Pathogenic role of (S)IgA in IgA nephropathy

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General Discussion and Summary

CHAPTER 8

Introduction

Primary IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis. The disease is characterized by mesangial deposits of IgA (1-3). These deposits are thought to be composed mostly of high molecular weight IgA1, sometimes together with IgM or complement components like C3 (4,5). The disease shows a broad spectrum of clinical presentations, leading to progressive renal failure in a substantial proportion of patients. Case reports have shown that IgA deposits disappear after transplantation of a kidney with IgA deposits into a non-IgAN patient (6). Furthermore, after renal transplantation recurrent mesangial IgA deposition is observed in about 50 % of the patients (7). These results strongly suggest that the basic abnormality of the disease lies within the IgA immune system rather than in the kidney.

In serum of IgAN patients, elevated levels of IgA and IgA immune complexes were observed (8,9). Furthermore, aberrantly glycosylated IgA, due to a reduced activity of the β1-3 galactosyltransferase, has been described in patients with IgAN. This aberrantly glycosylated IgA is also demonstrated in glomerular IgA deposits, suggesting a better binding of this IgA as compared to normal glycosylated IgA.

Another important factor is the binding of IgA to mesangial cells. Several findings point to an IgA receptor on mesangial cells (10,11). For most of the IgA receptors it has already been demonstrated that they are not present on mesangial cells (12,13). There are some new candidates like Fcα/μR (14) or transferrin receptor (15) but, there is only limited information available about these receptors and further research has to confirm their possible role in IgAN.

The present thesis focuses on different aspects of IgAN, namely IgA and the binding of IgA to its receptors. In the first part of this thesis (chapter 2,3) we focused on possible receptor-mediated mechanisms underlying mesangial IgA deposition. In the second part of the thesis (chapter 4 to 7) we focused our attention on the characterization of IgA and associated molecules present in the mesangium of IgAN patients and on the characterization of serum IgA from patients and controls.

Binding of IgA to IgA receptors

Several IgA receptors have been identified and characterized. One of these receptors is FcαRI/CD89. A number of studies already showed that CD89 is not present on mesangial cells. Still, there are suggestions for a role of this receptor in IgAN. Human CD89 transgenic mice spontaneously develop at old age renal disease with characteristics of IgAN (16). On the other hand, our group showed that injection of soluble human CD89 into mice does not induce deposition of IgA in kidneys (17). It remains unclear how human CD89 could affect IgA deposition, since we and others showed that human CD89 cannot react with murine IgA (17,18).

Complexes of CD89 with monomeric IgA are present in the circulation (19). However, it is also shown that especially high molecular weight IgA can interact with CD89 (20-22). Therefore, we investigated the binding of different molecular forms of IgA with CD89 with different methods. In chapter 2 we confirm data from literature (20-22), that high molecular weight IgA can interact with CD89. We however, using

surface plasmon resonance (SPR), a method in which the interaction in time can be measured, found that the initial interaction of monomeric IgA and CD89 is comparable to polymeric IgA-CD89 interactions. However, the interaction of polymeric IgA with CD89 is more stable as compared to monomeric IgA ultimately resulting in a higher final binding of polymeric IgA to CD89 than monomeric IgA. A recently published crystal structure of the IgA1Fc-CD89 complex showed that one IgA molecule is in complex with two CD89 molecules, confirming interactions of mIgA with CD89 (23). Furthermore, the initial interaction of mIgA with CD89 might also be sufficient for internalization of mIgA via CD89, thereby creating intracellular CD89-IgA complexes which then can be secreted in the circulation (19).

After cleavage of the N-glycans present on IgA we demonstrated enhanced association of IgA to CD89, suggesting an important role for the N-glycans on IgA in the initial interaction. In literature, it is still controversial whether N-glycans present on IgA do influence the interaction with CD89 (24,25). In the crystal structure of the IgA1Fc-CD89 complexes the observed N-glycans are close but do not directly contact the receptor (23). Some amino acid mutations in the CD89 molecule, which are not in direct contact with IgA, also hamper the binding of IgA-CD89. Because of the presence of the N-glycans close to the contact sites it could be that the N-glycans hamper the IgA/CD89 interaction.

Another recently identified IgA receptor is Fcα/μR. This receptor has been described to be present on B cells and macrophages (26). Furthermore, high expression in the kidney is observed (26). The Fcα/μR is also suggested to be present on mesangial cells, and thereby could play a role in IgAN. However there are no suitable reagents available to study this receptor. Therefore we made chimeric proteins to study IgA and IgM binding to this receptor. We demonstrated that IgA binds to Fcα/μR, likely through the proximal immunoglobulin like domain. The binding of IgA to Fcα/μR was equal for monomeric and polymeric IgA and was glycan dependent. Therefore these findings do not fit in the observations that it is mostly pIgA that is present in the renal mesangium of IgAN patients (27). Moreover, the glycan composition of IgA described in patients with IgAN (28-30), does not have any effect on the interaction of IgA with Fcα/μR. After removing the N-glycans and the sialic acids from the IgA molecule, the interaction with the Fcα/μR is hampered, whereas deglycosylation of IgA leads to an increase in binding to mesangial cells (31). We confirmed the presence of the Fcα/μR on mesangial cells. Furthermore, we showed an upregulation of Fcα/μR transcript in mesangial cells after IL-1 stimulation. However, high molecular weight IgA binds better to mesangial cells as compared to monomeric IgA and we could not detect differences in IgA binding to IL-1 stimulated mesangial cells. In conclusion, the Fcα/μR could play a role in the clearance of IgA in IgAN, but its in vitro characteristics do not favor a significant role for this receptor in IgAN.

Complement in IgA nephropathy

In IgAN deposits of IgA are commonly associated with complement components, most often C3, the membrane attack complex (C5b-9) and properdin (32,33). Furthermore, increased levels of split products of activated C3 have been observed in the circulation of IgAN patients (34-36). These findings suggest involvement of the

alternative pathway in IgAN. Indeed, in vitro as well as in vivo studies, have indicated that IgA can directly activate the alternative pathway of complement (37-39).

Recently, the lectin pathway of complement, with their recognition molecules MBL, H-ficolin and L-ficolin, has been described (40-43). MBL is able to bind directly to a number of microorganisms, via carbohydrates expressed on their surface (44,45). Upon binding, MBL activates the complement cascade via the lectin pathway, which plays a critical role in the first line of host defense against these pathogens. Furthermore, genetic mutations in the MBL gene, leading to low serum MBL levels and non functional MBL, have a negative impact on several chronic diseases (46-48). However evidence is increasing that MBL and the lectin pathway of complement can also be unfavorable for disease progression. This is proposed for rheumatoid arthritis, and is suggested by the presence of MBL in renal biopsies from patients with IgAN (49-51). Furthermore, it was shown that MBL is able to bind to polymeric IgA which leads to the activation of the lectin pathway in vitro (52).

In chapter 4 we investigated the presence of MBL in glomerular IgA deposits of IgA nephropathy patients. We showed that in 25 % of the cases co-deposition of IgA with MBL was present (Figure 1). Furthermore, patients with MBL deposition suffered from more severe renal injury as compared to the MBL negative cases, suggesting an important role for MBL in disease progression. In a recent Japanese study it has been suggested that MBL deposition was restricted to biopsies with IgA2 deposition (53). However, in the biopsies that we investigated we were not able to detect any IgA2. The difference with an earlier report might be due to ethnical differences of the patients.

In chapter 5 we investigated the differences between monomeric and polymeric IgA purified from patient and control. We confirmed that specifically polymeric IgA is able to bind to MBL and induce C4 activation. However, no differences between patients and controls were observed. Furthermore, we showed that on the heavy chain of polymeric IgA oligomannose structures are present which are absent on monomeric IgA. 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) (44). Therefore, the oligomannose structure present on the heavy chain of IgA could be a likely candidate to bind MBL, but this needs further confirmation

Serum IgA in IgA nephropathy

In IgAN, the glycosylation of the IgA1 molecule plays an important role. It has been described that the IgA1 molecule is aberrantly glycosylated leading to increased Tn antigen (GalNAcα1-Ser/Thr) residues (28,30). Allen et al. have reported that the β1-3 galactosyltransferase synthesis activity was remarkably lower in peripheral B lymphocytes of IgAN patients as compared to healthy controls (54). The β1-3 galactosyltransferase activity is dependent on the coexistence of a specific chaperone, Cosmc (core 1 β1-3-Gal-T-specific molecular chaperone) (55), and in IgAN patients it is suggested that downregulation of Cosmc is important for the aberrant O-glycosylation of IgA1 (56).

Serum IgA from IgAN patients was shown to have an increased binding to

mesangial cells (57), however this could not be confirmed by others (58). Furthermore, increased production of cytokines and chemokines was shown after stimulation of mesangial cells with serum IgA from IgAN patients (59-61). To mimic this aberrantly glycosylated IgA, IgA was treated with enzymes. In vitro studies with deglycosylated IgA suggested that the undergalactosylated IgA in IgAN patients might be involved in increased interaction with mesangial cells (31). Noteworthy, these in vitro studies made use of the lectin Jacalin (which specifically recognizes Galβ1-3GalNAc) for IgA purification, possibly leading to a selection of only a subpopulation of serum IgA1.

In chapter 5 we purified IgA from serum of patients and controls with an anti-human IgA affinity column and separated these IgA preparations in monomeric and polymeric IgA. With this method we did not select for typical IgA glycosylation structures. With these highly purified monomeric and polymeric IgA preparations we studied the interaction with mesangial cells and lectins. The interaction with mesangial cells, and the induction of IL-8 production was better for polymeric IgA than for monomeric IgA, but there was no difference between patients and controls. Stimulation of mesangial cells with IgA and IgA binding to mesangial cells has been studied before (31,60,62,63), but this is one of the first studies that a correlation between binding of IgA to mesangial cells and chemokine production after stimulation of mesangial cells with the same IgA preparations shows.

Another interesting finding in this chapter is the binding of Helix aspersa (HAA) to the IgA preparations. HAA is a lectin which recognizes terminal GalNAc. In an earlier report, size fractionation of total serum of an IgAN patient showed HAA reactivity in the high molecular weight fractions suggesting interaction with high molecular weight IgA (31). In our study, we confirmed this better binding of polymeric IgA to HAA as compared to monomeric IgA. Binding of HAA was strongly increased by neuraminidase treatment, suggesting a high frequency of non-galactosylated O-linked glycans on IgA, that expose terminal GalNAc after enzymatic removal of sialic acid.

Polymeric serum IgA contains more IgA2 as compared to monomeric serum IgA (chapter 5). Mucosal IgA is largely polymeric IgA whereas serum IgA contains mainly monomeric IgA. Furthermore, the IgA subclass distribution varies with different mucosal locations (5) depending on the site, the total IgA amount consists of 15 to 65 % IgA2. Therefore, an increased polymeric IgA2 fraction may suggest its production at the mucosal sites. Another explanation could be the glycosylation of the IgA molecule. IgA2 contains additional N-glycans as compared to IgA1. At present it is unknown whether the glycans present on IgA are important for the polymerization process. The observation of a difference in N-glycosylation of polymeric IgA versus monomeric IgA, together with the presence of two to three additional N-glycans on IgA2 as compared to IgA1, may suggest a more efficient polymerization of IgA2, and therefore could explain that more IgA2 is found in the polymeric IgA fraction.

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 (64), but also occasionally other infections have been implicated, including gastrointestinal and urinary tract infections (65,66). Furthermore, mucosal immunization with a neo-antigen in healthy individuals leads to an antigen-specific immune response in plasma, suggesting a relation between mucosa and bone marrow. However, this response is reduced after immunization of patients with IgAN (67). SIgA is the major immunoglobulin responsible for protecting the mucosal surfaces. However, at present it is not clear whether a mucosal challenge also regulates levels of circulating SIgA.

Generation of secretory IgA (SIgA) is a specific process taking place at mucosal surfaces (68), after binding of dimeric IgA (dIgA) to the polymeric Ig receptor (pIgR) followed by a process of transcytosis. In addition also a process of transepithelial transport has been described in which SIgA adheres selectively to microfold (M) cells irrespective of their antigen-binding specificity (69,70), and that SIgA is subsequently transported across the epithelium and targeted to dendritic cells (DC) (71,72). In vitro it has been demonstrated that DC can bind and endocytose SIgA (73). It could be hypothesized that not all SIgA is internalized by DC and this SIgA may end up in the circulation. Indeed, small amounts of SIgA can also be found in human serum (74,75). Moreover, increased serum levels of SIgA have been reported in various diseases (76-78) indicating that SIgA may be a marker of clinical interest.

In the final section of this thesis we addressed the role of SIgA in IgA nephropathy (Figure 1). First, we investigated the presence of SIgA in serum (chapter 6) and in purified IgA preparations (chapter 5). We showed that in purified IgA preparations SIgA is restricted to the polymeric IgA fractions and that the relative concentration of SIgA is higher in patients with IgAN as compared to controls. In serum we measured low concentrations of SIgA, but we could not observe differences in SIgA concentrations between patients and controls. However, we did find a relation between hematuria in patients with IgAN and the SIgA concentration in serum.

Tonsils in IgA nephropathy

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 a first line of defence against viral, bacterial and food antigens. Tonsils of IgAN patients contain more IgA secreting B cells as compared to other diseases (79). This increase is paralleled by an increase of the number of dimeric IgA secreting cells (79). Furthermore, SIgA can adhere to M cells and transported into the lymphoid tissues. Stimulation, by ultra short wave, of the tonsils of IgAN patients leads to deterioration in renal function as compared to patients with other kidney diseases (80). In addition, previous episodes of gross hematuria following upper respiratory tract infections and the level of serum secretory IgA were higher in IgAN patients with deterioration of renal function after tonsil stimulation that those without deterioration (80). Tonsillar IgA1 from IgAN patients contains more asialo IgA1 as compared to serum IgA1 (81,82), suggesting that underglycosylated tonsillar IgA is produced in this lymphoid organ. This abnormally produced IgA reach the peripheral blood, and because of their size or composition, fails to be properly filtered by the kidneys but rather accumulate in the mesangium. In this respect the role of SIgA in this process

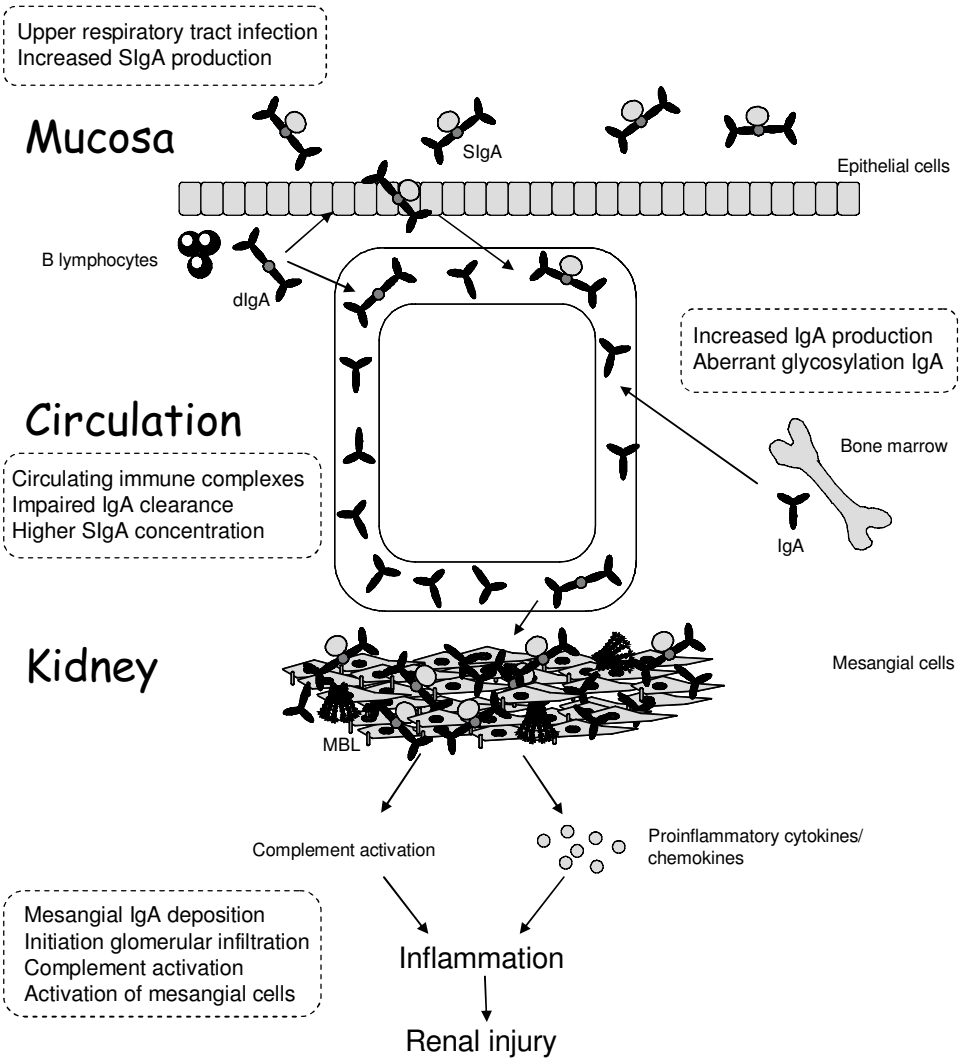

Figure 1: Hypothetical overview of different factors involved in IgA Nephropathy.
In IgAN three compartments are important, the mucosa, the circulation and the kidney. After upper respiratory tract infection 40 % of the IgAN patients have recurrent episodes of hematuria, suggesting a role for the mucosal compartment, whereby may be some SIgA is leaking in the circulation. Furthermore, the B lymphocytes present at the mucosal surface will produce dIgA which will be present in the circulation and via transcytosis in the epithelial cells secreted as SIgA in the mucosa. Via the circulation the SIgA is in contact with the mesangial cells to which it can bind. At the same time, the bone marrow is producing increasing amounts and aberrantly glycosylated IgA which is also present in the circulation. The binding of IgA to mesangial cells will lead to activation of the mesangial cells and the complement pathways. This mesangial cell activation will attract immune cells, and thereby inducing inflammation leading to renal injury.

was not studied. Tonsillectomy can improve the renal function and keep the renal function stable in some IgAN patients (83,84). Tonsillectomy had a favorable effect on long term renal survival in IgAN patients (85).

SIgA and mesangial cells

The activation of mesangial cells by IgA1 immune complexes is considered as an initiating event in the pathogenesis of IgA nephropathy (Figure 1). Therefore, we analyzed the interaction of serum IgA and SIgA with mesangial cells (chapter 6). We demonstrated a higher binding of SIgA to mesangial cells as compared to the binding of serum IgA. Passive protection by SIgA, secreted by the mucosal immune system, plays a central role in the protection of mucosal surfaces in general. However, after stimulation of mesangial cells with SIgA, the mesangial cells start to produce even more IL-6 than after exposure to serum IgA. Finally, we demonstrate in chapter 6 that immunoglobulins eluted from glomeruli of a patient show a strong accumulation of SIgA in the renal mesangium as compared to other immunoglobulins. These data suggest a pathogenic role for SIgA in IgA nephropathy.

SIgA glycosylation in IgAN

As mentioned before, it is assumed that glycosylation of IgA is an important factor in IgAN (Figure 1). Previous studies have shown a role for glycosylation of IgA and its subsequent interaction with mesangial cells (31,60,62,63). The glycosylation of SIgA is different as compared to serum IgA. Secretory IgA consists of two IgA molecules together with the J chain and the secretory component. Modeling SIgA has shown that secretory component masks the heavy chain of IgA and thereby prevents interaction with glycan structures (86). Furthermore, it was shown that N-glycans present on SIgA contain other structures as compared to serum IgA (86). In IgAN, especially the O-glycosylation of IgA was studied. The O-glycan structure on the hinge region of SIgA1 contains many different carbohydrate structures. These structures might be differently exposed because of the presence of secretory component. Taken together, this may lead to increased interaction of SIgA with mesangial cells. In this respect, the specific alterations in glycosylation of SIgA from IgAN patients would be very interesting to study.

SIgA in IgAN biopsies

To confirm the presence of SIgA in glomeruli of a patient with IgAN, we stained kidney biopsies from IgA nephropathy patients for SIgA (chapter 7). In 15 % of the cases positive staining of SIgA in biopsies was observed. In a Japanese study all IgAN biopsies studied were positive for secretory component, whereas normal kidneys were negative (87). However, the relation with the presence of secretory component and other molecules in the glomeruli or clinical parameters of the patients were not studied. Interestingly, this study (87) showed an association with single nucleotide polymorphisms (SNPs) in the pIgR and the presence of IgAN.

In chapter 7 we demonstrate a clear correlation between the presence of SIgA and MBL in renal biopsies. Earlier studies had already demonstrated an interaction of SIgA with MBL (86). Royle et al. suggested that disruption of the non-covalent interactions between secretory component and the IgA heavy chain may, for instance at low pH, lead to MBL binding and subsequent complement activation via the lectin pathway (86). The presence of SIgA with MBL in glomeruli of a subpopulation of IgAN patients suggests unmasking of the IgA heavy chain leading to MBL deposition. However, MBL was found in 20 % of the cases in the glomeruli, where-

as glomerular SIgA is detected in 15 % of the cases. This indicates that MBL most probably is not only binding to deposited SIgA but also to other high molecular weight forms of IgA.

Immunization studies in IgAN patients

Several immunization studies, both after systemic and oral secondary immunization, showed conflicting results with respect to the specific serum and mucosal IgA concentrations in IgAN patients (88-94). After mucosal immunization with CTB, IgAN patients exhibit a decreased mucosal and systemic antigen specific IgA response, whereas simultaneous systemic immunization with KLH did not lead to a difference in antigen specific IgA response between patients and controls (67). Unpublished results from our group showed the size distribution of the antigen specific IgA response in serum and nasal washes after immunization with CTB and KLH. The nasal washes contain mainly SIgA whereas serum IgA showed a typical serum IgA profile (mainly mIgA). Furthermore, SIgA is restricted to fractions containing high molecular weight IgA. The antigen specific IgA response is mostly of high molecular weight. Unfortunately, with the available reagents, we were not able to detect antigen specific SIgA. However, the partly overlapping profile would be in line with a role for antigen specific SIgA.

The systemic antigen specific IgA response we found was mainly associated with high molecular weight IgA. We hypothesize that this IgA may be partly derived from mucosal sites via production of dimeric IgA by mucosal B cells and leakage of SIgA from the mucosa (Figure 1). However, also in the bone marrow antigen specific IgA1 antibody producing cells were demonstrated (67). IgA from the circulation is mainly produced in the bone marrow and is monomeric, whereas the response we tested was mainly high molecular weight IgA. Therefore it would be very interesting to investigate the size distribution of bone marrow synthesized antigen specific IgA. In literature, up-regulation of pIgA synthesis in the bone marrow and an increase of pIgA-producing plasma cells have been described in IgAN patients (79,95). However, after intramuscular immunization with inactivated influenza virus mainly a monomeric IgA response is observed (88). It seems that the route of immunization is important for the size distribution of the antigen specific IgA response. Therefore, it would be very interesting to investigate the size of the antigen specific IgA after different immunization routes.

Conclusion

In summary, we have demonstrated in the first part of this thesis that monomeric IgA is able to interact with different IgA receptors and that this interaction is glycosylation dependent. Furthermore, we investigated the binding of IgA to a recently identified IgA receptor, thereby showing clear IgA binding to this receptor. However, the binding of monomeric and polymeric IgA were similar, suggesting that this receptor is probably not the mesangial receptor responsible for the deposition of IgA in the mesangium of IgAN patients.

In the second part of this thesis we showed clear differences between polymeric and monomeric IgA, which could finally lead to deposition of IgA (Figure 1). We

demonstrated a better interaction of polymeric IgA with mesangial cells than that of monomeric IgA. Furthermore, polymeric IgA is able to interact with MBL and thereby activates the lectin pathway of complement, in addition to the alternative pathway. We also showed that binding of MBL to IgA can lead to MBL deposition in the glomeruli of a subpopulation of IgAN patients. Moreover, we showed that this deposition of MBL is associated with more severe renal injury (Figure 1).

IgAN patients often have macroscopic hematuria following upper respiratory tract infections. Although, we could not observe differences in SIgA concentrations in serum of IgAN patients and controls, we showed a clear relation with the SIgA concentration in serum and hematuria. Furthermore, significantly more SIgA was present in affinity purified IgA preparations from IgAN patients as compared to controls. Moreover, we showed a strong accumulation of SIgA, as compared to other immunoglobulins, in the glomeruli, and this was in agreement with the positive SIgA staining in kidney biopsies of a subpopulation of IgAN patients (Figure 1). Altogether, the data presented in this thesis support a role for SIgA in the pathogenesis of a subpopulation of IgAN patients. Further research to define such a pathogenic role is warranted.

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