Immune regulation in IgA nephropathy

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Chapter 5

The role of SIgA and complement in IgA-nephropathy

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Summary

IgA nephropathy (IgAN) is characterized by glomerular deposition of IgA, often together with complement components. This deposited IgA is mainly polymeric in nature. Although early studies suggested a role for local complement activation in the development of glomerular injury in IgAN, recent attention has focussed on the involvement of the lectin pathway of complement activation in the progression of renal disease in IgAN. Additionally, we have found that glomerular secretory IgA deposition may be one of the initiators of local complement activation in the kidney. In the present review we will discuss recent developments in this area and provide a model of how mucosal immunity and renal inflammation may be interconnected.

IgA Nephropathy

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide. The hallmark of this disease is the deposition of IgA1 in the glomerular mesangium [1;2]. These deposits are thought to be composed mainly of high molecular weight IgA1, sometimes together with IgG or complement components like C3 [3]. The disease shows a broad spectrum of clinical presentations, leading to progressive renal failure in a substantial proportion of patients. It has been reported that IgA deposits disappear after transplantation of a kidney from an IgAN patient into a non-IgAN patient [4]. Furthermore, after renal transplantation recurrent mesangial IgA deposition is observed in about 50% of patients [5]. These results strongly suggest that the basic abnormality of the disease lies within the IgA immune system rather than within the kidney. It is likely that several factors contribute to the development of IgAN, including the nature, glycosylation pattern and composition of IgA, dysregulation of the IgA immune response, and changes in the clearance of IgA from the circulation [6].

Serum levels of IgA are increased in approximately 50% of patients with IgAN [7]. Importantly, in other diseases associated with increased serum IgA, such as IgA myeloma, mesangial IgA deposition is not seen suggesting there is something particular about the IgA molecule in IgAN that promotes mesangial deposition. One of the most consistent changes seen in the circulating pool of IgA is aberrant IgA1 glycosylation, possibly due to a reduced activity of core 1 ß1,3-galactosyltransferase (reviewed by Jan Novak in this issue). This aberrantly glycosylated IgA has also been demonstrated in glomerular IgA deposits, suggesting such molecules have a predisposition to mesangial deposition compared to normally glycosylated IgA [8].

The increase in circulating IgA1 levels appears to be the result of an increased production of this isotype by the bone marrow [9] and a low clearance rate by the liver [9;10]. Mucosal pIgA plasma cell numbers are normal or even reduced in IgAN [11], and pIgA antibody levels in mucosal secretions are not increased and are sometimes lower than controls [12].

Interestingly, patients with IgAN often present with macroscopic hematuria following upper respiratory tract infections. Mucosal infection

or presentation of live micro-organisms leads to excess amounts of IgA in the systemic compartment with the propensity to induce glomerular injury. Immunization studies in IgAN, using different antigens and routes of administration, have produced conflicting results with respect to the systemic IgA response [12-14]. Mucosal and systemic IgA hypo-responsiveness to mucosal immunization with the neoantigen cholera toxin subunit B (CTB) has been reported [12]. Furthermore systemic antigen [12;15;16] challenge results in normal or increased titers of circulating pIgA1 antibodies [15;16] with increased levels of IgA in mucosal secretions of IgAN patients [17].

The fundamental result of these changes in the IgA immune system is a qualitative and quantitative alteration in circulating IgA. We believe these changes have a direct impact on the systemic clearance of IgA and the interaction of systemic IgA with glomerular mesangial cells. These events are the driving force for the local inflammatory response. Based on recent developments, this review will primarily focus on the role of secretory IgA (SIgA) and mannose binding lectin (MBL) in the pathogenesis of IgAN.

Immunoglobulin A

Immunoglobulin A (IgA) is the most abundantly produced immunoglobulin isotype, and plays a critical role in protecting the host against environmental pathogens at mucosal surfaces [18]. In humans, IgA in the circulation primarily consists of monomeric IgA (mIgA), and only 10-20% of the IgA is dimeric or polymeric IgA (pIgA). Furthermore, IgA consists of two subclasses namely IgA1 and IgA2. IgA1 has ten potential O-glycosylation sites and two N-glycosylation sites. IgA2 has no O-glycosylation sites but has two to three additional N-glycosylation sites. In vitro deglycosylation of IgA leads to self-aggregation, suggesting that underglycosylation of IgA may contribute to the generation of high molecular weight IgA [19].

In secretions, secretory IgA (SIgA) is generated during transcytosis of dimeric IgA (dIgA) by epithelial cells, ultimately leading to its association with the extracellular part of the polymeric Ig receptor (secretory component). Besides the presence of SIgA in the mucosa, low levels (10 µg/ml) of SIgA can be detected in serum [20;21].

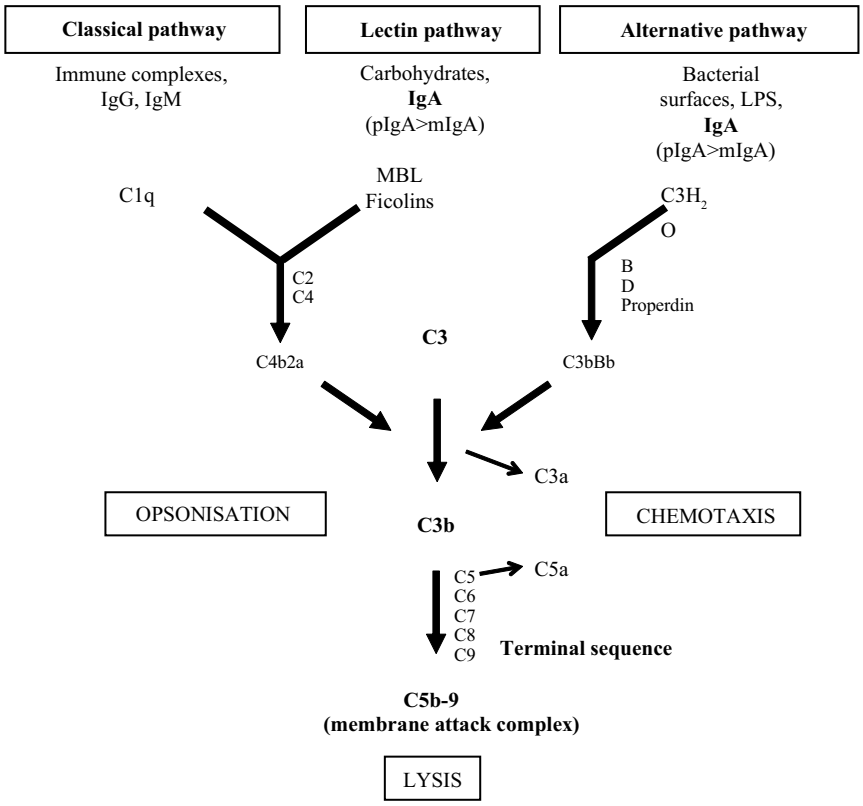

Figure 1. The three complement pathways of complement activation. IgA can activate both the lectin pathway and the alternative pathway. For both pathways polymeric IgA is a more potent activator than monomeric IgA. (MAC: membrane attack complex).

Complement in IgA Nephropathy

In IgAN deposits of IgA are commonly associated with the deposition of complement components, most often C3, the membrane attack complex (C5b-9) and properdin [22;23]. Furthermore, increased levels of split products of activated C3 have been observed in the circulation of patients with IgAN and associated with increased proteinuria and hematuria, suggesting involvement of the alternative pathway in IgAN [24]. Indeed, *in vitro* as well as *in vivo* studies, have shown that polymeric IgA can directly activate the alternative

pathway of complement, whereas monomeric IgA is a poor activator of the complement system [25;26] (Figure 1). The molecular basis for this difference between monomeric and polymeric IgA is not clear. Recently, the lectin pathway of complement, with the recognition molecules MBL, H-ficolin and L-ficolin, has been described [27;28]. MBL is able to bind directly to a number of micro-organisms, via carbohydrates expressed on their surface [29]. Upon binding to an activator, MBL activates the complement cascade via the lectin pathway, which plays a critical role in the first line of host defence against these pathogens. Furthermore, genetic polymorphisms in the MBL gene, resulting in low serum MBL levels and non-functional MBL, have a negative impact on several chronic diseases [30].

Evidence is accumulating that MBL and activation of the lectin pathway of complement can also be unfavourable for disease progression. This has been suggested for rheumatoid arthritis, and also for IgAN, based on renal biopsy studies demonstrating the presence of excess MBL in glomeruli in IgAN [31-33]. Furthermore, it has been shown that MBL is able to bind polymeric IgA, leading to activation of the lectin pathway *in vitro* [34]. However, there is no difference in the binding of MBL to IgA from healthy subjects or patients with IgAN [35]. The carbohydrate recognition domain of MBL is able to bind in a calcium-dependent way to a number of saccharides, such as D-mannose, L-fucose, and N-acetylglucosamine (GlcNAc). The binding of MBL by IgA is likely to be through the oligomannose structures present in the N-linked sugars of the heavy chains of polymeric IgA however this requires further confirmation [35].

Deposition of MBL in association with IgA, as a marker for lectin pathway activation, has been reported in a subpopulation of IgAN patients [31;32;36], but these findings have been questioned by others [37]. Furthermore, the relationship of glomerular MBL deposition with parameters of renal damage and complement activation via the lectin pathway is inconsistent between different studies. Recently our group described a renal biopsy series in which IgA was co-deposited with MBL in 25% of examined patients [38]. Furthermore, patients with MBL deposition exhibited more severe renal disease as compared to MBL-negative cases, suggesting an important role

for MBL in disease progression [38]. These results indicate that activation of the lectin pathway, initiated via MBL and possibly also L-ficolin, occurs in a subpopulation of IgAN patients, implicating MBL as a biomarker for disease progression in these cases [38]. There have been indications that MBL can be expressed by intrisic renal cells, but at present the relative contribution of these cells to deposited MBL is unclear. These results indicate that activation of the lectin pathway, initiated via MBL and possibly also L-ficolin, occurs in a subpopulation of IgAN patients, implicating MBL as a biomarker for disease progression in these cases [38].

These findings at the biopsy level emphasise the importance of further delineating the precise composition of IgA in mesangial deposits as ultimately this data will inform us about the mechanisms involved in IgA deposition and complement activation in IgAN. Local complement activation will result in cell injury and induction of an inflammatory cascade that contributes to disease progression. The impact of glycosylation has been reviewed in detail elsewhere [39] and will not be discussed further in this review. We concentrate on recent findings concerning the presence of SIgA in renal deposits.

Secretory IgA in IgA nephropathy

About 40% of patients with IgAN have recurrent episodes of macroscopic hematuria frequently preceded, one or two days earlier, by infections. Upper respiratory tract infections occur most frequently [40], but occasionally other infections have been implicated, including gastrointestinal and urinary tract infections. Mucosal immunization with a neo-antigen in healthy individuals leads not only to a localised mucosal immune response but also to an antigen-specific immune response in plasma, suggesting a close relationship between the mucosa and bone marrow. This response is reduced after immunization of patients with IgAN [12].

Production of secretory IgA (SIgA) is a specific process taking place at mucosal surfaces and occurs following binding of dimeric IgA (dIgA) to the polymeric Ig receptor (pIgR) and transcytosis of this IgA across the mucosal epithelium [41]. Epithelial IgA transport in the opposite direction has also been described, where SIgA binds selectively to microfold (M) cells

irrespective of their antigen-binding specificity, followed by transport of SIgA across the epithelium and targeting to subepithelial dendritic cells (DC) [42]. *In vitro* it has been demonstrated that human DC can bind and endocytose SIgA [43]. It has been suggested that this targeting of SIgA to DC may play an important role in mucosal immune regulation through modulation of DC activation [42].

Importantly, not all retrograde transport seems to be associated directly with DC uptake, because small amounts of SIgA can also be found in human serum [20;44]. Moreover, increased serum levels of SIgA have been reported in various diseases [45-48] indicating that SIgA may be a marker of clinical interest. Recently our group showed that in purified serum IgA preparations SIgA is found in high molecular weight IgA fractions and that the relative concentration of SIgA is higher in patients with IgAN as compared to controls [35]. In serum low concentrations of SIgA were measured but there were no differences in SIgA concentrations between patients with IgAN and healthy subjects. However, there was a correlation between hematuria in patients with IgAN and the serum SIgA concentration [21]. There also is evidence that systemic clearance of SIgA may be defective in IgAN [49].

Glycosylation of SIgA in IgA nephropathy

As mentioned previously, it is has been suggested that the glycosylation of IgA is an important pathogenic factor in IgAN. The predominance of IgA1 in mesangial deposits and the unusual hinge region of IgA1 with multiple O-linked glycosylation sites has stimulated a great deal of interest in changes to IgA1 glycosylation in IgAN. Indeed, both in serum but more importantly, also in the eluate of isolated glomeruli, a specific reduction of O-linked galactosylation has been observed [50-52]. Furthermore, with size fractionation of eluted proteins from kidney sections, it has been found that these deposits contain predominantly high molecular weight forms of IgA [53]. Recently, we demonstrated a 120-fold accumulation of SIgA, based on a comparison of the composition of serum and glomerular immunoglobulins, in IgA eluted from isolated glomeruli in IgAN [21].

Previous studies have shown a role for glycosylation of IgA in the binding and activation of mesangial cells [54-57]. Stimulation of mesangial cells with high molecular weight IgA leads to enhanced production of chemokines and cytokines, including IL-6, TGF-β, TNF-α, MCP-1, IL-8 and MIF [55;58;59]. Interestingly, SIgA binds better to mesangial cells than serum IgA, and binding of SIgA results in mesangial cell synthesis and release of increased amounts of IL-6 [21]. At present, it is unclear which mesangial cell IgA receptor binds SIgA.

The glycosylation of SIgA is different to serum IgA in several respects. Firstly, SIgA is a tetramolecular complex consisting of two IgA molecules, J chain and SC wrapped around the four α heavy chains. Modelling of SIgA suggests that the N-glycans of the α heavy chains can be masked by SC [60]. This may also result in altered exposure of the hinge region O-glycans. Moreover, specific analysis of the glycosylation of the IgA heavy chains present in SIgA, has demonstrated different N-glycan structures compared to that of serum IgA. Specifically, terminal GlcNAc residues are present in the majority of the N-glycans of SIgA [60]. The O-glycans of the hinge region of the α heavy chain of SIgA1 display a wide range of glycan structures, which for the major part are now characterized [60]. It would be very interesting to have precise information on the glycosylation of the α heavy chains of SIgA in IgAN, but at present such information is not available.

SIgA in IgAN biopsy specimens

To confirm the presence of SIgA in glomeruli in IgAN, our group stained kidney biopsy specimens from patients with IgAN for SIgA deposition. In 15% of cases positive staining for mesangial SIgA was observed [61]. In a separate study secretory component deposition was identified in 13 out of 191 IgAN renal biopsies, while all control biopsies were negative for secretory component [62]. In a Japanese study all IgAN biopsies studied were positive for secretory component, whereas normal kidneys were negative [63]. Interestingly, in this study there was an association between single nucleotide polymorphisms (SNPs) in the pIgR and the presence of IgAN [63]. The relationship between secretory component deposition and other molecules in

the glomeruli, and clinical parameters of the patients, has not been studied. In our study, double staining and confocal microscopy demonstrated remarkable co-localization of SIgA and MBL, supporting the previously reported strong correlation between SIgA and MBL in a subgroup of patients [61]. In addition, there was strong co-localization with C4d, suggesting local complement activation. An association between SIgA and MBL has been reported by other investigators [60]. Royle et al. suggested that disruption of the non-covalent interactions between secretory component and the IgA heavy chain, for instance at low pH, may lead to MBL binding and subsequent complement activation via the lectin pathway [60].

The notion of a pathogenic role of SIgA deposition in IgAN and the knowledge that mucosal sites are critical for the generation of SIgA raises interesting questions concerning the involvement of mucosal immune responses in IgAN.

The role of the tonsils in IgA nephropathy

The tonsils are located at the gateway of the respiratory and alimentary tract and belong to the mucosa-associated lymphoid tissue. The major function of the tonsils is as a first line of defence against viral, bacterial and food antigens. In IgAN tonsillar tissue contains more IgA-secreting B cells than healthy subjects and this increase is matched by a parallel increase in the number of dimeric IgA secreting cells [64]. Tonsils from patients with IgAN contain more IgA-producing cells compared to controls [65], and synthesise IgA1 which is less sialylated than serum IgA1 [66;67], suggesting that the tonsils may be a source of the IgA that deposits in IgAN.

Stimulation of the tonsils by ultra short wave has been shown to trigger acute changes in the urinary sediment in a subgroup of patients with IgAN but not patients with other renal diseases [68]. These patients had suffered more frequent episodes of macroscopic hematuria following upper respiratory tract infections and had higher levels of serum SIgA preceding tonsillar stimulation than those IgAN patients who did not respond to tonsillar stimulation [68].

Although no randomised controlled trials of tonsillectomy in IgAN patients have been reported it has been suggested that tonsillectomy can

improve renal outcome in some patients [69;70]. There is some retrospective evidence from Japan that tonsillectomy was associated with a favourable effect on long term renal survival in IgAN patients supporting the notion that the mucosal IgA immune system may have an important role in the pathogenesis of IgAN [71].

Immunization studies in IgAN patients

Immunization studies examining both systemic and mucosal (oral) secondary immune responses in IgAN have generated conflicting results with respect to serum and mucosal antigen-specific IgA responses [15;17;72-75]. In IgAN mucosal immunization with CTB resulted in an impaired mucosal and systemic antigen-specific IgA response compared with healthy subjects, whereas there was no difference in antigen-specific IgA responses following simultaneous systemic immunization with the neoantigen Keyhole Limpet Haemocyanin (KLH) [12].

Recently, Smith et al. described the O-glycosylation pattern of antigen-specific serum IgA1 against the systemic antigen tetanus toxoid (TT) and the mucosal antigen *Helicobacter pylori* (HP) [76]. In this study higher Vicia Villosa (lectin recognizing the Tn-antigen) binding was observed for IgA1 specific for HP as compared to TT. There were no differences between patients and controls. This suggests that IgA1 O-glycosylation may vary in different immune responses and may be determined at the site of antigen encounter. This also would imply that an altered balance in O-glycosylation pattern of IgA1 in IgAN patients could potentially be a consequence of a mucosal immune response rather than a generic defect in B cell O-glycosylation [76].

Recently we investigated the size distribution of antigen-specific IgA in serum and nasal washes after mucosal and systemic immunization [77]. Nasal washes contained mainly SIgA whereas serum IgA displayed the usual size distribution of serum IgA, being mainly monomeric. SIgA was detectable in these sera, and as expected restricted to the high molecular weight IgA fractions. We found that the antigen-specific IgA was found predominantly in the high molecular weight fractions, irrespective of the route of administration. Importantly, we were also able to demonstrate low but significant levels of

antigen specific SIgA in serum after intranasal vaccination, strongly suggesting a link between mucosal immune responses and circulating SIgA (figure 2).

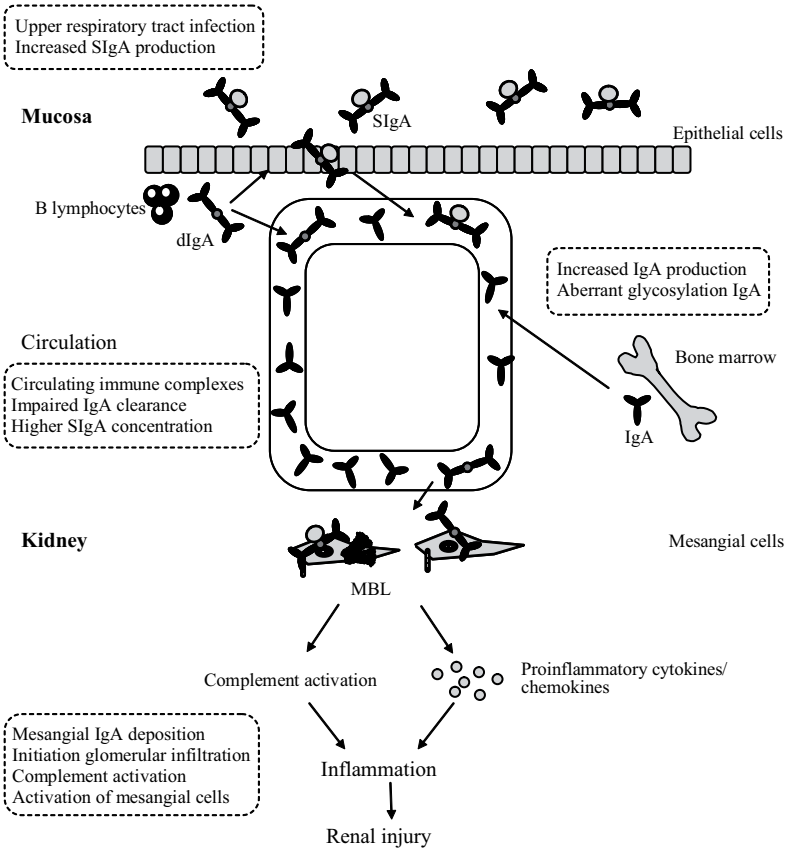

Figure 2. A model to link mucosal immune responses with deposition of SIgA in the renal mesangium. In IgAN three compartments are important, the mucosa, the circulation and the kidney. B lymphocytes present at mucosal sites will, upon activation, produce dIgA which will be predominantly secreted as SIgA across mucosal epithelial surfaces. Via an unknown mechanism, small amounts of SIgA enter the circulation and ultimately come into contact with mesangial cells. At the same time, both mucosal B cells and plasma cells in the bone marrow are producing increasing amounts of aberrantly glycosylated IgA which enters the circulation. The binding of SIgA and aberrantly glycosylated IgA1 leads to mesangial cell activation. SIgA deposition is also associated with mesangial deposition of MBL and activation of the complement system, resulting in more severe glomerular injury.

Conclusion

In this review we have discussed the potential role for MBL and SIgA in the pathogenesis of IgAN. Polymeric IgA is able to activate the alternative pathway but also by binding MBL activate the lectin pathway of complement. We also have discussed the mesangial deposition of MBL in IgAN and that this deposition is associated with more severe renal injury.

Patients with IgAN often experience macroscopic hematuria following upper respiratory tract infections. Although there is no obvious increase in serum SIgA in IgAN we have described a clear relationship between serum SIgA concentrations and risk of hematuria in IgAN. Moreover, compared to other serum immunoglobulins SIgA appears to deposit preferentially within glomeruli in IgAN and this is reflected by the SIgA staining observed in kidney biopsies of patients with IgAN. The presence of SIgA is strongly associated with co-deposition of MBL and the complement activation product C4d. Taken together, the data presented in this review support a role for SIgA and MBL in the pathogenesis of IgAN in a subpopulation of patients.

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