# Immune regulation in IgA nephropathy

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# Chapter 7

## IgA1 glycosylation in IgA Nephropathy, as sweet as it can be

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The pathogenesis of IgA nephropathy (IgAN) is still not clear, but it is now well accepted that an aberrant glycosylation pattern of IgA is involved. This is supported by the fact that in IgAN mesangial deposits of IgA contain high concentrations of abnormally O-glycosylated IgA1, characterized by undergalactosylation. In the current issue Buck et al. [1] investigated whether this aberrant glycosylation pattern of IgA in IgAN patients is due to diminished activity or reduced gene expression of specific glycosyltransferases. They investigated the activity of the enzyme β-galactosyltransferase (C1Gal-T1) and its molecular chaperone (Cosmc) in purified B cells isolated from peripheral blood (PB) and bone marrow (BM) of IgAN patients and controls. They also measured gene expression in PB and BM cells and related this to the enzyme GalNAc-T2, which synthesizes the core O-glycan. Using this approach, no differences in O-galactosylation activity between IgAN patients and controls could be detected.

IgAN is a heterogeneous disease with a wide range in clinical presentations, varying from a-symptomatic microscopic hematuria to gross macroscopic hematuria and can have a variable clinical outcome ranging from normal glomerular filtration rate to end stage renal disease in up to 30% of cases. The histological hallmark of the disease is the presence of mesangial deposition of polymeric IgA1, in combination with complement factor C3 and often IgG or IgM. In IgAN patients the serum concentration of IgA is frequently increased compared with that in controls, but in addition IgA present in the circulation was shown to be abnormally O-glycosylated. Importantly, this undergalactosylated form of the IgA1 hinge region was also over-represented in biopsies of patients with IgAN [2]. Therefore, it is likely that this abnormally glycosylated IgA is involved in the pathogenesis of IgAN. The reduced galactosylation leads to increased exposure of the internal GalNAc (Tn antigen) that can be detected with specific lectins. Recently it has been suggested to use this specific characteristic for the development of an ELISA-based diagnostic test in IgAN [3]. This abnormal glycosylated IgA has a higher tendency to self-aggregate and form complexes with IgG antibodies directed against epitopes in the hinge region of IgA1. Besides, in patients with

Henoch-Schönlein purpura, a disease closely related to IgAN with a similar renal histological pattern, only those patients who had abnormal glycosylated IgA had renal involvement, whereas patients with normal glycosylated IgA did not have renal involvement [4].

Despite the importance of aberrantly glycosylated IgA in IgAN, the molecular mechanisms underlying these differences are not known. With the identification and characterization of the enzyme system responsible for O-linked glycosylation, including C1Gal-T1 and its molecular chaperone Cosmc, novel avenues of research have opened up. However, an important and intriguing observation is the fact that aberrant O-glycosylation seems to be absent in other serum proteins, whereas even for IgA it is generally accepted that only a small proportion of the IgA molecules show these alterations (Figure 1). For instance, GalNac specific lectins specifically react with high molecular weight fractions of serum IgA, which only constitute 10% of circulating IgA [5].

Since IgA1 glycosylation is determined within B lymphocytes, and only a fraction of the IgA1 seems to be affected, this suggest that alterations in glycosylation are only present in a subset of B cells. In humans, besides IgA the only other immunoglobulin that is O-glycosylated is IgD. Smith et al. have studied the glycosylation patterns of IgA1 and IgD in IgAN patients and in controls and observed that the abnormal underglycosylated pattern of IgA1 was not present in IgD [6]. As IgD is a marker of naïve B cells, this might suggest that deficiencies are restricted to certain differentiation stages of B cells. In the study of Buck et al. [1], enzyme activities were determined in purified B cells isolated by positive selection using anti-CD19 coated magnetic beads. As plasma cells are CD19 negative, this analysis might exclude the contribution of plasma cells. Although the number of plasma cells in the circulation will be low, in BM their number is expected to be higher. It is thought that IgA present in the circulation is produced mainly by plasma cells derived from the BM, suggesting that the absence of plasma cells in the analysis could be of importance.

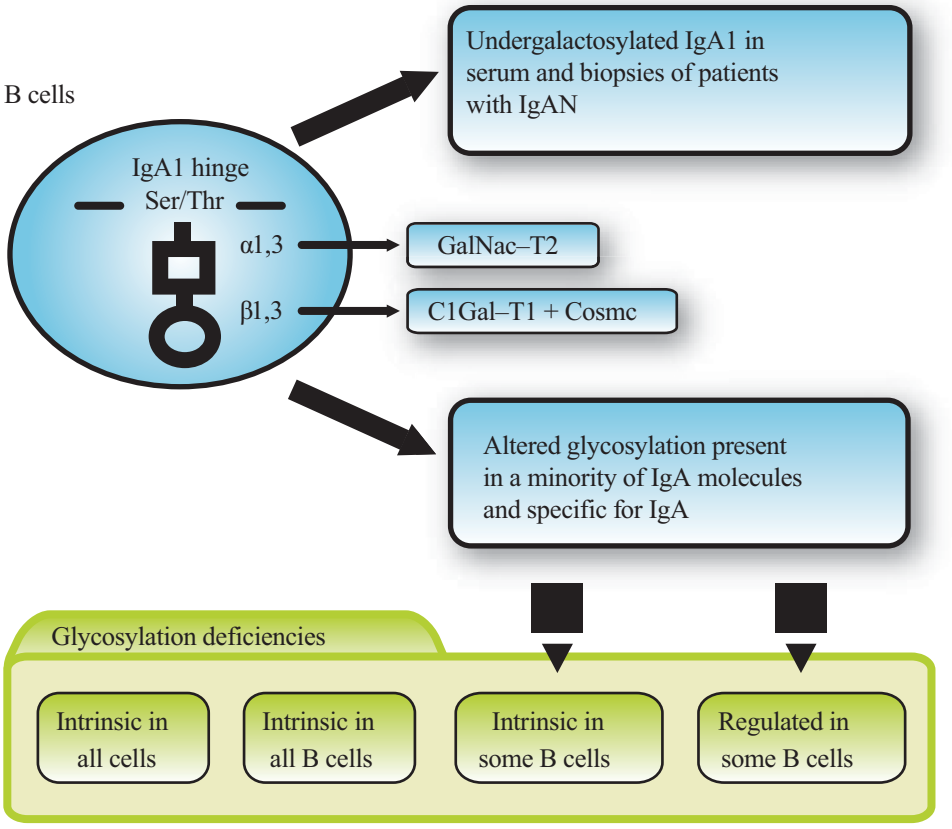


**Figure 1. Schematic view on potential mechanisms leading to aberrantly glycosylated IgA.** Undergalactosylated IgA1 is present in serum and biopsies of patients with IgAN. This glycosylation is controlled during the synthesis of IgA in B cells by the glycosyltranferases C1Gal-T1 and its molecular chaperone Cosmc and GalNAc-T2. As other proteins seem not to be affected and only some of the B cells in IgAN produce undergalactosylated IgA, generalized intrinsic defects in B cells or other cells can be excluded. Therefore, it is postulated that either the glycosylation is disturbed intrinsically in some B cells, or regulatory processes affect only some B cells during an ongoing immune response. In the latter case, environmental or cellular signals involved in B cell activation and IgA production might be involved.

The fact that the proposed deficiencies are only present in a subset of B cells (Figure 1), might suggest that genetic factors are not likely to be involved. Nevertheless, it was recently shown that polymorphisms in the gene for C1Gal-T1 were associated with susceptibility for the development of IgAN in a Chinese population [7]. A disease in which aberrant glycosylation patterns are present due to genetically determined factors is the so called Tn syndrome. This is a rare autoimmune disorder in which a somatic mutation in the Cosmc gene leads to incompletely glycosylated proteins in subpopulations of all cell lineages [8]. It is unclear whether such a mosaic pattern can contribute to the aberrant glycosylation in IgAN and so far no correlation between IgAN and the Tn syndrome has been described.

IgA present in the circulation is derived not only from the BM, but also from mucosal tissues. Interestingly, most IgA produced at the mucosa will be dimeric in nature, and can either be transported over epithelial barriers to generate secretory IgA (SIgA), or contributes to the high-MW IgA fraction in the circulation. Therefore, both high-MW IgA and SIgA, which can also be present in the circulation and in renal deposits [9], might represent a recent mucosal challenge. Buck et al. [1] investigated the activity of C1Gal-T1 and Cosmc and GalNAc-T2 in PB and BM samples taken from IgAN persons who had no macroscopic hematuria or intercurrent illness at the time of sampling. This suggests that no active immune reaction was present at the time of investigation, which might have masked a difference in glycosylation activity.

In a recent vaccination study, a direct comparison of glycosylation has been made of IgA directed against a mucosal antigen, *Helicobacter pylori* (HP), and against a systemic adjusted antigen, tetanus toxoid (TT) [10]. Both in IgAN patients and in controls the IgA1 against HP showed higher lectin binding, reflecting more undergalactosylated IgA than the IgA against TT. This strongly suggests that an aberrant glycosylation pattern in IgAN could not only be the result of an intrinsic defect in glycosylation mechanisms, but also might reflect differences in regulation of glycosylation (Figure 1). As immunoglobulin production is the result of a complex process, involving B cells, T cells, antigen presenting cells and local and systemic factors like

cytokines, each of these factors can potentially influence the glycosylation profile. Dendritic cells (DC) are professional antigen presenting cells which have direct effects on B cells, and under the influence of the mucosal environment can promote the generation of IgA producing cells [11]. We have shown a reduced capacity of DC of IgAN patients to induce IgA switch [12]. Whether the abnormal DC function also affects the glycosylation pattern in IgAN patients is so far unknown.

In conclusion it is clear that aberrantly glycosylated IgA plays an important role in the pathogenesis of IgAN. Glycosylation is a complex process, influenced by many factors, that can either be intrinsic or can differ in time and change due to locally determined circumstances. The study of Buck et al. [1] shows that overall there is no difference between IgAN patients and controls in C1Gal-T1 and Cosmc activity nor in gene expression, related to GalNAc-T2. Although this study's results might appear to be negative, it clearly brings IgAN research at a next level. It is conceivable that more detailed molecular analysis will be required to unravel the mechanisms contributing to undergalactosylation of IgA1. This should probably include developments in the direction of single cell analysis of IgA producing B cells or longitudinal analysis of actively developing IgA immune responses upon mucosal or systemic challenges.

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