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Inherited IgA glycosylation pattern in IgA nephropathy and HSP nephritis: where do we go next?

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New data from Kiryluk *et al.* show the importance of genetic factors in determining the profile of serum IgA1 *O*-glycoforms in IgA nephropathy and Henoch–Schönlein purpura nephritis. Elevated serum levels of poorly galactosylated IgA1 *O*-glycoforms do not, however, appear sufficient in themselves to cause nephritis in these two diseases, and a 'second hit' is necessary before changes in IgA1 glycosylation translate into clinical disease. The challenge now is to determine what these genetic factors are.

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IgA nephropathy (IgAN) and Henoch–Schönlein purpura (HSP) are both diseases characterized by deposition of circulating IgA1-containing immune complexes (IgA-ICs).[1] Unlike IgAN, in which IgA-IC deposition occurs solely within the glomerulus, HSP is a small-vessel systemic vasculitis characterized by IgA-IC deposition in affected blood vessels. Up to 40% of patients with HSP will develop a mesangial proliferative glomerulonephritis, HSP nephritis (HSPN), which is usually indistinguishable from IgAN. There is much indirect evidence to suggest a close relationship between IgAN and HSP. Monozygotic twins have been described, one of whom developed IgAN and the other HSP at the same time. HSP developing on a background of proven IgAN has been described in both adults and children. Many abnormalities of the IgA immune system, including changes in the profile of circulating IgA1 *O*-glycoforms, have been described in both IgAN and HSP. Significantly, an increase in the levels of poorly galactosylated IgA1 *O*-glycoforms appears to be specifically associated with development of nephritis in HSP.[2] The study by Kiryluk *et al.*[3] (this issue) confirms that children with IgAN and HSPN have raised levels of poorly galactosylated IgA1 *O*-glycoforms in their serum. Importantly, they also show that these children frequently have a parent with a similar serum IgA1 *O*-glycoform profile, highlighting the importance of genetic factors in determining the constitution of serum IgA1 *O*-glycoforms in IgAN and HSPN.

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ARE CHANGES IN IgA1 *O*-GLYCOSYLATION ALONE SUFFICIENT TO CAUSE DISEASE?

As Kiryluk *et al.*[3] discuss, an increase in the levels of poorly galactosylated IgA1 *O*-glycoforms appears insufficient in itself to cause IgAN or HSPN, as first-degree relatives with high serum levels of poorly galactosylated IgA1 *O*-glycoforms exhibited no signs of either IgAN or HSPN. These observations are similar to those reported in cohorts of white, Asian, and African-American families with IgAN.[4–6] These new data add weight to the suggestion that a 'second hit' is required before high levels of poorly galactosylated IgA1 translate into overt disease. Current evidence would suggest that this second hit is the formation of glycan-specific IgG (and IgA) antibodies that drive formation of large circulating immune complexes prone to deposition. Poorly galactosylated IgA1 *O*-glycoforms might act either as autoantigens driving the formation of glycan-specific antibodies in susceptible individuals, or as antigens for crossreactive anti-microbial antibodies.

WHAT ARE THE GENETIC FACTORS CONTROLLING IgA1 *O*-GLYCOSYLATION IN IgAN AND HSPN?

Not surprisingly, significant efforts have been made to try and establish the genetic basis for IgAN; however, little work has been undertaken in HSP. Four genome-wide scans (three in familial and one in sporadic IgAN) have identified susceptibility loci on chromosomes 6q22, 6p, 4q26–31, 17q12–22, and 2q36. None of these loci correspond to genes directly involved in IgA1 *O*-glycosylation, and therefore the genes involved in determining the composition of serum IgA1 *O*-glycoforms remain unknown. A number of small case-control association studies have looked at single-nucleotide polymorphisms in genes for specific glycosyltransferases involved in IgA1 *O*-glycosylation (Figure 1). Polymorphisms in the *C1GALT1* and *ST6GALNAC2* genes, encoding core 1 β1,3-galactosyltransferase (C1β3Gal-T) and α2,6-sialyltransferase, respectively, are associated with a genetic susceptibility to IgAN in a Chinese population. Genomic analyses in European and Chinese populations have identified no *COSMC* (core 1 β1-phosphate uridyltransferase (Gal-T)-specific molecular chaperone) gene variants in sporadic or familial IgAN. Cosmc is a molecular chaperone involved in the folding and stability of C1β3Gal-T. Without Cosmc, translated C1β3Gal-T is lost and there is deficiency of galactose on glycoprotein acceptors. The single-nucleotide polymorphism in the promoter region of ST6GALNAC2 that has been linked with development of IgAN has been shown *in vitro* to significantly reduce promoter activity

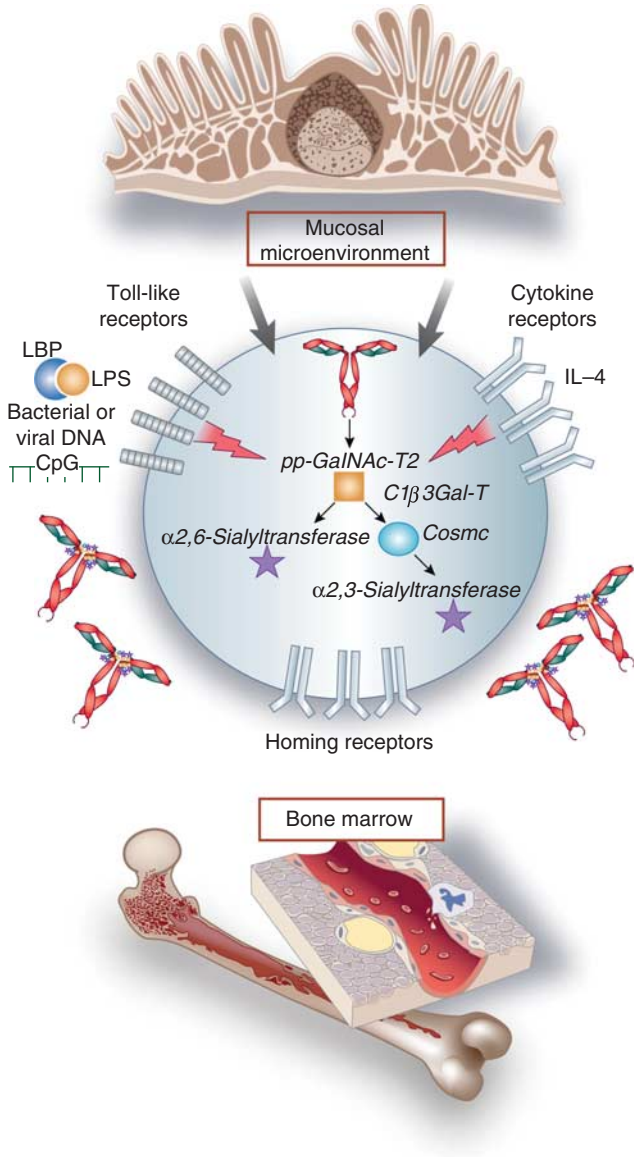

Figure 1 | Potential pathways responsible for determining the composition of serum IgA1 O-glycoforms in IgA nephropathy and Henoch–Schönlein purpura nephritis. The initial event in *O*-glycosylation of the IgA1 hinge region is the addition of *N*-acetylgalactosamine (GalNAc) to threonine or serine residues, which is under the control of UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase 2 (pp-GalNAc-T2). GalNAc may then be either sialylated in an α2,6-linkage or galactosylated via a β1,3-linkage. It has been proposed that sialylation of GalNAc prevents further extension of the core 1 structure with galactose, and that therefore the balance between the activity of α2,6-sialyltransferase and the activity of core 1 β1,3-galactosyltransferase (C1β3Gal-T) determines the synthesis of poorly galactosylated IgA1 *O*-glycoforms. The function of C1β3Gal-T is dependent on the presence of a molecular chaperone, Cosmc. α2,3-Sialyltransferase catalyzes the terminal sialylation of galactosylated GalNAc. Polymorphisms within the genes encoding these *O*-glycosyltransferases could result in production of poorly galactosylated IgA1; however, there is little convincing evidence to suggest this is the case in IgAN and HSPN. External factors, which are also likely to be subject to genetic variation, may influence glycosyltransferase activity and drive IgA1-committed plasma cells to synthesize poorly galactosylated IgA1. These include B-cell imprinting at the time of mucosal antigen encounter, cytokine expression within the local microenvironment, and Toll-like receptor activation by bacterial lipopolysaccharide (LPS) and bacterial DNA sequences with unmethylated cytosine–guanine dinucleotide (CpG) motifs. Alternatively, it is possible that plasma-cell glycosyltransferase activity is completely normal in IgAN and HSPN, but because of translocation of mucosally imprinted B cells to systemic sites due to faulty homing signals, these plasma cells release normal 'mucosal-type' polymeric poorly galactosylated IgA1 directly into the circulation rather than into the submucosa for transport across the mucosal epithelium and into mucosal secretions. IL-4, interleukin-4; LBP, LPS binding protein.

and α2,6-sialylation of IgA1 compared with the more common haplotype. Data from the United States would, however, suggest that reduced galactosylation of IgA1 in IgAN is due to enhanced α2,6-sialyltransferase activity, with sialylation of *N*-acetylgalactosamine (GalNAc) preventing the further addition of galactose. It could be that these reported differences in IgA1 α2,6-sialylation reflect different pathogenic processes operating in different races; alternatively, the impact of these polymorphisms on the functional activity of α2,6-sialyltransferase may vary in specific B-cell subsets *in vivo*.

Studies examining the expression of mRNA for the different glycosyltransferases have also been reported. One study showed reduced mRNA levels of Cosmc in peripheral blood B cells in IgAN, while C1β3Gal-T mRNA levels were unaltered. Furthermore, this reduction in Cosmc mRNA correlated with the level of poorly galactosylated IgA1 in the serum at the time of sampling. How these changes in Cosmc mRNA levels related to functional activity of C1β3Gal-T was not assessed. A separate study reported reduced sialylation of serum IgA1 and decreased expression of ST6GALNAC2 in peripheral blood B cells in Chinese patients.

DO FACTORS OTHER THAN THE GLYCOSYLTRANSFERASES DETERMINE IgA1 *O*-GLYCOSYLATION IN IgAN AND HSPN?

What is clear from the available evidence is that patients with IgAN can produce IgD and IgA1 that is highly galactosylated. It is also important to acknowledge that only a small fraction of serum IgA1 is poorly galactosylated; therefore, only a small proportion of IgA1-committed plasma cells are synthesizing these glycoforms of IgA1. It is therefore unlikely that there is an overarching 'defect' in immunoglobulin *O*-glycosylation in IgAN or HSP. We believe that it is more likely that factors outside the immediate *O*-glycosylation machinery of the plasma cell are responsible for the changes seen in the composition of serum IgA1 *O*-glycoforms in IgAN and HSPN and that this is where the genetic origins of IgAN and HSPN are to be found. A number of external factors have been shown to

modulate IgA synthesis and glycosyltransferase expression and could be subject to genetic variation (Figure 1). These include B-cell programming at the time of antigen encounter, cytokine expression within the local microenvironment, and Toll-like receptor (TLR) activation.

Mucosal imprinting of B cells and IgA1 *O*-glycosylation

Secretory IgA comprises a quite different spectrum of *N*- and *O*-glycoforms than serum IgA, suggesting that mucosal IgA synthesis may at least in some way be associated with site-specific IgA1 glycosylation. Consistent with this observation, we have shown that IgA1 *O*-glycoforms with specificity for mucosal antigens are poorly galactosylated compared with IgA1 *O*-glycoforms against systemic antigens, both in healthy subjects and in IgAN.[7] This suggests that during normal priming and maturation of mucosal B cells there is imprinting for a specific pattern of IgA1 *O*-glycosylation characterized by poor galactosylation of the IgA1 hinge region. External mucosal factors driving this process might include the local cytokine milieu and co-ligation of Toll-like receptors. We have hypothesized that the excessive amounts of poorly galactosylated IgA1 *O*-glycoforms in the circulation in IgAN result from mis-homing of normal mucosally primed plasma cells to systemic sites, with secretion of normal, but nephritogenic, 'mucosal-type' IgA1 into the circulation. If this is the case, then genetic variation in the expression of B-cell homing receptors or endothelial addressins could underlie the increased levels of poorly galactosylated IgA1 characteristic of IgAN and HSPN.

The cytokine milieu and IgA1 *O*-glycosylation

Recent work using a surface IgA1-positive human B lymphoma cell line has shown that the T-helper 2 cytokine interleukin-4 may play a key role in controlling *O*-glycosylation of the IgA1 hinge region. Interleukin-4 stimulation significantly decreased the mRNA levels of both C1β3Gal-T and Cosmc. In parallel, C1β3Gal-T activity was reduced and the synthesized IgA1 *O*-glycoforms were poorly galactosylated.[8] Studies in animal models of IgAN have similarly shown that IgA *N*-glycosylation can be influenced by locally synthesized cytokines, in particular the T-helper 2 cytokines.

Toll-like receptors, mucosal pathogens, and IgA1 *O*-glycosylation

Toll-like receptors play key roles in innate immunity to microbial pathogens via recognition of a diverse range of pathogen-associated molecular patterns, such as bacterial lipopolysaccharide, RNAs, and DNAs. TLR-9 is expressed by B lymphocytes and binds bacterial DNA sequences with unmethylated cytosine–guanine dinucleotide (CpG) motifs. Ligation of TLR-9 leads to polyclonal activation of B cells, class switching, and immunoglobulin production. IgA secretion by mucosal lamina propria and tonsillar B cells is increased after TLR-9 stimulation, implying that mucosal B cells can recognize pathogen-associated molecular patterns and secrete IgA in a T cell-independent manner. Nasal challenge with CpG oligodeoxynucleotides has been shown to increase serum IgA levels and accentuate mesangial IgA deposition and renal injury in an IgAN-prone mouse model.[9] At present there are no data on expression of TLR-9 in human IgAN or the effect of ligation on *O*-glycosylation of IgA1; however, as TLR-9 is expressed on mucosal B cells, it would not be surprising if ligation resulted in increased release of poorly galactosylated polymeric IgA1. TLR-4 is found principally on myeloid cells, but it is also expressed on mature B cells. TLR-4 can bind a large variety of exogenous and endogenous agonists, including Gram-negative bacterial lipopolysaccharide and heat-shock proteins of bacterial and host origin. Ligation of B-cell TLR-4 by bacterial lipopolysaccharide induces methylation of the Cosmc gene, leading to reduced activity of C1β3Gal-T and reduced galactosylation of IgA1.[10] Whether B-cell TLR-4 expression is altered in IgAN is not known; however, these *in vitro* data provide early evidence that specific mucosal pathogens might be able to influence the *O*-glycosylation of mucosal IgA1 antibodies.

WHERE DO WE GO NEXT?

The study of Kiryluk *et al.*[3] reinforces the notion that changes in the profile of serum IgA1 *O*-glycoforms are not enough in themselves to cause IgAN, or HSPN. An increase in serum levels of poorly galactosylated IgA1 does, however, remain the most consistent finding in patients with IgAN and HSPN and is likely to be the trigger for the subsequent development of nephritogenic IgA immune complexes. There is increasing evidence that genetic factors strongly influence the *O*-glycosylation profile of serum IgA1 in IgAN and HSPN; however, the genes responsible for this are at present unknown. An increased realization of the importance of extrinsic factors in control of IgA1 *O*-glycosylation means investigators will need to widen their net when searching for these genetic factors, as the primary abnormality could lie in biochemical pathways far distant from the glycosyltransferases.

DISCLOSURE

The authors declared no competing interests.

REFERENCES

1. Davin JC, Ten Berge IJ, Weening JJ. What is the difference between IgA nephropathy and Henoch-Schonlein purpura nephritis? *Kidney Int* 2001; **59**: 823–834.
2. Allen AC, Willis FR, Beattie TJ *et al.* Abnormal IgA glycosylation in Henoch-Schonlein purpura restricted to patients with clinical nephritis. *Nephrol Dial Transplant* 1998; **13**: 930–934.
3. Kiryluk K, Moldoveanu Z, Sanders JT *et al.* Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch–Schönlein purpura nephritis. *Kidney Int* 2011; **80**: 79–87.
4. Lin X, Ding J, Zhu L *et al.* Aberrant galactosylation of IgA1 is involved in the genetic susceptibility of Chinese patients with IgA nephropathy. *Nephrol Dial Transplant* 2009; **24**: 3372–3375.
5. Hastings MC, Moldoveanu Z, Julian BA *et al.* Galactose-deficient IgA1 in African Americans with IgA nephropathy: serum levels and heritability. *Clin J Am Soc Nephrol* 2010; **5**: 2069–2074.
6. Gharavi AG, Moldoveanu Z, Wyatt RJ *et al.* Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. *J Am Soc Nephrol* 2008; **19**: 1008–1014.
7. Smith AC, Molyneux K, Feehally J *et al.* O-glycosylation of serum IgA1 antibodies against mucosal and systemic antigens in IgA nephropathy. *J Am Soc Nephrol* 2006; **17**: 3520–3528.
8. Yamada K, Kobayashi N, Ikeda T *et al.* Down-regulation of core 1 β1,3-galactosyltransferase and Cosmc by Th2 cytokine alters O-glycosylation of IgA1. *Nephrol Dial Transplant* 2010; **25**: 3890–3897.
9. Suzuki H, Suzuki Y, Narita I *et al.* Toll-like receptor 9 affects severity of IgA nephropathy. *J Am Soc Nephrol* 2008; **19**: 2384–2395.
10. Qin W, Zhong X, Fan JM *et al.* External suppression causes the low expression of the Cosmc gene in IgA nephropathy. *Nephrol Dial Transplant* 2008; **23**: 1608–1614.