Pathogenic role of (S)IgA in IgA nephropathy

Oortwijn, B.D.

General Introduction

CHAPTER 1

Clinical presentation of IgA nephropathy

Primary IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis. The disease shows a broad spectrum of clinical presentations, leading to progressive renal failure in a substantial proportion of patients. The hallmark of this disease is deposition of IgA1 in the glomerular mesangium (1-3). In the glomeruli of patients with IgAN mostly high molecular weight IgA1 is detected, sometimes together with IgM and/or C3 (4,5). After renal transplantation recurrent mesangial IgA deposition is observed in 50 % of the patients (6). Case reports have shown that IgA deposits disappear after transplantation of a kidney with IgA deposits into a non-IgAN patient (7). These results strongly suggest that IgAN is not only a disease of the kidney, but also dependent on systemic factors.

Interestingly, patients with IgAN often present macroscopic hematuria following upper respiratory tract infections. Mucosal immune challenge leads to an increased production of IgA in the systemic compartment, probably based on the migration of B cells (the mucosa-bone marrow axis) (8). This mucosa-bone marrow traffic has been confirmed by challenging healthy individuals intranasally with the neoantigen cholera toxin subunit B (CTB) (9). In patients with IgAN a mucosal IgA hyporesponse to mucosal immunization with this neoantigen was observed (9).

Since differences in circulating IgA together with IgA binding to mesangial cells have been proposed to play an important role in the pathogenesis of IgA nephropathy, detailed analysis of circulating IgA and its interactions with cellular receptors is important.

Histopathology of IgA nephropathy

The most common histological lesion seen in renal biopsies from patients with IgAN are focal or diffuse mesangial proliferative glomerulonephritis (10). The initial phase of IgAN is characterized by increase in mesangial matrix but no segmental sclerosis. Focal proliferative lesions comprise the largest subgroup of IgA nephropathy. The histologic changes range from focal and segmental mesangial proliferative glomerulonephritis to focal glomerulonephritis with segmental endocapillary cell proliferation, with or without crescent formation. Associated with these variable expressions of glomerular pathology are variable degrees of tubular atrophy, interstitial fibrosis, and interstitial inflammation comprised of lymphocytes, monocytes/macrophages, and plasma cells. The number of macrophages in the glomeruli correlates with the presence of crescents and proteinuria (11). Furthermore, more monocytes and T-cells were found in biopsies of patients with active disease as compared to those without disease activity (12).

Immunohistochemistry has revealed that IgA deposits mainly consist of the IgA1 subclass (13,14), and commonly occur with co-deposits of C3, IgG, and, less common, IgM (15). The predominance of IgA1 deposits and the specific hinge region of IgA1 with potential O-linked glycosylation sites have initiated a directed search for alterations in glycosylation. Indeed, in the eluate of renal deposits, a specific reduction of O-linked galactosylation has been observed (16,17). Furthermore, with size fractionation of eluted proteins from kidney sections, it was shown that deposited

IgA is mostly high molecular weight of nature (18). Additional evidence for the deposition of high molecular weight IgA was obtained from immunohistochemical analysis of renal tissue (14,19,20).

Composition of IgA

IgA is the most abundant class of immunoglobulin synthesized in humans, with 66 mg of IgA/kg of body weight produced daily compared to 34 mg of IgG and 8 mg of IgM. The half life of IgA in the circulation is 6 days. Almost all circulating IgA (2 mg/ml) is produced in the bone marrow and the liver is involved in the catabolism of the circulating IgA. Only negligible amounts (1 mg/ kg bodyweight/ day) of the total IgA produced in the bone marrow, spleen and lymph nodes (20 mg/ kg bodyweigth/ day) reach the external secretions (21). The other part of the IgA (46 mg/ kg bodyweight/ day) is produced at the mucosal sites and is secreted efficiently as secretory IgA (SIgA).

General composition

Human IgA exists as two isotypes, IgA1 and IgA2, with IgA2 having two allotypic variants: IgA2m(1) and IgA2m(2). The human IgA subclasses differ at 14 amino acid (aa) positions in the α-chain sequence. α-Chains have three constant region domains Cα1 to Cα3 (Figure 1A). IgA2 of the A2m(2) allotype differs from A2m(1) and IgA1 in 6 positions; 2 in Cα1 and 4 in Cα3. The Cα3 domain is the same for IgA1 and IgA2m(1). The Cα2 domain is the same for both A2 allotypes and differs from IgA1.

A major difference between IgA1 and IgA2 occurs in the hinge region. IgA2 molecules lack a 13-aa segment found in the hinge region of IgA1 molecules, which contain 5 potential O-linked carbohydrate sites.

Serum IgA

Human IgA in serum exists with an IgA1: IgA2 ratio of about 9:1 (22,23) and is found in different molecular forms: monomeric IgA (mIgA), composed of two heavy and two light chains; dimeric IgA (dIgA), consists of two IgA molecules linked with a joining (J-) chain. Finally, in serum also additional high molecular weight forms of IgA can be recognized, generally described as polymeric IgA (pIgA). The composition of human serum polymeric IgA is diverse and may include CD89/IgA complexes, IgA immune complexes and IgA-fibronectin complexes (24). In humans, circulating IgA primarily consists of monomeric IgA (mIgA), and only 10-20% of the IgA is found in high molecular weight IgA (dIgA and pIgA) forms. In contrast, in rodents IgA is mostly present in a high molecular weight form (25).

Secretory IgA

Secretory IgA (SIgA) is the major immunoglobulin responsible for protecting the mucosal surfaces. To generate SIgA, dimeric IgA with the attached J-chain is produced in plasma cells close to the epithelium. The epithelial cells express on the basolateral side the polymeric Ig receptor (pIgR) that binds to dIgA; this complex is translocated through the epithelial cell (transcytosis). During transcytosis the extra-

cellular part of the pIgR, the secretory component (SC), is covalently linked to dIgA. At the mucosal surface the secretory component is cleaved from the pIgR and secretory IgA is secreted. Besides the presence of SIgA in the mucosa, low levels (10 µg/ ml) of SIgA can be detected in serum (Figure 1B) (26,27).

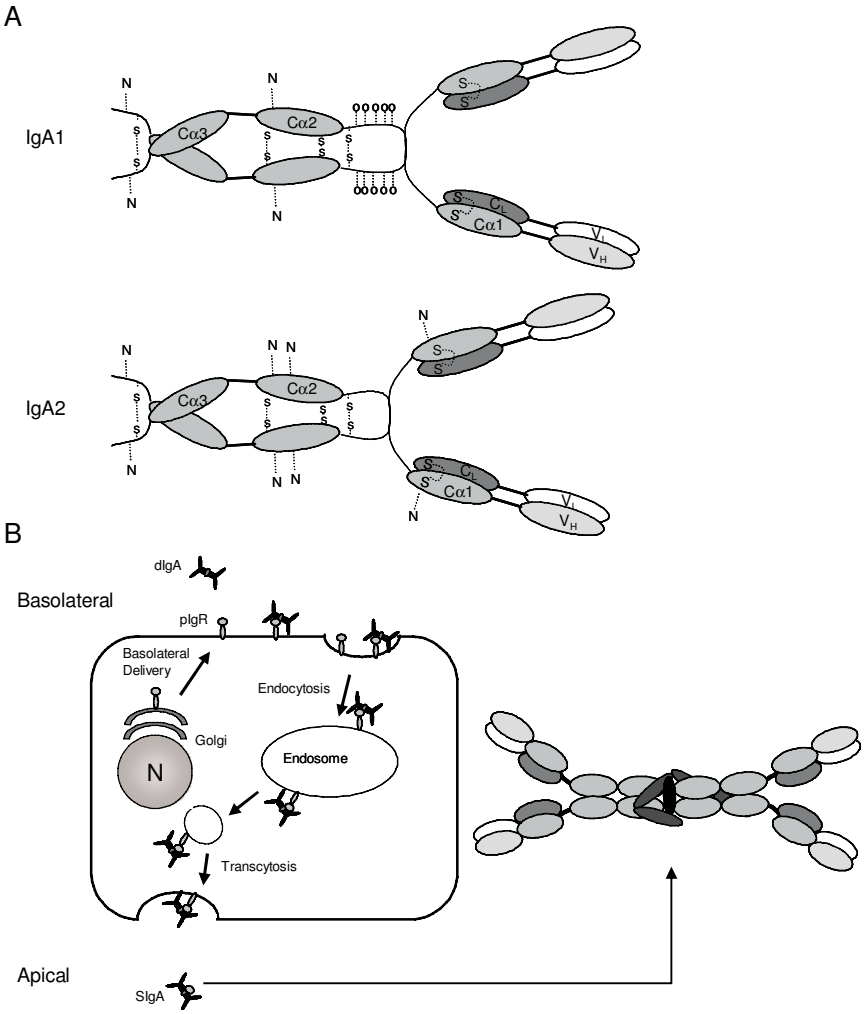

Figure 1: proposed domain structure of human monomeric IgA
A) The α heavy chain contains 3 constant domains Cα1, Cα2 and Cα3 and 1 variable domain VH. The light chain contains 1 constant domain CL and 1 variable domain VL. Positions of disulfide bonds (S), N- (N) and O-(O) linked glycosylation sites are indicated. (adapted from (30)). B) Pathway of pIgR through an epithelial cell (adapted from (104))

Glycosylation of serum IgA

Glycans contribute 6 to 7 % of the total molecular mass of IgA1 and 8 to 10% of the total mass of IgA2 proteins. The higher carbohydrate content in IgA2 proteins is the result of additional N-linked oligosaccharide side chains (28). Human IgA1 contains two conserved N-glycosylation sites in each α-chain (Asn263 and Asn459), while the IgA2 subclass contains an additional two (IgA2m(1)) or three (IgA2m(2)) conserved N-glycans (29). The number, the type and the terminal sugar residues vary between proteins of IgA subclasses but also within one subclass (Figure 2) (30). Serum IgA contains complex type N-linked carbohydrate moieties. Biantennary structures accounted for 86 % of the N-linked glycans on IgA whereas 14 % of the oligosaccharides were multiantennary or extended (31).

IgA1 is one of the few serum proteins and unique among circulating immunoglobulins in having O-glycosylation as well as N-glycosylation sites. These O-glycosylation sites are restricted to the hinge region of IgA1, which contains four to five short chains. The O-glycans are relatively simple sugars in which N-acetylgalactosamine (GalNAc) is O-linked to a serine or threonine residue. The glycan is completed with a terminal galactose (Gal) with or without additional sialic acid residues (NeuNAc) (Figure 2).

Glycosylation of SIgA

The glycosylation of SIgA is different compared to that of serum IgA in several aspects (Figure 2). Modelling of SIgA suggests that the N-glycans on the heavy chain can be masked by the SC (32). This may also result in a different exposure of the O-glycans. Moreover, specific analysis of the glycosylation of the IgA heavy chain present in SIgA, demonstrated different N-glycan structures compared to that of serum IgA. Specifically, terminal GlcNAc residues are present on the majority of the N-glycans of SIgA (32). The O-glycans on the hinge region of the heavy chain of SIgA1 presented a wide range of glycan structures, of which the major part is now characterized (32). Finally, also SC itself is heavily glycosylated.

The J chain (16 kDa) contains a single carbohydrate side chain linked to asparagine. This N-linked glycan is approximately 8 % of the molecular mass of J chain. This chain consists of fucose, mannose, galactose, N-acetylglucosamine and sialic acid. The N-glycan appears to be critical to polymer formation between J chain and IgA monomer subunits (Figure 2) (33).

Free secretory component (SC) was isolated from mucosal secretions as well as associated with SIgA. SC (70 kDa) consists of five immunoglobulin-like domains with approximately 22 % of the total molecular mass of SC contributed by carbohydrates. The 5 to 7 site chains contain N-acetylglucosamine, fucose, mannose, galactose and sialic acid, N-glycosidic linked to the protein backbone (Figure 2) (34).

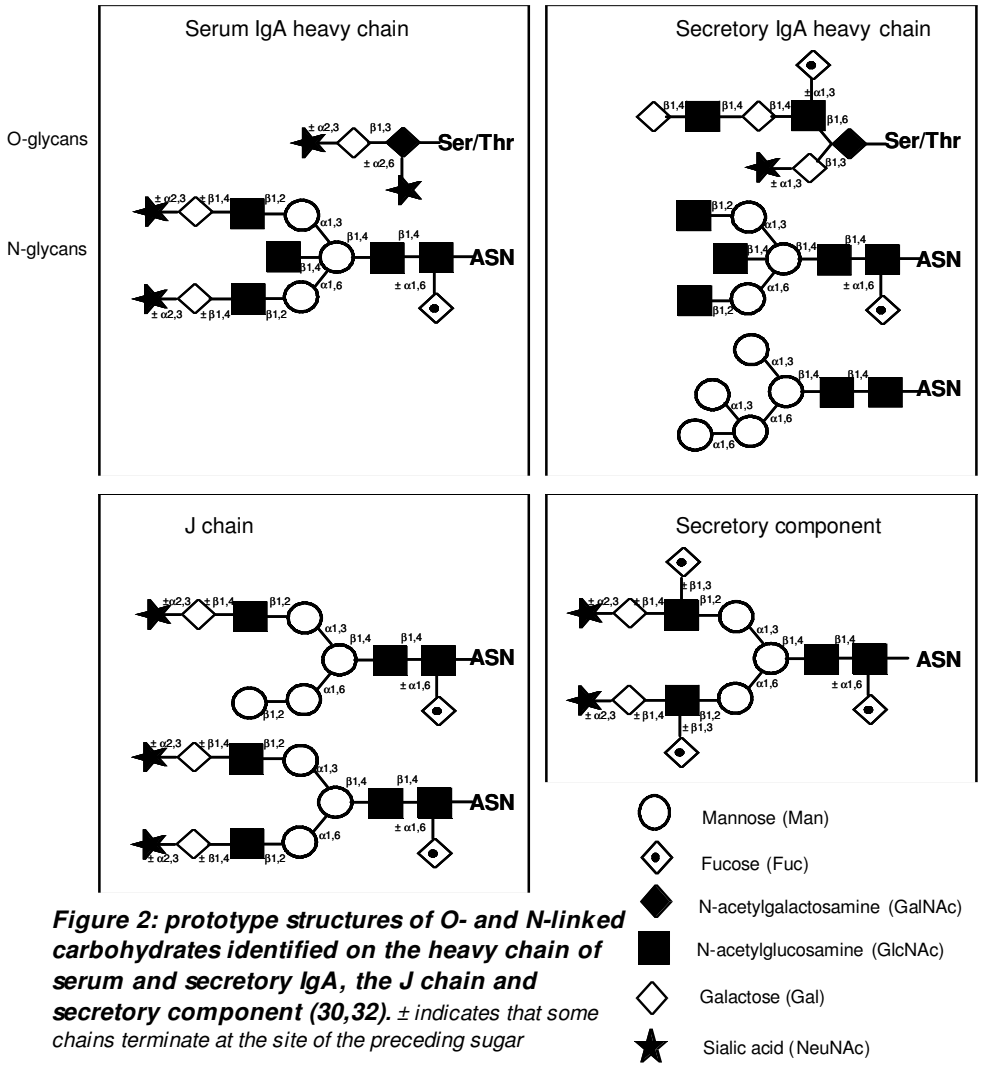

Figure 2: prototype structures of O- and N-linked carbohydrates identified on the heavy chain of serum and secretory IgA, the J chain and secretory component (30,32). *± indicates that some chains terminate at the site of the preceding sugar*

Effector functions of IgA

IgA plays an important role in providing protection at mucosal surfaces. Passive protection by SIgA, secreted by the mucosal immune system, plays a central role in the protection of mucosal surfaces in general. Mechanisms of protection by SIgA at mucosal surfaces are: inhibition of adherence (SIgA appears to surround a microbe and other particulate antigens with a hydrophilic shell that repels attachment to a mucosal surface), agglutination, mucus trapping (SIgA diffuses freely through mucus (35)), neutralization of enzymes and toxins, and interaction with innate antimicrobial factors. On the other site there is increasing evidence that serum IgA is able to trigger effector functions that have the potential to destroy micro-organ-

isms, including: interaction with the complement pathway (although the level of activation differs between isotypes), interaction with Fc receptors on leukocytes, and epithelial cells (Figure 3).

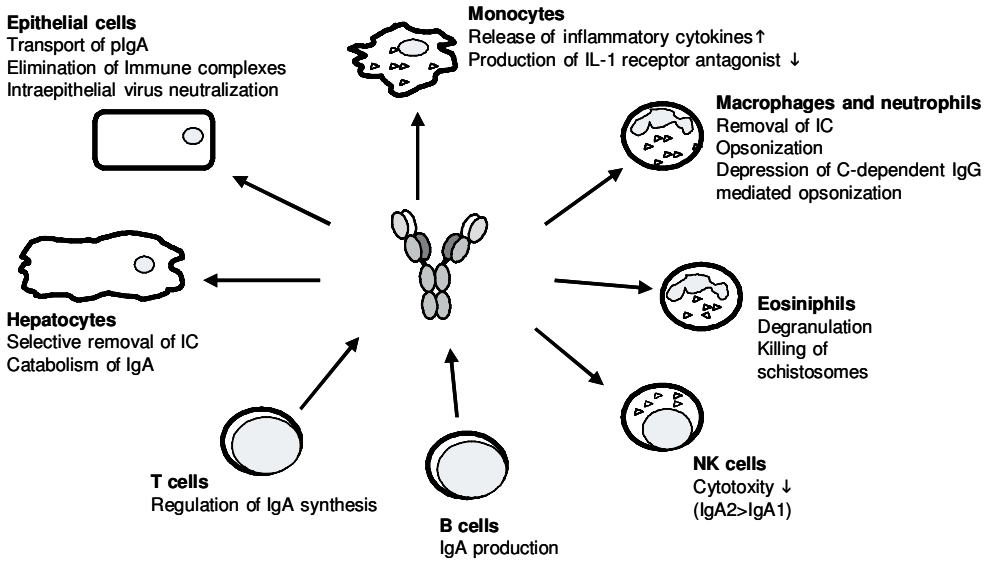

Figure 3: Biological consequences of the interaction of IgA with various cell types (adapted from (30)).

Cells of the myeloid lineage (neutrophils, eosinophils, monocytes, and macrophages) express CD89 through which these cells can be activated by IgA. B cells produce IgA, whereas T cells are important for the regulation of the IgA production. The interaction of IgA with Natural Killer (NK) cells may be mediated by lectin-like receptors for carbohydrate determinants. Epithelial cells transport dIgA to the apical surface where it release SIgA. Hepatocytes are important in the clearance of IgA from the circulation.

Complement activation

The complement system is a key component of our innate immune system and is comprised of a complex of at least 30 proteins and regulators. The liver is the main source of complement synthesis. The complement molecules constitute approximately 5 % of the total serum proteins. Three principle pathways are involved in complement activation, the classical pathway, the alternative pathway and the lectin pathway, each with their own recognition mechanism. These pathways converge at the central component of the complement system, C3. The final common pathway leads to the formation of a protein complex on a complement-activating surface, named the membrane attack complex (MAC) (Figure 4). IgA can activate complement via the alternative pathway and the lectin pathway (36,37). The lectin pathway can be activated via the recognition molecules mannose-binding lectin (MBL), H-ficolin and L-ficolin. IgA can interact with MBL and thereby activate the lectin pathway as demonstrated by activation and deposition of C4 (37), whereas for the ficolins no IgA binding data are available yet.

IgA receptors

Different IgA receptors are described in literature (Table 1). These receptors belong to two major families of receptors, namely the Ig superfamily and the lectin family. The known receptors for IgA in the Ig superfamily are the FcαRI (CD89), the Fcα/μR and the polymeric Ig receptor. The polymeric Ig receptor is present on epithelial cells and is important for the transcytosis of IgA to the mucosal surfaces (38). The known receptors for IgA in the lectin family are the asialoglycoprotein receptor (ASGPR) and the mannose receptor. The ASGPR is present on hepatocytes and is important for clearance of IgA (39). The ligand specificity for ASGPR is terminal galactose. The other IgA receptor in the lectin family, the mannose receptor, is present on dendritic cells and macrophages, and can bind and internalize SIgA without inducing maturation in dendritic cells (40). This binding of SIgA to the mannose receptor is sugar dependent and can be blocked with mannose, fucose and N-acetylglucosamine.

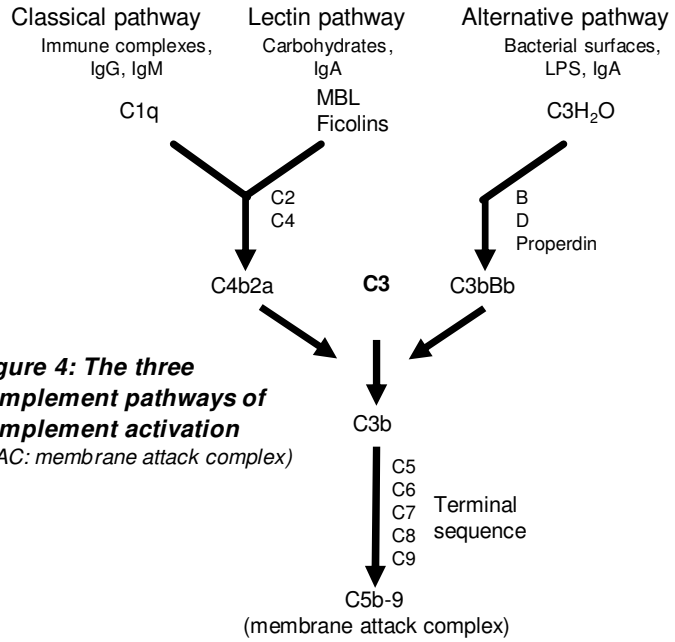

Figure 4: The three complement pathways of complement activation
(MAC: membrane attack complex)

FcαRI (CD89)

FcαRI (CD89) is an IgA receptor, which is constitutively expressed on polymorphonuclear leukocytes (PMN), monocytes, eosinophils, and selected macrophages (41). CD89 is also expressed on Kupffer cells in the liver. It was suggested that CD89 on Kupffer cells provides a second line of defence in mucosal immunity (42). Initially it has been suggested that CD89 might be an IgA receptor at the surface of mesangial cells (43), however it is now widely accepted that CD89 is not expressed by mesangial cells (44-47). CD89 consists of two extracellular Ig-like domains with potential N- and O-linked glycosylation sites, followed by a stretch of hydrophobic

amino acids representing the predicted transmembrane domain, with a positively charged arginine which is essential for association of CD89 with the FcR γ-chain homodimer-signalling subunit (48), and a short cytoplasmic tail devoid of recognition signalling motifs (Figure 5). The protein core of CD89 has a predicted molecular mass of 30 kDa with differential glycosylation at six potential N-linked sites, and the probability of additional O-glycosylation contributing to the variable size observed for the mature receptor, 55-75 kDa on monocytes and neutrophils, 70-100 kDa on eosinophils. The site of interaction between CD89 and IgA was identified in the first extracellular domain of CD89 (49,50) and in the Cα2/Cα3 junction of IgA (51,52).

CD89 participates in different aspects in host defence. CD89 induces phagocytosis of IgA complexed antigens (53), initiates antibody-dependent cellular cytotoxity (54) and CD89 is important for the clearance of IgA from the circulation (48). Upon activation, a soluble form of CD89 is released from the surface of monocytes and monocytic cell lines (55). These soluble CD89 molecules circulate in a complex, covalently linked with IgA, in the high molecular weight fractions of serum IgA (56). Binding studies with different molecular forms of IgA have shown that pIgA binds better to CD89 than mIgA (54,57,58). Furthermore one study suggested that SIgA can only interact with CD89 if MAC-1 (CD11b/ CD18) is present (59).

To study the role of the IgA-CD89 interaction mouse models were used. Although CD89 is described on human myeloid cells, no murine homolog has yet been defined. Therefore transgenic mouse models have been created, including a model in which the CD11b promoter was used (60). In this model human CD89 was highly expressed on macrophages/ monocytes. These transgenic mice develop spontaneously massive mesangial IgA deposits after 12 weeks, suggesting a role for CD89 in IgA nephropathy (60).

Table 1: Receptors with IgA binding capacities

	Receptor	*Ligand*	*Cellular distribution*
Ig family	FcαRI (CD89)	pIgA> mIgA	Myeloid cells
	Fcα/μR	IgA/ IgM	B cells,Macrophages,Mesangial cells
	pIgR	dIgA	Epithelial cells
C-type lectins	ASGPR	IgA	Hepatocytes
	Mannose receptor (CD205)	SIgA	Macrophages,Dendritic cells
	Transferrin receptor (CD71)	IgA1	Mesangial cells

Fcα/μR

The Fcα/μR, located on chromosome 1, is a newly identified receptor for IgA. Transcription of the receptor is demonstrated in several tissues including thymus, spleen (B cells and macrophages, but not on granulocytes, T cells or NK cells), liver, kidney, small and large intestines, testis and placenta (61,62). Furthermore, transcription of the Fcα/μR was described on mesangial cells and was upregulated after stimulation of mesangial cells with IL-1 (63).

The Fcα/μR is a type 1 transmembrane protein with a 32-aa leader sequence, a

423-aa extracellular domain, a 20-aa transmembrane domain and a 60-aa cytoplasmic region. The extracellular domain has four potential sites for NH_2-linked glycosylation (61), leading to a mature protein of 60- 70 kDa. In the extracellular domain of the receptor cysteine residues are identified and it is flanked by the consensus sequence for immunoglobulin-like domains, indicating that this molecule is a member of the immunoglobulin super family. The Fcα/μR shows no significant homology with other proteins. However, in the immunoglobulin like domain there is a motif that is conserved in the first immunoglobulin like domain of the polymeric Ig receptor (Figure 5) (61).

The Fcα/μR mediates endocytosis of immune complex composed of Staphylococcus aureus and IgM anti-S. aureus antibody by primary B lymphocytes (61). The underlying mechanism of this internalization is not yet known. However, experiments with Fcα/μR mutants suggest that the di-leucine motif is important in this process (61). Furthermore, the Fcα/μR acquires the ability to bind IgM and IgA antibodies after stimulation of B cells.

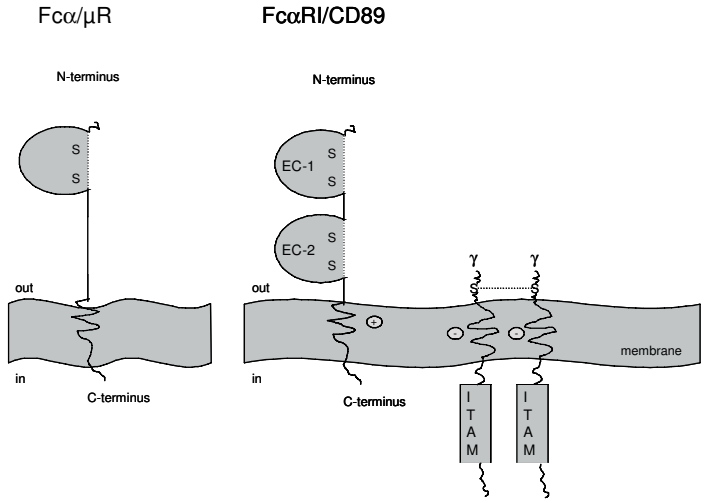

Figure 5: structure of Fcα/μR and of CD89 with the γ-chain.
The extracellular, membrane and cytoplasmic domains of the Fcα/μR and CD89 as well as the γ-chain with its signalling motifs are depicted.

IgA receptors and mesangial cells

The binding of high molecular weight IgA is better to mesangial cells than that of monomeric IgA. However the specific mechanism for the binding and retention of IgA1 remains uncertain. Several findings point to an IgA-specific receptor(s) on mesangial cells (41,64). However none of the known IgA receptors (CD89, ASGPR, pIgR) is expressed on mesangial cells (45,47). Two other receptors have recently emerged as candidate receptors for binding IgA; CD71 (transferrin receptor) and the Fcα/μR (63,65,66). CD71 expression is enhanced in the glomeruli of IgA nephropathy patients and co-localizes with IgA1 deposits. Mesangial cells bind both IgA sub-

classes, whereas CD71 binds only IgA1, suggesting involvement of an additional receptor. Theoretically, it may be the Fcα/μR that can be transcribed by mesangial cells in vitro (63). In contrast, another group found that polymeric IgA1 from IgA nephropathy patients induces macrophage migration inhibitory factor (MIF) and TNF-α in mesangial cells. This induction is probably through an unidentified IgA receptor, as shown by failure to suppress IgA-induced MIF synthesis by blocking IgA receptors with specific antibodies or various ligands to IgA receptors (67).

IgA in IgA nephropathy

Several studies using lectin interactions (Table 2) and fluorophore-assisted carbohydrate electrophoresis (FACE) focused on the analysis of IgA glycosylation, showing aberrant O-glycosylation in circulating IgA from IgAN patients, resulting in increased Tn antigen (GalNAcβ1-Ser/Thr) residues (68-71). This undergalactosylated IgA1 may lead to recognition by IgG antibodies and generation of circulating IgG-IgA1 complexes (72). Furthermore, altered interaction with mesangial cells has been described (73). This aberrantly O-glycosylated IgA is suggested to be dependent on a hampered function of the β1-3 galactosyltransferase (74). Furthermore, it is suggested that downregulation of the β1-3 galactosyltransferase chaperone (Cosmc) is important for the aberrant O-glycosylation in patients with IgAN (75).

Table 2: Reactivity of lectins used for analysis of IgA glycosylation

Name	*Abbreviation*	*Sugar specificity*
Artocarpus integrifolia	Jacalin	Galβ1-3GalNAc
Helix aspersa	HAA	GalNAc
Vicia villosa	VV	GalNAc
Helix pomatia	HPO	GalNAc
Erythrina crystagalli	ECL	Galβ1-4GlcNAc
Ricinus communis	RCA	βGal
Sambucus nigra	SNA	NeuNAc
Peanut agglutinin	PNA	Galβ1-3GalNAc

Levels of plasma IgA1 are elevated in about half of the patients with IgAN (76-78), which appears to be the result of an increased production of this isotype by the bone marrow (79-82) and by a low elimination rate by the liver. Mucosal pIgA plasma cell numbers are normal or even reduced in IgAN (83,84), whereas pIgA antibody levels in mucosal secretions are not elevated and are sometimes lower than controls (9). Furthermore, systemic antigen challenge results in increased titers of circulating pIgA1 antibodies (85,86) with normal levels in mucosal secretions (87).

IgA and mesangial cells

Functional studies with purified IgA from IgAN patients showed that IgA from IgAN patients binds better to mesangial cells than IgA healthy individuals (73),

although this is still controversial (88). The binding of polymeric and aggregated IgA to mesangial cells was stronger as compared to monomeric IgA. Moreover, polymeric IgA with the highest net negative charge is superior in binding to mesangial cells (73). In IgAN circulating aberrantly glycosylated IgA1 has been described. To mimic this IgA, IgA1 was purified with Jacalin and in vitro degalactosylated. The removal of galactose residues from IgA1 isolated with Jacalin increases binding to mesangial cells in vitro (89).

The activation of mesangial cells by IgA1 immune complexes is considered the initiating event in the pathogenesis of IgA nephropathy. Mesangial cell activation was observed in vitro in many instances (90-93). Exposure of mesangial cells to IgA is capable of initiating a proinflammatory cascade involving mesangial cell secretion of IL-1β, TNF-α , IL-6, TGF-β and MIF and the release of the chemokines MCP-1 (CCL2), IL-8, and IP-10 (91,94-97). After stimulation of mesangial cells with degalactosylated IgA the production of these factors is higher as compared to control IgA. In vivo, urinary IL-6 (98), the tubular and interstitial expression of intercellular adhesion molecule type 1 (99), and the intrarenal expression of proinflammatory cytokines and chemokines (100) correlated with renal injury and may have prognostic value.

IgA is also capable of altering mesangial cell-matrix interactions by modulating integrin expression, and this could have an important role in remodeling of the mesangium following glomerular injury (101). There is also evidence that activation of mesangial cells by co-deposited IgG could synergistically contribute to the development of a proinflammatory mesangial cell phenotype and thereby influence the degree of glomerular injury (102). It is not yet clear which specific physicochemical properties of mesangial IgA affect mesangial cell activation; however, there is some in vitro evidence that undergalactosylated IgA glycoforms from patients with IgAN reduce proliferation, increase nitric oxide synthesis and the rate of apoptosis, and enhance integrin synthesis in cultured mesangial cells (101,103). This, together with the overrepresentation of aberrantly glycosylated IgA1 in mesangial IgA, suggests that IgA1 O-glycosylation plays a role in both the deposition of IgA and the subsequent injury.

Scope of this thesis

For a better understanding of the role of IgA and mesangial cells in IgA nephropathy, we focused on different questions in the course of the disease. In chapter 2 and 3 we focused on the possible receptor mechanisms underlying mesangial IgA deposition. Therefore we studied the interaction of IgA with CD89 in different binding assays. We showed a similar association to CD89 for monomeric and polymeric IgA (chapter 2). CD89 is described not to be present on mesangial cells, whereas the recently identified Fcα/μR is suggested to be expressed by mesangial cells. To investigate the role of the Fcα/μR in IgA nephropathy we produced fusion proteins of this receptor and used these fusion proteins for IgA binding studies (chapter 3).

Because it is suggested that IgAN is not only a disease of the kidney, but also dependent on systemic factors we investigated in chapter 4 to chapter 8 which changes in IgA lead to the deposition of IgA in the glomeruli. Therefore, we investi-

gated in chapter 4 the activation of the lectin pathway of complement via IgA in glomeruli of IgAN patients. We show that activation of the lectin pathway of complement in the glomeruli of patients with IgAN is associated with more severe renal disease. In chapter 5 we isolated IgA from patients and controls and separated this IgA in monomeric and polymeric IgA. With these IgA preparations we investigated the differences between monomeric and polymeric IgA between patients and controls including the interaction with lectins and mesangial cells. In this study we observed clear differences between monomeric and polymeric IgA for lectin and mesangial cell interactions, but there were no differences between patients and controls. However, the concentration of SIgA in the polymeric IgA preparations was significantly higher in patients as compared to controls. This suggests that only a minor part of the IgA from patients might be different from controls. Finally, we demonstrated that SIgA is able to bind stronger to mesangial cells than serum IgA, and that SIgA is present in glomerular IgA deposits (chapter 6). To confirm the presence of SIgA in the glomerular IgA deposits we studied the presence of SIgA in biopsies from IgAN patients. We showed in chapter 7 that in 15 % of the cases SIgA is detectable in the glomerular IgA deposits. Finally, chapter 8 summarizes the studies described in this thesis and discusses the relevance of these new findings.

REFERENCES

1. Berger J, Hinglais N: Les depots intercapillaires d'IgA-IgG. J Urol Nephrol (Paris) 74:694-695, 1968
2. Donadio JV, Grande JP: IgA nephropathy. N Engl J Med 347:738-748, 2002
3. Feehally J: IgA nephropathy--a disorder of IgA production? QJM 90:387-390, 1997
4. Floege J, Feehally J: IgA nephropathy: recent developments. J Am Soc Nephrol 11:2395-2403, 2000
5. Van Es LA, de Fijter JW, Daha MR: Pathogenesis of IgA nephropathy. Nephrology 3:3-12, 1997
6. Sanfilippo F, Croker BP, Bollinger RR: Fate of four cadaveric donor renal allografts with mesan gial IgA deposits. Transplantation 33:370-376, 1982
7. Silva FG, Chander P, Pirani CL et al.: Disappearance of glomerular mesangial IgA deposits after renal allograft transplantation. Transplantation 33:241-246, 1982
8. Russell MW, Lue C, van den Wall Bake AW et al.: Molecular heterogeneity of human IgA anti bodies during an immune response. Clin Exp Immunol 87:1-6, 1992
9. de Fijter JW, Eijgenraam JW, Braam CA et al.: Deficient IgA1 immune response to nasal cholera toxin subunit B in primary IgA nephropathy. Kidney Int 50:952-961, 1996
10. Haas M: Histology and immunohistology of IgA nephropathy. J Nephrol 18:676-680, 2005
11. Arima S, Nakayama M, Naito M et al.: Significance of mononuclear phagocytes in IgA nephropathy. Kidney Int 39:684-692, 1991
12. Li HL, Hancock WW, Hooke DH et al.: Mononuclear cell activation and decreased renal func tion in IgA nephropathy with crescents. Kidney Int 37:1552-1556, 1990
13. Conley ME, Cooper MD, Michael AF: Selective deposition of immunoglobulin A1 in immunoglobulin A nephropathy, anaphylactoid purpura nephritis, and systemic lupus erythe matosus. J Clin Invest 66:1432-1436, 1980
14. Valentijn RM, Radl J, Haaijman JJ et al.: Circulating and mesangial secretory component-bind ing IgA-1 in primary IgA nephropathy. Kidney Int 26:760-766, 1984
15. Russell MW, Mestecky J, Julian BA et al.: IgA-associated renal diseases: antibodies to envi

ronmental antigens in sera and deposition of immunoglobulins and antigens in glomeruli. J Clin Immunol 6:74-86, 1986
16. Allen AC, Bailey EM, Brenchley PE et al.: Mesangial IgA1 in IgA nephropathy exhibits aberrant O-glycosylation: observations in three patients. Kidney Int 60:969-973, 2001
17. Hiki Y, Odani H, Takahashi M et al.: Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. Kidney Int 59:1077-1085, 2001
18. Monteiro RC, Halbwachs-Mecarelli L, Roque-Barreira MC et al.: Charge and size of mesangial IgA in IgA nephropathy. Kidney Int 28:666-671, 1985
19. Egido J, Sancho J, Mampaso F et al.: A possible common pathogenesis of the mesangial IgA glomerulonephritis in patients with Berger's disease and Schonlein-Henoch syndrome. Proc Eur Dial Transplant Assoc 17:660-666, 1980
20. Komatsu N, Nagura H, Watanabe K et al.: Mesangial deposition of J chain-linked polymeric IgA in IgA nephropathy. Nephron 33:61-64, 1983
21. Conley ME, Delacroix DL: Intravascular and mucosal immunoglobulin A: two separate but relat ed systems of immune defense? Ann Intern Med 106:892-899, 1987
22. Delacroix DL, Dive C, Rambaud JC et al.: IgA subclasses in various secretions and in serum. Immunology 47:383-385, 1982
23. Conley ME, Koopman WJ: Serum IgA1 and IgA2 in normal adults and patients with systemic lupus erythematosus and hepatic disease. Clin Immunol Immunopathol 26:390-397, 1983
24. van der Boog PJ, van Kooten C, de Fijter JW et al.: Role of macromolecular IgA in IgA nephropathy. Kidney Int 67:813-821, 2005
25. Endo T, Radl J, Mestecky J: Structural differences among serum IgA proteins of chim panzee, rhesus monkey and rat origin. Mol Immunol 34:557-565, 1997
26. Delacroix DL, Vaerman JP: A solid phase, direct competition, radioimmunoassay for quantita tion of secretory IgA in human serum. J Immunol Methods 40:345-358, 1981
27. Thompson RA, Asquith P, Cooke WT: Secretory IgA in the serum. Lancet 2:517-519, 1969
28. Endo T, Mestecky J, Kulhavy R et al.: Carbohydrate heterogeneity of human myeloma proteins of the IgA1 and IgA2 subclasses. Mol Immunol 31:1415-1422, 1994
29. Mattu TS, Pleass RJ, Willis AC et al.: The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fc alpha recep tor interactions. J Biol Chem 273:2260-2272, 1998
30. Mestecky J, Moro B, Underdown BJ: Mucosal Immunoglobulins, chap. 9, in Mucosal Immunology, 2 ed., edited by Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR, San Diego, Academic Press, 1999, pp 133-152
31. Field MC, Amatayakul-Chantler S, Rademacher TW et al.: Structural analysis of the N-glycans from human immunoglobulin A1: comparison of normal human serum immunoglobulin A1 with that isolated from patients with rheumatoid arthritis. Biochem J 299 (Pt 1):261-275, 1994
32. Royle L, Roos A, Harvey DJ et al.: Secretory IgA N- and O-Glycans Provide a Link between the Innate and Adaptive Immune Systems. J Biol Chem 278:20140-20153, 2003
33. Krugmann S, Pleass RJ, Atkin JD et al.: Structural requirements for assembly of dimeric IgA probed by site-directed mutagenesis of J chain and a cysteine residue of the alpha-chain CH2 domain. J Immunol 159:244-249, 1997
34. Mizoguchi A, Mizuochi T, Kobata A: Structures of the carbohydrate moieties of secretory com ponent purified from human milk. J Biol Chem 257:9612-9621, 1982
35. Saltzman WM, Radomsky ML, Whaley KJ et al.: Antibody diffusion in human cervical mucus. Biophys J 66:508-515, 1994
36. Hiemstra PS, Gorter A, Stuurman ME et al.: Activation of the alternative pathway of comple

ment by human serum IgA. Eur J Immunol 17:321-326, 1987
37. Roos A, Bouwman LH, Gijlswijk-Janssen DJ et al.: Human IgA activates the complement sys tem via the mannan-binding lectin pathway. J Immunol 167:2861-2868, 2001
38. Kaetzel CS, Robinson JK, Chintalacharuvu KR et al.: The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. Proc Natl Acad Sci U S A 88:8796-8800, 1991
39. Stockert RJ: The asialoglycoprotein receptor: relationships between structure, function, and expression. Physiol Rev 75:591-609, 1995
40. Heystek HC, Moulon C, Woltman AM et al.: Human immature dendritic cells efficiently bind and take up secretory IgA without the induction of maturation. J Immunol 168:102-107, 2002
41. Monteiro RC, van de Winkel JG: IgA Fc Receptors. Annu Rev Immunol 21:177-204, 2003
42. van Egmond M, van Garderen E, van Spriel AB et al.: FcalphaRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. Nat Med 6:680-685, 2000
43. Gomez-Guerrero C, Gonzalez E, Egido J: Evidence for a specific IgA receptor in rat and human mesangial cells. J Immunol 151:7172-7181, 1993
44. Barratt J, Greer MR, Pawluczyk IZ et al.: Identification of a novel Fcalpha receptor expressed by human mesangial cells. Kidney Int 57:1936-1948, 2000
45. Westerhuis R, van Zandbergen G, Verhagen NA et al.: Human mesangial cells in culture and i in kidney sections fail to express Fc alpha receptor (CD89). J Am Soc Nephrol 10:770-778, 1999
46. Diven SC, Caflisch CR, Hammond DK et al.: IgA induced activation of human mesangial cells: independent of FcalphaR1 (CD 89). Kidney Int 54:837-847, 1998
47. Leung JC, Tsang AW, Chan DT et al.: Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. J Am Soc Nephrol 11:241-249, 2000
48. Morton HC, van den Herik-Oudijk IE, Vossebeld P et al.: Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. J Biol Chem 270:29781-29787, 1995
49. Wines BD, Hulett MD, Jamieson GP et al.: Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. J Immunol 162:2146-2153, 1999
50. Morton HC, van Zandbergen G, van Kooten C et al.: Immunoglobulin-binding sites of human FcalphaRI (CD89) and bovine Fcgamma2R are located in their membrane-distal extracellular domains. J Exp Med 189:1715-1722, 1999
51. Carayannopoulos L, Hexham JM, Capra JD: Localization of the binding site for the monocyte immunoglobulin (Ig) A-Fc receptor (CD89) to the domain boundary between Calpha2 and Calpha3 in human IgA1. J Exp Med 183:1579-1586, 1996
52. Pleass RJ, Dunlop JI, Anderson CM et al.: Identification of residues in the CH2/CH3 domain interface of IgA essential for interaction with the human fcalpha receptor (FcalphaR) CD89. J Biol Chem 274:23508-23514, 1999
53. Gorter A, Hiemstra PS, Leijh PC et al.: IgA- and secretory IgA-opsonized S. aureus induce a respiratory burst and phagocytosis by polymorphonuclear leucocytes. Immunology 61:303-309, 1987
54. Fanger MW, Shen L, Pugh J et al.: Subpopulations of human peripheral granulocyes and monocytes express receptors for IgA. Proc Natl Acad Sci U S A 77:3640-3644, 1980
55. van Zandbergen G, Westerhuis R, Mohamad NK et al.: Crosslinking of the human Fc recep tor for IgA (FcalphaRI/CD89) triggers FcR gamma-chain-dependent shedding of soluble CD89. J Immunol 163:5806-5812, 1999

56. van der Boog PJ, van Zandbergen G, de Fijter JW et al.: Fc alpha RI/CD89 circulates in human serum covalently linked to IgA in a polymeric state. J Immunol 168:1252-1258, 2002
57. Reterink TJ, van Zandbergen G, van Egmond M et al.: Size-dependent effect of IgA on the IgA Fc receptor (CD89). Eur J Immunol 27:2219-2224, 1997
58. van Zandbergen G, van Kooten C, Mohamad NK et al.: Reduced binding of immunoglobulin A (IgA) from patients with primary IgA nephropathy to the myeloid IgA Fc-receptor, CD89. Nephrol Dial Transplant 13:3058-3064, 1998
59. van Spriel AB, Leusen JH, Vile H et al.: Mac-1 (CD11b/CD18) as Accessory Molecule for FcalphaR (CD89) Binding of IgA. J Immunol 169:3831-3836, 2002
60. Launay P, Grossetete B, Arcos-Fajardo M et al.: Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice. J Exp Med 191:1999-2009, 2000
61. Shibuya A, Sakamoto N, Shimizu Y et al.: Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes. Nat Immunol 1:441-446, 2000
62. Sakamoto N, Shibuya K, Shimizu Y et al.: A novel Fc receptor for IgA and IgM is expressed on both hematopoietic and non-hematopoietic tissues. Eur J Immunol 31:1310-1316, 2001
63. McDonald KJ, Cameron AJ, Allen JM et al.: Expression of Fc alpha/mu receptor by human mesangial cells: a candidate receptor for immune complex deposition in IgA nephropathy. Biochem Biophys Res Commun 290:438-442, 2002
64. Novak J, Julian BA, Tomana M et al.: Progress in molecular and genetic studies of IgA nephropathy. J Clin Immunol 21:310-327, 2001
65. Moura IC, Centelles MN, Arcos-Fajardo M et al.: Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. J Exp Med 194:417-425, 2001
66. Haddad E, Moura IC, Arcos-Fajardo M et al.: Enhanced Expression of the CD71 Mesangial IgA1 Receptor in Berger Disease and Henoch-Schonlein Nephritis: Association between CD71 Expression and IgA Deposits. J Am Soc Nephrol 14:327-337, 2003
67. Leung JC, Tang SC, Chan LY et al.: Polymeric IgA increases the synthesis of macrophage migration inhibitory factor by human mesangial cells in IgA nephropathy. Nephrol Dial Transplant 18:36-45, 2003
68. Allen AC, Bailey EM, Barratt J et al.: Analysis of IgA1 O-glycans in IgA nephropathy by fluorophore-assisted carbohydrate electrophoresis. J Am Soc Nephrol 10:1763-1771, 1999
69. Allen AC: Methodological approaches to the analysis of IgA1 O-glycosylation in IgA nephropathy. J Nephrol 12:76-84, 1999
70. Hiki Y, Tanaka A, Kokubo T et al.: Analyses of IgA1 hinge glycopeptides in IgA nephropathy by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Am Soc Nephrol 9:577-582, 1998
71. Coppo R, Amore A: Aberrant glycosylation in IgA nephropathy (IgAN). Kidney Int 65:1544-1547, 2004
72. Tomana M, Novak J, Julian BA et al.: Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. J Clin Invest 104:73-81, 1999
73. Leung JC, Tang SC, Lam MF et al.: Charge-dependent binding of polymeric IgA1 to human mesangial cells in IgA nephropathy. Kidney Int 59:277-285, 2001
74. Allen AC, Topham PS, Harper SJ et al.: Leucocyte beta 1,3 galactosyltransferase activity in IgA nephropathy. Nephrol Dial Transplant 12:701-706, 1997

75. Qin W, Zhou Q, Yang LC et al.: Peripheral B lymphocyte beta1,3-galactosyltrans ferase and chaperone expression in immunoglobulin A nephropathy. J Intern Med 258:467-477, 2005
76. van den Wall Bake AW, Daha MR, van der AA et al.: Serum levels and in vitro production of IgA subclasses in patients with primary IgA nephropathy. Clin Exp Immunol 74:115-120, 1988
77. Delacroix DL, Elkom KB, Geubel AP et al.: Changes in size, subclass, and metabolic proper ties of serum immunoglobulin A in liver diseases and in other diseases with high serum immunoglobulin A. J Clin Invest 71:358-367, 1983
78. Peterman JH, Julian BA, Kirk KA et al.: Selective elevation of monomeric IgA1 in IgA nephropathy patients with normal renal function. Am J Kidney Dis 18:313-319, 1991
79. Harper SJ, Allen AC, Layward L et al.: Increased immunoglobulin A and immunoglobulin A1 cells in bone marrow trephine biopsy specimens in immunoglobulin A nephropathy. Am J Kidney Dis 24:888-892, 1994
80. van den Wall Bake AW, Daha MR, Evers-Schouten J et al.: Serum IgA and the production of IgA by peripheral blood and bone marrow lymphocytes in patients with primary IgA nephropa thy: evidence for the bone marrow as the source of mesangial IgA. Am J Kidney Dis 12:410-414, 1988
81. van den Wall Bake AW, Daha MR, Radl J et al.: The bone marrow as production site of the IgA deposited in the kidneys of patients with IgA nephropathy. Clin Exp Immunol 72:321-325, 1988
82. van den Wall Bake AW, Daha MR, Haaijman JJ et al.: Elevated production of polymeric and monomeric IgA1 by the bone marrow in IgA nephropathy. Kidney Int 35:1400-1404, 1989
83. Westberg NG, Baklien K, Schmekel B et al.: Quantitation of immunoglobulin-producing cells in small intestinal mucosa of patients with IgA nephropathy. Clin Immunol Immunopathol 26:442-445, 1983
84. Harper SJ, Pringle JH, Wicks AC et al.: Expression of J chain mRNA in duodenal IgA plasma cells in IgA nephropathy. Kidney Int 45:836-844, 1994
85. van den Wall Bake AW, Beyer WE, Evers-Schouten JH et al.: Humoral immune response to influenza vaccination in patients with primary immunoglobulin A nephropathy. An analysis of isotype distribution and size of the influenza-specific antibodies. J Clin Invest 84:1070-1075, 1989
86. Layward L, Allen AC, Harper SJ et al.: Increased and prolonged production of specific polymer ic IgA after systemic immunization with tetanus toxoid in IgA nephropathy. Clin Exp Immunol 88:394-398, 1992
87. Layward L, Finnemore AM, Allen AC et al.: Systemic and mucosal IgA responses to systemic antigen challenge in IgA nephropathy. Clin Immunol Immunopathol 69:306-313, 1993
88. Wang Y, Zhao MH, Zhang YK et al.: Binding capacity and pathophysiological effects of IgA1 from patients with IgA nephropathy on human glomerular mesangial cells. Clin Exp Immunol 136:168-175, 2004
89. Novak J, Vu HL, Novak L et al.: Interactions of human mesangial cells with IgA and IgA-con taining immune complexes. Kidney Int 62:465-475, 2002
90. Chen A, Chen WP, Sheu LF et al. : Pathogenesis of IgA nephropathy: in vitro activation of human mesangial cells by IgA immune complex leads to cytokine secretion. J Pathol 173:119-126, 1994
91. Duque N, Gomez-Guerrero C, Egido J: Interaction of IgA with Fc alpha receptors of human mesangial cells activates transcription factor nuclear factor-kappa B and induces expression and synthesis of monocyte chemoattractant protein-1, IL-8, and IFN-inducible protein 10. J Immunol 159:3474-3482, 1997
92. Fujii K, Muller KD, Clarkson AR et al.: The effect of IgA immune complexes on the proliferation

of cultured human mesangial cells. Am J Kidney Dis 16:207-210, 1990
93. Haas CS, Schocklmann HO, Lang S et al.: Regulatory mechanism in glomerular mesangial cell proliferation. J Nephrol 12:405-415, 1999
94. Monteiro RC, Moura IC, Launay P et al.: Pathogenic significance of IgA receptor interactions in IgA nephropathy. Trends Mol Med 8:464-468, 2002
95. van den Dobbelsteen ME, van der Woude FJ, Schroeijers WE et al.: Binding of dimeric and polymeric IgA to rat renal mesangial cells enhances the release of interleukin 6. Kidney Int 46:512-519, 1994
96. Gomez-Guerrero C, Lopez-Armada MJ, Gonzalez E et al.: Soluble IgA and IgG aggregates are catabolized by cultured rat mesangial cells and induce production of TNF-alpha and IL-6, and proliferation. J Immunol 153:5247-5255, 1994
97. Lai KN, Tang SC, Guh JY et al.: Polymeric IgA1 from Patients with IgA Nephropathy Upregulates Transforming Growth Factor-beta Synthesis and Signal Transduction in Human Mesangial Cells via the Renin-Angiotensin System. J Am Soc Nephrol 14:3127-3137, 2003
98. Harada K, Akai Y, Kurumatani N et al.: Prognostic value of urinary interleukin 6 in patients with IgA nephropathy: an 8-year follow-up study. Nephron 92:824-826, 2002
99. Arrizabalaga P, Sole M, Abellana R et al.: Tubular and Interstitial Expression of ICAM-1 as a Marker of Renal Injury in IgA Nephropathy. Am J Nephrol121-128, 2003
100. Lim CS, Yoon HJ, Kim YS et al. : Clinicopathological correlation of intrarenal cytokines and chemokines in IgA nephropathy. Nephrology (Carlton) 8:21-27, 2003
101. Peruzzi L, Amore A, Cirina P et al.: Integrin expression and IgA nephropathy: in vitro modula tion by IgA with altered glycosylation and macromolecular IgA. Kidney Int 58:2331-2340, 2000
102. van Dixhoorn MG, Sato T, Muizert Y et al.: Combined glomerular deposition of polymeric rat IgA and IgG aggravates renal inflammation. Kidney Int 58:90-99, 2000
103. Amore A, Cirina P, Conti G et al.: Glycosylation of circulating IgA in patients with IgA nephropa thy modulates proliferation and apoptosis of mesangial cells. J Am Soc Nephrol 12:1862-1871, 2001
104. Mostov KE, Kaetzel CS: Immunoglobulin transport and the polymeric immunoglobulin recep tor, chap.12, in Mucosal Immunology, 2 ed., edited by Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR, San Diego, Academic Press, 1999, pp 181-211