagine residues, are by far the most common, and usually consist of complex, branched chains [8]. O-linked sugars are more commonly associated with membrane bound proteins and are less frequently found in serum proteins. They are usually simpler moieties linked to serine or threonine residues. Both types of sugars can carry terminal sialic acid, a highly negatively charged carbohydrate that is much more labile than the core structure, and is important in determining the clearance of many proteins.

IgA1 is one of the very few serum proteins to have both N-linked and O-linked sugars. These lie in the hinge region of the molecule, between the CH1 and CH2 domains of the heavy chain (Figure 1). The hinge region is made up of 17 amino acids, of which at least six are O-linked glycosylation sites [9]. The O-linked sugar chains are core 1 structures based on N-acetylgalactosamine (GalNAc) in O-linkage with serine (usually) or threonine (Figure 2). This core GalNAc is usually further extended with galactose (Gal) in the β1,3 configuration, and one or two sialic acid units (N-acetylneuraminic acid, NeuNAc) in an α2,6 and/or α2,3 configuration. Thus, each O-glycan may be in one of four different forms, A through D, illustrated in
Figure 2. When the carbohydrate moiety B is associated with membrane proteins it is known as the Thomsen-Friedenrich antigen (T antigen). The IgA2 gene has a deletion in this region, so IgA2 has no hinge region and does not carry O-linked sugars.

An IgA1 monomer, consisting of two $\alpha_1$ heavy chains, each with up to nine potential O-glycosylation sites (although they may not all be occupied), therefore carries multiple closely adjacent O-linked sugars in the hinge region, providing a tight clustering of NeuNAc, Gal and GalNAc residues, variations in which, may exert significant effects on the overall physicochemical property of the IgA1 molecule [3]. Normal serum consists of a mixture of IgA1 O-glycoforms due to the heterogeneity afforded both by varied site occupancy and by occurrence of a mixture of GalNAc-Gal-NeuNAc structures at each of the O-glycosylation sites (Figure 2).

Control of IgA1 O-glycosylation

IgA1 O-glycosylation takes place in the Golgi apparatus and is effected by the sequential actions of highly specific glycosyltransferases, which have only recently begun to be recognised and understood. The initial event is the addition of GalNac to threonine or serine in the protein backbone, mediated by a UDP-N-acetyl-$\alpha$-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase (pp-GalNAc-T). A family of these enzymes has been described, of which only one, named pp-GalNAc-T2, appears to have significant function in human IgA1 B cells [10]. The galactosylation of the core 1 structure is then completed by core 1 $\beta_1$-3 galactosyltransferase (C1Gal-T1) [11, 12]. Core 1 $\beta_1$,3-galactosyltransferase 1 is found in human IgA1 B cells and transfers Gal from UDP-Gal to GalNAc via a $\beta_1$,3-linkage. Its function is dependent on the presence of a molecular chaperone Cosmc, which is involved in the folding and stability of C1Gal-T1 [13]. Without Cosmc
translated C1Gal-T1 is lost and there is deficiency of Gal on glycoprotein acceptors. Indeed, mutation in the Cosmc gene has recently been shown to result in the Tn polyagglutinability syndrome, a rare autoimmune haematological disorder characterised by the presence of an incompletely glycosylated (agalactosyl) Thomsen-Friedenrich antigen (the Tn antigen) on the cell surface in subpopulations of blood cells [14]. Finally, O-sialylation may occur, catalysed by members of the sialyltransferase family [15].

Very little is known about the factors controlling IgA1 O-glycosylation. Mass spectroscopic analysis of hinge region glycopeptides has shown that in each individual serum sample, between ten and twenty differently glycosylated peptides are represented in significant proportions [9, 16]. This array of O-glycoforms suggests IgA1 plasma cells are capable of producing antibodies with vastly different O-glycan profiles. Whether different glycoforms are better suited to particular immune functions at particular immune sites is not clear at present. Interestingly, in vitro studies in animal systems have demonstrated that IgA glycosylation can be influenced by locally synthesised cytokines, in particular the Th2 cytokines although this work still needs to be reproduced in humans [17, 18]. Furthermore, there is evidence that secretory IgA has quite different N-linked and O-linked glycosylation profiles to serum IgA suggesting mucosal IgA synthesis may at least in some way be associated with site-specific IgA glycosylation [19, 20].

IgA1 O-glycosylation in IgAN

A number of experimental approaches to the study of IgA1 O-glycosylation in IgAN have been used: lectin binding to the O-glycans in situ on IgA1 [21-26]; mass spectroscopy of the isolated IgA1 hinge region complete with its O-glycans [16, 27-
and chromatographic separation of the released O-glycans, both in their intact form and as free monosaccharides [25, 32-34] (Figure 3). All of these techniques have shown abnormal IgA1 O-glycoform patterns in IgAN, but as yet none has been able to precisely elucidate the nature of the abnormality. The basic principles, strengths and weaknesses of each of these methodological approaches have been reviewed previously [35, 36].

The principle O-glycosylation abnormality found with all of these techniques in IgAN is reduced galactosylation of the IgA1 hinge region O-glycans, leading to increased frequency of truncated O-glycans (Figure 2, A). Altered sialylation of the IgA1 O-glycans in IgAN is more contentious with increased and decreased O-sialylation being reported [28, 31-33, 37-42].

IgA1 O-glycan galactosylation in IgAN

Early reports of defective galactosylation of IgA1 O-glycans were confined to studies of serum IgA [16, 21, 22, 25, 32], but two studies of IgA1 eluted from isolated glomeruli have now identified the same O-galactosylation abnormalities in mesangial IgA1, strongly implicating altered IgA1 galactosylation in the mechanism of IgA deposition [31, 43]. A similar abnormality in galactosylation has been demonstrated for IgA1 produced in vitro by tonsillar lymphocytes suggesting that the tonsils may contribute to the circulating pool of undergalactosylated IgA1 in IgAN [27, 44]. The presence of aberrantly galactosylated IgA-containing immune complexes [IgA-IC] has also been reported in the urine of patients with IgAN but not in patients with non-IgAN proteinuric glomerular disease [45].

Understanding the origin of this defect in IgAN is clearly integral to our overall understanding of the pathogenesis of IgAN and has raised a number of important questions which we and others have tried to answer over the past decade.
What are the functional consequences of defective IgA1 O-galactosylation?

A number of different lines of experimental evidence support the importance of IgA1 undergalactosylation in the pathogenesis of IgAN.

IgA-immune complex formation and glomerular deposition of IgA1: Aberrantly galactosylated IgA1 molecules have an increased tendency both to self-aggregate [46-51] and form antigen-antibody complexes with IgG antibodies directed against IgA1 hinge epitopes [26, 52-55], favouring the generation of IgA-IC [56]. It has long been recognised in animal models of glomerulonephritis that macromolecular Ig-containing complexes are particularly prone to mesangial trapping and the formation of circulating IgA-IC may therefore promote mesangial IgA deposition. It has been shown in animal models of IgAN that IgA-IC formation, glomerular IgA deposition and initiation of glomerular inflammation are influenced by the composition of the IgA1 hinge region and the degree of IgA glycosylation [57-59]. In addition, IgA1 molecules lacking terminal Gal have increased in vitro affinity for the extracellular matrix components fibronectin and type IV collagen and this may synergistically promote the mesangial trapping and glomerular accumulation of circulating IgA-IC [23, 48, 60].

Mesangial cell activation and initiation of glomerular injury: It has been established for some time that mesangial cells (MC) preferentially bind IgA-IC in vitro and that this binding results in the MC adopting a pro-inflammatory phenotype [61-65]. There is now increasing evidence for enhanced binding of IgA-IC containing undergalactosylated IgA1 to human MC [66-69], including specific binding to CD71, the mesangial transferrin receptor [70]. Binding of undergalactosylated IgA-IC is associated with increased MC proliferation and apoptosis [38, 71]; reduced synthesis
of VEGF (vascular endothelial growth factor) [72]; altered integrin expression [73] and increased synthesis of extracellular matrix components [66].

**Complement activation:** Glomerular complement activation is believed to enhance the inflammatory cascade and potentiate glomerular injury in IgAN. IgA-IC and mesangial IgA1 can activate complement both via the alternative pathway and the mannan binding lectin pathway [74, 75]. Furthermore, the degree of IgA1 glycosylation and the molecular weight of IgA complexes (dimeric IgA, pIgA and IgA-IC) has been shown to be important in the ability of IgA1 to activate complement [74-76].

Consistent with these laboratory findings clinical studies have shown an increase in circulating IgA-IC in IgAN [56, 77] and that undergalactosylated IgA1 is almost exclusively found complexed in IgA-IC and is mostly associated with J chain containing pIgA [52, 65, 78]. Furthermore, the extent of the IgA1 glycosylation defect may correlate with the degree of glomerular injury at the time of renal biopsy [41, 79, 80].

**Are there similar defects in N-glycosylation of IgA1 in IgAN?**

There have been far fewer studies examining IgA N-linked glycosylation in IgAN, however, those reported have failed to show any consistent defect in N-linked glycosylation of either IgA1 or IgA2 in IgAN [21, 38, 81]. A recent report has demonstrated significant differences in the composition of N-linked sugars on mIgA and pIgA but these were not altered in IgAN [65]. Changes in IgA N-linked glycosylation have however been shown to result in increased levels of IgA-IC and mesangial IgA deposition in a transgenic mouse lacking the N-glycosylating enzyme β 1,4-galactosyltransferase suggesting N-linked sugars may play a role in IgAN, although this work needs to be confirmed in humans [82].
Is defective O-galactosylation common to all serum proteins in IgAN?

Of the few serum proteins which are O-glycosylated, two have been studied in detail: C1 inhibitor (C1inh) and IgD. In both instances there was no alteration of the O-glycosylation profile of either C1inh or IgD in IgAN although both proteins were differentially O-glycosylated when compared to IgA1 [21, 83]. These observations argue against there being a generic defect of O-glycosylation of serum proteins in IgAN, and suggests that the defect is either restricted to specific B cell populations, or arises from abnormal control of IgA1 synthesis and secretion.

Is defective O-galactosylation common to all B lymphocyte subsets in IgAN?

The only O-glycosylated human immunoglobulin isotype other than IgA1 is IgD [84], which like IgA1, has a hinge region, and although the amino acid sequences of the two isotypes differ they both carry approximately five O-glycan moieties per heavy chain [85, 86]. IgD mainly exists as a membrane-bound immunoglobulin, acting as the antigen receptor of mature but naïve circulating B cells before class-switching [87]. During primary immune responses, some IgD-secreting plasma cells are generated and these are responsible for the small quantities of IgD found in serum [88]. However, unlike IgA-producing B cells, IgD cells are not found among the memory B cell population characterised by extensive somatic hypermutation and affinity maturation [89].

We have recently reported that in healthy subjects IgD is more heavily galactosylated but less sialylated than IgA1 suggesting galactosylation is downregulated in the more mature IgA1-secreting cells [83]. Alternatively, it is possible that the rate of IgA1 synthesis exceeds the galactosylating capacity of the plasma cells, and it is intriguing to speculate that the availability of the chaperone protein Cosmc may be a factor in this. In contrast, IgA1 is more sialylated than IgD, indicating upregulation of
sialyltransferases with class switching. The pattern of IgD O-glycosylation is identical in patients with IgAN implying that the abnormality of IgA1 O-glycosylation in IgAN is not shared by IgD, and is therefore not due to defective expression or function of glycosylating enzymes affecting the entire B cell lineage. It appears immunoglobulin O-glycosylation is differentially controlled at different stages of B cell development, and suggests that the alterations seen in IgAN only occur after class switching to IgA synthesis and may be secondary to aberrant immunological control mechanisms.

**Is defective O-galactosylation due to changes in the amino acid sequence of the IgA1 hinge region?**

Changes in the sequence of the α1 heavy chain hinge region, in particular loss or substitution of serine and/or threonine residues, could account for the observed changes in IgA1 O-glycosylation in IgAN as there would be an associated loss of potential O-glycosylation sites (Figure 1). There is however no evidence for this; genetic sequence analysis of the IgA1 hinge region and precise measurement of deglycosylated IgA1 hinge region peptides by mass spectrometry are normal in IgAN [16, 90].

**Is defective O-galactosylation common to all IgA1 committed B lymphocytes in IgAN?**

Common to all studies examining IgA1 O-glycosylation in IgAN is the heterogeneity of IgA1 O-glycoforms in the serum at any one time suggesting that any defect in B cell O-glycosylation is not uniformly expressed in all IgA1-committed B lineage cells [9, 16]. How then is it possible to identify those IgA1-committed B lineage cells responsible for secreting undergalactosylated IgA1 in IgAN?

In common with the O-glycosylation defect of IgA1 there are a number of other features characteristic of the IgA immune response in IgAN. These include persistent
systemic overproduction of pIgA1 against a variety of systemic and mucosal antigens [91-94], failure of affinity maturation of serum IgA1 antibodies [95] and systemic IgA1 immune responses that tend to be exaggerated and prolonged [96]. Some of these features are also characteristic of a mucosal IgA immune response where the IgA secreted is predominantly polymeric, of low affinity and can be differentially O-glycosylated [19, 20, 97]. Furthermore, studies of serum IgA-IC have shown that undergalactosylated IgA1 is predominantly associated with J chain containing pIgA [52]. While we know that mucosal pIgA1 plasma cell numbers [98, 99] and mucosally-secreted IgA antibody responses [100, 101] are reduced in IgAN it is becoming evident that the site of antigen encounter heavily influences the phenotype of both T and B lymphocytes both through their subsequent expression of cell surface homing receptors and ultimately their functional capabilities [102, 103]. It is possible therefore that the pattern of IgA1 O-glycosylation is programmed at the time of antigen encounter, along with the production of J chain and synchronous with affinity maturation.

We have therefore studied the pattern of O-glycosylation of IgA1 generated following an antigen encounter within the systemic compartment (tetanus toxoid, TT) and IgA1 generated following encounter with an antigen at the mucosal surface (Helicobacter pylori, Hp) [20]. We found the characteristic increase in undergalactosylated IgA1 in total serum IgA1 in IgAN, however the galactosylation of the antigen-specific IgA1 antibodies to TT or to Hp did not differ from controls. This suggests that the O-glycosylation of antigen-specific IgA1 antibodies is normal in IgAN. However, we found that in both IgAN and controls, the galactosylation of IgA1 anti-Hp was strikingly and significantly reduced, while that of IgA1 anti-TT was normal,
indicating that antibodies of different specificities have markedly different O-glycosylation profiles. This observation indicates that the production of the alternative IgA1 O-glycoforms is differentially controlled in different immune responses. The undergalactosylated glycoforms of IgA1 may in fact not represent “defective” O-glycosylation at all but be part of the expected spectrum of IgA1 O-glycoforms generated after antigen encounter at mucosal surfaces. Consistent with a mucosally programmed response these antibodies are also likely to be polymeric and of low affinity-features characteristic of the pathogenic serum IgA1 fraction [3]. Their overexpression in the serum of patients with IgAN may therefore simply reflect the displacement of mucosally activated IgA1-committed B cells from the mucosal to systemic compartment [98, 99, 104, 105]. Clearly much more work needs to be undertaken to evaluate this hypothesis but there is evidence emerging of altered homing of B and T lymphocyte subsets in IgAN [106, 107].

**Is there evidence for changes in the activity of C1Gal-T1 or Cosmc in IgAN?**

It may be predicted that the generation of undergalactosylated IgA1 can be the result of a functional change in C1Gal-T1 activity either through direct modulation of C1Gal-T1 or its molecular chaperone Cosmc. Few studies have been performed that have directly assessed the function of these proteins in IgAN. Those that are reported have studied unfractionated B cells which may not reveal changes in enzyme activity restricted to specific B cell subsets. We reported some time ago evidence for a functional change in C1Gal-T1 in peripheral blood B cells [108], however, we have since found no overt alteration in the activity of this enzyme in bone marrow B cells in IgAN [109]. Functional studies have been hampered for some years by lack of information on the molecular genetics of glycosyltransferases, however, such work is now possible as C1Gal-T1 and Cosmc, have now been cloned [11, 13].
Are genetic studies going to help in identifying the basis for IgA1 undergalactosylation?

With the genetic characterisation of C1Gal-T1 and its molecular chaperone Cosmc it has become possible to study changes in the O-glycosylation of IgA1 at a genetic level. The C1Gal-T1 gene lies on chromosome 7p14-p13, with a 1794bp cDNA encoding a protein of 363 amino acids [11] and the Cosmc gene lies on chromosome Xq23, with a 1471bp cDNA sequence encoding a protein of 318 amino acids [13]. The predominance of IgAN in males in certain populations and the fact that the Cosmc gene lies on the X chromosome has further increased interest in the possibility of a genetic susceptibility to IgAN, although neither gene lies within the previously identified IGAN1 locus on chromosome 6q22-23[110].

A recent case-control association study looking at single-nucleotide polymorphisms (SNPs) in C1Gal-T1 has reported that polymorphisms of the C1Gal-T1 gene are associated with a genetic susceptibility to IgAN in a Chinese population [111]. How these polymorphisms relate to changes in the functional activity of C1Gal-T1 is at present uncertain. There has also been a recent report of a severe but partial loss-of-function mutation in the mouse C1Gal-T1 gene which results in the development of kidney disease but not IgAN [112]. The lack of an IgAN phenotype is perhaps not surprising as mouse IgA lacks a hinge region and therefore also O-linked glycosylation.

What is clear from the available evidence is that patients with IgAN can produce IgD and IgA1 which is normally galactosylated; specifically those IgA1 antibodies directed against systemic antigens[20]. Also, patients with IgAN do not develop the Tn polyagglutinability syndrome and therefore it is unlikely that there is a common, functionally significant, somatic mutation in genes encoding for the O-galactosylation.
of all proteins, and in particular IgA1, in IgAN. Somatic mutations in the gene for Cosmc have been described that cause a functional abnormality of C1Gal-T1 and lead to the Tn polyagglutinability syndrome and generation of a tumour-specific glycopeptidic neoantigen [14, 113]. The effect of this mutation on IgA1 O-glycosylation and the development of IgAN have not yet been fully elucidated.

Likewise, studies examining the expression of mRNA for the different glycosyltransferases are now being reported. One study showed reduced mRNA levels of Cosmc in peripheral blood B cells in IgAN while C1Gal-T1 mRNA levels were unaltered [114]. Furthermore, this reduction in Cosmc mRNA correlated with the level of undergalactosylated IgA1 in the serum at the time of sampling. How these changes in Cosmc mRNA levels related to functional activity of C1Gal-T1 was not assessed. We would argue that studying peripheral blood B cells or even IgA1-committed B cells, most of which will be inactive memory B cells not synthesising IgA1, will not necessarily reveal the subtle changes that occur in IgA1 O-glycosylation in IgAN. Investigators have to take into account that any changes in C1Gal-T1 activity and/or Cosmc levels may be restricted to specific IgA1-committed B cell lineage subsets which will almost certainly be resident in systemic sites such as the bone marrow. Ideally the cells that need to be studied are those actually synthesising IgA1- the plasmablasts and plasma cells, however, these cells remain difficult to isolate and are only found in the peripheral circulation in very small numbers. It is important that only a small fraction of serum IgA is undergalactosylated, therefore, only a small proportion of IgA1-committed B cells are synthesising this glycoform of IgA1, and patients with IgAN can still galactosylate IgA1 perfectly normally in specific circumstances. Mass sampling of the entire peripheral B cell lineage may therefore miss the “pathogenic” B cell subsets secreting
undergalactosylated IgA1 as the majority of B cells are likely to be synthesising IgA1 in an appropriate manner under normal immunological control mechanisms.

**IGA1 O-glycan sialylation in IgAN**

Sialylation is a common and functionally important post-translational modification of many glycoproteins[15]. Sialic acid is a large, hydrophilic, negatively charged sugar which is principally found at the terminal ends of O-linked and N-linked oligosaccharide chains. Sialylation plays a significant role in both maintaining the conformational stability of glycoproteins and systemic clearance of circulating glycoproteins through interaction with the hepatic asialoglycoprotein receptor (ASGP-R)[115]. The significance of sialylation of the O-linked sugars of IgA1 in IgAN is not clear. Increased α2,3-linked [40, 81] and α2,6-linked [38, 40] sialylation of serum IgA1 have been reported in IgAN. It has been suggested that increased sialylation of IgA1 in IgAN inhibits interaction with the ASGP-R resulting in a reduced rate of systemic clearance of IgA-IC and persistence of these IgA-IC in the circulation [37, 40, 116]. IgA-IC with a high sialic acid content have been shown to bind MC more strongly [117], differentially upregulate MC integrins and nitric oxide synthesis [38, 73], and induce apoptosis and suppress MC proliferation [38]. Furthermore, it has been postulated that sialylated IgA1 is more likely to bind extracellular matrix components because of its net negative charge [48].

In contrast, a number of studies have reported reduced sialylation of serum IgA1 in IgAN [28, 31, 33, 39, 41, 42, 49, 79, 118] and tonsillar IgA1 [44]. The significance of reduced IgA1 sialylation is equally contentious, with studies reporting that reduced sialylation plays a variable role in IgA1 self-aggregation and IgA-IC formation [46, 48, 50], MC proliferation and activation [4, 38, 73] and histopathological phenotype in IgAN [41, 49, 79].
Crucial to the interpretation of all these studies is the reliability and specificity of the lectins used to analyse the $\alpha_2,3$-linked and $\alpha_2,6$-linked sialic acid content of IgA1 [119]. Current mass spectrometry data supports IgA1 being undersialylated in IgAN, whether this relates to the $\alpha_2,3$- or $\alpha_2,6$-linked sialic acid or both is unknown [16, 28, 31].

In parallel with studies examining IgA1 $O$-galactosylation patients with IgAN are able to normally sialylate IgD suggesting there is not a generic change in sialylation within B cells. Sialylation of IgA1 is known to vary between mucosal and systemic sites and changes in sialylation appear to be most pronounced in IgA1 contained within IgA-IC [20, 79]. Therefore it may be that the pattern of IgA1 sialylation is also programmed following antigen encounter and that it may be linked with galactosylation. This requires investigation.

**Conclusions**

A change in the $O$-glycosylation profile of serum IgA1 in IgAN is undoubtedly of integral importance to the development of IgAN. It is now becoming apparent that changes in the extent of IgA1 galactosylation and sialylation are closely regulated and dependent on the circumstances of antigen encounter. We propose that B cells encountering antigen at mucosal sites are programmed to synthesise preferentially IgA1 glycoforms which are undergalactosylated, and probably also undersialylated. These glycoforms exist predominantly as J chain containing pIgA1 and are destined for mucosal secretion where perhaps they offer some functional advantage over alternative glycoforms. The well described displacement of IgA secreting plasma cells from mucosal to systemic sites in IgAN may reflect a mis-homing of mucosally-primed B lineage cells to sites such as the bone marrow. These displaced cells have no
option but to secrete their “mucosal” pIgA1 glycoforms into the systemic circulation. It is this IgA1 that has the propensity for the mesangial deposition and triggering of glomerular injury characteristic of IgAN. Much more work is needed to understand these processes, which should allow precise definition of the origin of the O-glycosylation changes that drive the development of IgAN.
References


acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase


25. Mestecky J, Tomana M, Crowley-Nowick PA, Moldoveanu Z, Julian BA, Jackson S: Defective galactosylation and clearance of IgA1 molecules as a
possible etiopathogenic factor in IgA nephropathy. *Contributions to Nephrology* 104:172-182, 1993


subclass bound preferentially to asialo-, agalactoimmunoglobulin

Kokubo T, Sano T, Kobayashi Y: Detection of gender difference and epitope
specificity of IgG antibody activity against IgA1 hinge portion in IgA
nephropathy patients by using synthetic hinge peptide and glycopeptide
probes. Nephrology (Carlton) 9:26-30, 2004

Kobayashi Y: Humoral immunity against the proline-rich peptide epitope of
the IgA1 hinge region in IgA nephropathy. Nephrol Dial Transplant 15:28-33,
2000

56. van der Boog PJ, van Kooten C, de Fijter JW, Daha MR: Role of
macromolecular IgA in IgA nephropathy. Kidney Int 67:813-821, 2005

57. Sano T, Hiki Y, Kokubo T, Iwase H, Shigematsu H, Kobayashi Y:
Enzymatically deglycosylated human IgA1 molecules accumulate and induce
inflammatory cell reaction in rat glomeruli. Nephrol Dial Transplant 17:50-
56, 2002

58. Oida E, Nogaki F, Kobayashi I, Kamata T, Ono T, Miyawaki S, Serikawa T,
Yoshida H, Kita T, Muso E: Quantitative trait loci (QTL) analysis reveals a
close linkage between the hinge region and trimeric IgA dominancy in a high
IgA strain (HIGA) of ddY mice. Eur J Immunol 34:2200-2208, 2004

Kobayashi Y: Underglycosylation of IgA1 hinge plays a certain role for its
glomerular deposition in IgA nephropathy. J Am Soc Nephrol 10:760-769,
1999


with tetanus toxoid in IgA nephropathy. *Clinical & Experimental Immunology* 88:394-398, 1992


Legends to figures

Figure 1
The IgA1 molecule showing the position of the hinge region O-glycans. The hinge region of IgA1 lies between the CH1 and CH2 domains of the α1 heavy chain. The hinge region is made up of 17 amino acids and the amino acid sequence is displayed to the right of the figure. Serine and threonine residues provide nine potential O-linked glycosylation sites [arrows]; although to date only six are known to be occupied by O-glycans. It is still not known which amino acids are occupied by O-glycans and whether it is the same amino acids for all O-glycoforms of IgA1.

Figure 2
The four major O-glycan forms of human IgA1. The IgA1 O-glycans are all based on a core 1 structure with N-acetylgalactosamine (GalNAc) units in O-linkage with serine or threonine. This may occur alone (form A) or may be extended with β1,3-linked galactose (Gal) (form B). Further extension with sialic acid (NeuNAc) in α2,3-linkage with Gal can then occur (form C), and finally, addition of sialic acid in α2,6-linkage with GalNAc makes form D.

Figure 3
Three approaches to IgA1 O-glycosylation analysis.
A: Lectin binding studies to the O-glycans in situ on the whole IgA1 molecule.
B: Analysis of the free O-glycans following release from IgA1.
C: Mass profiling of isolated hinge region glycopeptides by mass spectrometry.
HPLC, high performance liquid chromatography; FACE, fluorophore-assisted carbohydrate electrophoresis; SDS-PAGE, sodium dodecylsulphate polyacrylamide
gel electrophoresis; MALDI TOF MS, matrix-assisted laser desorption ionisation time of flight mass spectrometry; SELDI TOF MS, surface enhanced laser desorption ionisation TOF MS; FT-ICR MS, fourier transform-ion cyclotron resonance MS.
Figure 1
Figure 2

A  Ser/Thr -O- GalNac

B  Ser/Thr -O- GalNac -β1,3- Gal

C  Ser/Thr -O- GalNac -β1,3- Gal
     α2,3
     NeuNAc

D  Ser/Thr -O- GalNac -β1,3- Gal
     α2,6
     NeuNAc
     α2,3
     NeuNAc
Method involves:

- Enzymatic digestion of IgA1
- Isolation of hinge region peptides (HPLC, jacalin affinity chromatography)
- Treatment of glycopeptides with exoglycosidases
- MS of desialylated, degalactosylated and deglycosylated peptides (MALDI-TOF MS, SELDI-TOF MS, FT-ICR MS)

Lectin binding ELISA:

- Jacalin recognizes Gal-β1,3-GalNAc*
- Peanut agglutinin (PNA) recognizes Gal-β1,3-GalNAc*
- Vicia villosa (VV) recognizes GalNAc
- Helix aspersa (HA) recognizes GalNAc
- Elderberry bark lectin (SNA) recognizes α2,6 NeuNAc
- Maackia amurensis agglutinin (MAA) recognizes α2,3 NeuNAc

*Jacalin binding unaffected by glycan sialylation
PNA binding strongly inhibited by glycan sialylation

Method involves:

- O-glycan release (chemically or enzymatically)
- Separation of released O-glycans (HPLC or SDS-PAGE)
- Measurement of separated O-glycans (FACE or spectrophotometry)