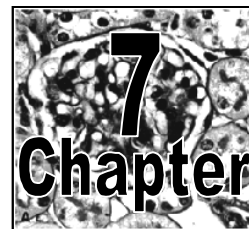


***GENERAL DISCUSSION
AND CONCLUSIONS***



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I. INTRODUCTION

A multitude of pathogenetic mechanisms has been suggested to be relevant and may be crucial during the course of IgA nephropathy (IgAN). Defective B cell activation after mucosal infection leads to an aberrant immunoglobulin production especially for the IgA-isotype and may result in increased levels of IgA in the circulation of patients with IgAN. Accordingly clinical relapses of IgAN, indicated by (macro-) hematuria, are associated with mucosal infections (1-3). On the other hand in the last years interest has focussed on abnormal IgA1 glycosylation of the hinge region of this immunoglobulin subclass. Structural investigations to analyze IgA1 O-glycans in IgA nephropathy and controls demonstrate that in IgAN, the IgA1 O-glycan chains are truncated, with increased terminal GalNAc (4). Other investigators found that the deficiency of galactose in the hinge region of the IgA1 molecule results in the generation of antigenic determinants containing GalNAc residues that are recognized by naturally occurring IgG and IgA1 antibodies. Nevertheless the relevance of immune complexes is not clear (5,6). Elevated plasma IgA1 in patients with IgAN could also be related with its different glycosylation of the hinge region. This is suggested to be important for interaction of IgA1 with receptors such as the asialoglycoproteinreceptor (7). Reduced binding of IgA1 to the asialoglycoproteinreceptor expressed on hepatocytes could be responsible for a slower clearance of IgA from the circulation and could result in higher serum IgA-levels (8). The other main clearance pathway is via Fc receptors for IgA expressed on circulating myeloid cells, which are downregulated in IgAN (9). This could lead to deposition of IgA in the renal mesangium. Furthermore it has been speculated that deficient glycosylation could lead to a higher capacity of IgA1 to aggregate to macromolecular forms of IgA1. Also its capacity to interact with extracellular matrix proteins as well as its capability in activating complement could be impaired and could therefore be an explanation of chronic inflammatory processes during IgAN (10,11).

The deposition of IgA in the glomerulus considered, as the major characteristic of IgA-nephropathy is still not explained (12,13). Deposition of IgA leads to complement activation via the alternative pathway followed by influx of inflammatory cells increased cytokine release by resident and infiltrating cells. This inflammatory response is followed by upregulation of mesangial matrix-production and mesangial cell proliferation (10,14,15).

II. RECEPTOR-MEDIATED BINDING OF IGA TO MESANGIAL CELLS

Increasing evidence on the existence of a mesangial receptor for IgA is supported by the observation that IgA is able to bind to rat and human mesangial cells and that bound IgA can be internalized and degraded by these cells (16,17,17,18,18,19,20). Binding of 125I-IgA to quiescent human MC showed 2.55×10^5 sites/cell with an affinity (K_a) of $3.2 \times 10^7 \text{ M}^{-1}$. Addition of selected recombinant cytokines had no significant influence on K_a , but increased the number of sites/cell relative to unstimulated cells (24). MC incubation with aggregated IgA (aIgA) elicited a dose-dependent increase in cytosolic Ca^{2+} . This effect was dependent on the Fc region of IgA, because Fc, but neither Fab fragment nor carbohydrates, inhibited the Ca^{2+} rise. The initial induction of Ca^{2+} rise was due to Ca^{2+} mobilization from inositol trisphosphate (IP3)-sensitive intracellular stores, while sustained levels were maintained through extracellular Ca^{2+} influx. Protein tyrosine kinase inhibitors abolished Ca^{2+} rise, indicating that tyrosine phosphorylation of some substrates is required for Ca^{2+} mobilization (21). Furthermore binding of IgA to MC leads to activation of MC and subsequently to increased expression of the transcriptional factor NF- κ B (22), c-jun (23), interleukin-6 (IL-6) (16-20,20), IL-8 (22), MCP-1 (22) and Tumor Necrosis Factor- α (TNF- α) (16).

Also most of these effects of IgA seems to be related to the Fc part of IgA: Investigations of Duque et al. (22) shows that aggregated IgA induces activation of the transcriptional factor NF- κ B and increases the expression of monocyte chemoattractant-protein 1, interleukin-8 and gamma-interferon induced protein 10. This effect was absent after preincubation of mesangial cells with Fc-fragments of IgA indicating that binding of IgA to mesangial cells is mediated via the Fc portion of this immunoglobulin. These findings strongly indicate the existence of a Fc-receptor for IgA on human MC.

The involvement of the known myeloid Fc receptor for IgA (CD89) on mesangial cells in the pathogenesis of IgA-nephropathy has been controversial (22-25). In chapter 4 a study is presented, investigating the expression of CD89 on human mesangial cells *in vivo* and *in vitro*. IgA blocking experiments demonstrated that IgA do not bind to this cell type via CD89. Even more immunohistochemical and flowcytometric experiments revealed no protein expression of CD89 neither on mesangial cells *in vitro* nor in human kidney section *in vivo* using known antibodies for CD89 as well as new generated monoclonal and polyclonal antibodies against this receptor. Furthermore also mRNA expression was not

detectable using RT-and nested RT-PCR. In summary the known Fc-receptor for IgA as well as other potentially IgA-binding proteins as stated in the introduction are not expressed on human mesangial cells.

Recently, a British group showed expression of mRNA transcripts with partial identity to CD89 on human MC in vitro using RT-PCR(26). The detected transcripts were located in the area of the extracellular domain 2 of CD89 which makes it unlikely that binding of IgA is mediated via these transcripts because it has been shown that binding of IgA to CD89 is mediated via the extracellular domain 1(27). Even more there was no protein expression detectable on the surface of mesangial cells as well as in culture medium using Western blot. However, the transcript fragments they identified may encode a new protein with partial homology to CD89. This hypothesis originates from the recent discovery of genes mapping to chromosome 19Q13.4 and encoding a family of type I transmembrane proteins with significant homology: CD89, paired Ig-like receptors (PIRs), killer inhibitor receptors (KIRs), leukocyte Ig-like receptor (LIRs), and Ig-like transcripts (ILTs) (28).

To identify the putative IgA-Fc receptor we generated an antibody directed against an IgA-binding protein expressed on human neutrophils as well as on human mesangial cells (Chapter 5). This monoclonal mouse antibody designated 72G12 is able to block binding of IgA to human mesangial cells. The recognized antigen is furthermore expressed on monocytes and even more on monocyte derived dendritic cells. Precipitation studies of membrane surface proteins from human mesangial cells demonstrated an 83 KD protein recognized by 72G12, which is also precipitated by human IgA. Further characterization of this potential IgA-receptor is needed to clarify its potential role during IgAN. Most important questions are possible difference in expression of this receptor in the glomerulus of patients with IgA-nephropathy and controls. It is of great interest to investigate its ability in binding of IgA, especially those with defective glycosylation of the hinge region and IgA-immunocomplexes as described in IgAN (5,6).

III. SHEDDING OF CD89/FC α RI

Soluble forms have been identified for various Fc receptors for IgG (Fc γ RII/CD32 and Fc γ RIII/CD16) and IgE (Fc ϵ RII/CD23) (29-32). Furthermore, it has been proposed that these soluble Fc receptors have a pathophysiological role in several diseases (33-35).

We investigated mechanisms involved in release of soluble CD89 from the surface of monocytic cell lines (Chapter 6). It was found that upon activation, myeloid cells could release a soluble form of Fc α RI/CD89. Western blot analysis

showed this soluble receptor to represent a 30-kD glycosylated protein that is capable of binding IgA. Both IgA and anti-CD89 antibodies induced the release of sCD89, which suggests that IgA antibodies produced during a mucosal immune response might have a regulatory effect on the CD89 effector functions. The 30 kD soluble CD89 molecule is released in a FcR γ -chain dependent manner from monocytic cell lines as indicated by experiments done with the CD89 transfected mouse cell line IIA1.6 which was alternatively cotransfected with the FcR γ -chain. Shedding of sCD89 was dependent on signaling via the γ -chain and prevented by addition of inhibitors of protein kinase C (staurosporine) or protein tyrosine kinases. Next to ligand-induced release of CD89 also pharmacologic activation by ionomycin and phorbol-myristate acetate as well as activation of the cell by tumor necrosis factor α leads to shedding of this receptor.

Recently Monteiro and coworkers reported about the presence of circulating soluble CD89-IgA complexes in patients with IgAN. Soluble CD89 was identified as a glycoprotein with a 24-kD backbone that corresponds to the expected size of CD89 extracellular domains. To demonstrate their pathogenic role, transgenic (Tg) mice expressing human CD89 on macrophage/monocytes were generated. These mice spontaneously developed massive mesangial IgA deposition, glomerular and interstitial macrophage infiltration, mesangial matrix expansion, hematuria, and mild proteinuria. The molecular mechanism was shown to involve soluble CD89 released after interaction with IgA. In contrast to our own results release of CD89 was independent of CD89 association with the FcR γ chain in this mouse model. The disease was induced in recombination activating gene mice by injection of serum from CD89-Tg mice, and in severe combined immunodeficiency -Tg mice by injection of IgA from patients. Depletion of soluble CD89 from serum abolished this effect. These results could reveal an important pathophysiologic aspect of soluble CD89 in the pathogenesis of IgAN (36).

IV. ROLE OF INTERLEUKIN-6 IN MEDIATING GLOMERULAR DAMAGE

Mesangial cell proliferation and mesangial matrix accumulation are key features of various human glomerular diseases, including IgA nephropathy (37,38). Interleukin-6 (IL-6) is produced in relatively large amounts by mesangial cells in response to a variety of stimuli, such as angiotensin II, lectins, matrix proteins, cytokines, as well as IgA-immunocomplexes and immune complexes of other isotypes of antibodies (16,19,39-44). It was described that IL-6 contributes to induction of matrix protein transcription and autocrine growth in mesangial cells in vitro (45,46). In vivo, glomerular IL-6 overexpression was detected in human

glomerulonephritis types characterized by mesangial hypercellularity such as IgA nephropathy and some types of lupus nephritis (47-51). Furthermore, IL-6 transgenic mice developed a mesangioproliferative glomerulonephritis (46) and the urinary excretion of IL-6 has been correlated with mesangial hypercellularity in patients with IgA nephropathy (46,52,53). It has therefore repeatedly been proposed that IL-6 is an important mediator of mesangial cell proliferation and matrix overproduction (38,41,54). The results described in chapter 2 analyze the role of interleukin 6 in mediating mesangial cell proliferation and matrix production *in vivo*. We demonstrated that IL-6 knock out mice develop a normal glomerular architecture and in particular a normal mesangium. Mesangioproliferative glomerulonephritis induced by Habu snake venom is equally severe in IL-6 knock out mice as in control mice. A continuous seven-day intraperitoneal infusion of 50 µg recombinant IL-6 into rats with a prior minimal (subnephritogenic) injury to mesangial cells does not induce glomerular cell activation, cell proliferation, matrix production, leukocyte influx, platelet influx or proteinuria. A continuous seven-day IL-6 infusion into rats with mesangioproliferative nephritis increases matrix protein transcription in the absence of detectable effects on matrix protein accumulation and otherwise has no effect on the natural course of the disease. Therefore we conclude that IL-6 is not a predominant mediator of mesangial cell proliferation and matrix overexpression *in vivo*.

Lots of other factors have been investigated in their opportunity to affect secretory/synthetic activity of mesangial cells. Change of their phenotype results in the acquisition of a proinflammatory and profibrotic phenotype of this cell. *In vitro* and animal data suggest that platelet-derived growth factor (PDGF) B-chain has a particular important role in this respect (55). MC produces PDGF and PDGF B-chain and its receptor are overexpressed in glomerular diseases. Infusion of PDGF-BB or glomerular transfection with PDGF B-chain cDNA induce mesangial proliferative changes *in vivo*. PDGF B-chain or β -receptor knock-out mice fail to develop a mesangium. Furthermore, antagonisation of PDGF B-chain reduce mesangial proliferation during experimental mesangial proliferative glomerulonephritis (56). Another factor playing a well-established role in experimental glomerulonephritis is transforming growth factor- β (TGF- β). Similar to PDGF, its release from the MC is induced by other growth factors, in particular angiotensin-II. TGF- β I is overexpressed in IgAN and transgenic overexpression of TGF- β in the kidney results in progressive fibrosis (57).

V. INFLAMMATORY CELLS DURING MESANGIOPROLIFERATIVE GLOMERULONEPHRITIS

The involvement of monocytes and macrophages in various glomerulonephritides has been clearly established (58). In particular their involvement has been associated with proliferative forms of glomerulopathies (59,60). Rodent models of glomerulonephritis (61-63) have provided experimental evidence. However, in some cases infiltrating monocytes may serve merely to remove immune complexes and do not contribute to the glomerular injury (64). Recent interest has focused on the role of macrophages in the pathogenesis of focal glomerulosclerosis (65,66). Both glomerular hypercellularity and expansion of the extracellular matrix are thought to be of primary importance in the development of capillary obsolescence and glomerulosclerosis. In the remnant kidney model in the rat Van Goor and colleagues (58) showed that macrophages play a central role in the development of focal glomerulosclerosis. As described by Floege et al., the development of glomerulosclerosis in this model is preceded by mesangial proliferation and mesangial matrix expansion (67).

To clarify the distinctive roles of inflammatory cells during mesangial cell proliferative glomerulonephritis we depleted selectively monocytes and neutrophils in a rat anti-Thy-1.1 model described in chapter 3. In this model PMN reduction was associated with enhanced hematuria, a higher percentage of glomeruli with micro-aneurysms, enhanced glomerular deposition of C3 and C6, and no significant change in proteinuria, suggesting that microaneurysm formation is an important factor in determining the degree of hematuria. So PMNs seem to contribute to the repair process during anti-Thy-1.1 nephritis. Maybe proteolytic enzymes released by these cells have a complement-clearing function, and consequently a reduction in PMN influx may be responsible for the observed pronounced and sustained C3 and C6 deposition in rats treated with ED7. In the literature a complement-cleaving function of proteases released from PMNs has been described (68-70). Because complement is the major pathogenic factor leading to MC lysis during anti-Thy-1.1 nephritis, this could be an explanation for the increased damage and hematuria observed in this study.

In contrast to our results obtained in a complement mediated anti-Thy-1.1 model earlier experiments investigated the influence of mAb against various adhesion molecules on glomerular neutrophil accumulation and proteinuria in rats with nephrotoxic serumnephritis (71). This model is characterized by upregulation of different adhesion molecules, rapid neutrophil infiltration into the glomerulus, and proteinuria. Treatment of animals with antibodies against CD18, CD11b, and ICAM-1 caused 63%, 46%, and 54% reduction, respectively, in proteinuria and

79%, 66%, and 54% reduction, respectively, in glomerular neutrophil counts, suggesting an important role for these adhesion molecules in this model of experimental nephritis (71). Moreover, these data indicate that neutrophils play a major pathogenetic role in experimental nephrotoxic nephritis, specifically in glomerular permselectivity. These findings are further supported by another study investigating nephrotoxic-serum nephritis (72). Recently Waddington and coworkers described a significant glomerular iNOS induction and high output nitric oxide production in the acute phase of neutrophil-dependent acute immune-complex nephritis. Selective iNOS inhibition in vitro inhibited nephritic glomerular and neutrophil NO₂ synthesis (73).

Concerning the role of macrophages, we found that selective depletion of macrophages lead to a reduced glomerular matrix expansion. In contrast to ED7 treatment, glomerular complement deposition, microaneurysm formation, and hematuria remained unaffected.

These data are in agreement with recently published investigations (51). It was found that, after induction of anti-Thy-1.1 nephritis, influx of macrophages is strain dependent. High amounts of infiltrating macrophages led to a highly significant increase of extracellular matrix in Lewis rats (74). The potential mechanisms by which macrophages mediate glomerular-cell proliferation and the development of mesangial matrix expansion could involve release of cytokines by the macrophage itself or could involve stimulation by the macrophage of other cell types to release cytokines, e.g. transforming growth factor β within the glomerulus (75-77). For transforming growth factor β involvement in mesangial matrix expansion has clearly been shown (77-79). Another macrophage-derived product with a possible high impact is nitric oxide, which is abundantly secreted by infiltrating monocytes in this model (64). Monocytes have been shown to induce increased transcription of TGF- β and fibronectin (80).

In coculture studies, Mosquera demonstrated that monocyte-derived culture supernatants could induce mesangial cells to synthesize fibronectin in vitro (81). As shown in Figure 3, the mesangial expansion in monocyte-depleted rats was not completely inhibited. This may be because 1) especially major histocompatibility complex-II negative resident macrophages are less sensitive to liposome-mediated elimination (E De Heer, V Cattell, unpublished results), and 2) cytokines from other sources, for instance platelet-derived growth factor, may be able to induce mesangial matrix expansion, albeit less effectively (57).

Taken together monocytes are specifically involved in the expansion of the mesangial extracellular matrix, whereas other immunopathological processes in the mesangium (complement activation, platelet aggregation, and mesangial

proliferation) remain unaffected. These findings indicate that the mesangial alterations occur through distinct signaling pathways.

VI. CONCLUDING REMARKS

The work described in this thesis has contributed to a better understanding of the differential role of infiltrating inflammatory cells as well as the relevance of interleukin-6 during the course of experimental mesangial cell proliferative glomerulonephritis. It was found that interleukin-6 does not play a major role in the pathogenesis of the mentioned form of glomerulonephritis, furthermore interleukin-6 is not crucial for the development of a normal mesangium. In contrast to other examinations in other forms of experimental nephritis neutrophils seems to have a complement clearing function during anti-Thy-1.1 nephritis. This function is contributed to worsening of this form of complement mediated glomerulonephritis after depletion of neutrophils. Infiltrating macrophages on the other hand are associated with an increase of mesangial matrix accumulation.

Concerning the role of the suggested IgA-receptor expressed on human mesangial cells CD89/Fc α RI we failed to show its expression neither at the protein nor at its mRNA-level in vitro and in vivo. However, recent observation described in this thesis demonstrates the existence of a novel IgA-binding moiety expressed on the surface of human renal mesangial cells. This finding could be an important connecting link between IgA-deposition in the renal mesangium and the defective glycosylation or/and the existence of IgA/IgG immunocomplexes in IgA-nephropathy.

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SAMENVATTING EN CONCLUSIES

Primaire Immuunglobuline A (IgA) nefropathie (IgAN) wordt veroorzaakt door depositie van IgA vanuit de circulatie in het mesangium van de nier. Waarom en hoe IgA neerslaat in de nier is nog steeds niet bekend. Een opmerkelijke bevinding is dat het IgA in de nier uitsluitend bestaat uit één subklasse, n.l. IgA1. Depositie van IgA in de nier leidt tot een ontsteking en gaat vaak gepaard met (macro) hematurie. Eerdere studies hebben aangetoond dat het neergeslagen IgA voor ten minste een deel bestaat uit dimeer- en polymeer IgA. Recente studies suggereren dat patiënten met IgAN, IgA produceren met een defect in O-glycosylering. Dit IgA zou in de eerste plaats slechter geklaard worden uit de circulatie en ook beter met mesangium cellen reageren. Het is al enige tijd bekend dat patiënten met IgAN gemiddeld een hogere concentratie van circulerend IgA hebben. De hypothese is dat abnormale glycosylering van het IgA en de relatief hogere concentraties van dimeer- en polymeer IgA bijdragen tot een verhoogde kans van depositie van IgA in het mesangium. Er is verder voldoende bewijs dat interactie van IgA met mesangiumcellen leidt tot binding van IgA aan deze cellen en activatie van de cellen met als gevolg productie van verschillende cytokinen en chemokines. Tevens kan door activatie van complement de ontsteking ter plaatse versterkt worden. De verhoogde mate van activatie van mesangium cellen leidt tenslotte tot verhoogde productie en neerslag van matrix eiwitten die weer de filtreerfunctie van de glomerulus verstoren. Op termijn leidt dit tot gradueel verlies van nierfunctie en uiteindelijk tot nierfalen.

In hoofdstuk 2 werden verschillende aspecten van activatie van mesangium cellen bestudeerd. Aangetoond werd dat interleukine (IL-6) geen cruciale rol speelt bij de proliferatie van mesangium cellen in de muis en de rat, in tegenstelling tot eerdere publicaties dat het instaat is om mesangium cellen te zetten tot verhoogde matrix productie. Omdat mesangium cellen na stimulatie zelf IL-6 produceren bestond de gedachte dat IL-6 en autocrien effect zou kunnen hebben in de nier. Gebaseerd op onze huidige vondsten dient een dergelijke conclusie echter afgezwakt te worden. Interessant was onze bevinding dat blokkering van granulocyten (hoofdstuk 3) in een model van membranoproliferatieve glomerulonefritis n.l. in het anti-Thy.1 model, leidt tot verergering van proteïnurie en schade in de glomerulus. Het bleek dat ratten met anti Thy.1 nefritis die anti-integrine antilichamen kregen toegediend, meer depositie van de complement componenten C3 en C6 vertoonden en verhoogde proteinurie. Wij veronderstellen dat onder condities van infiltratie en activatie van granulocyten, de mate van depositie en/of activatie van complement beïnvloedt wordt door proteolytische enzymen van de geactiveerde granulocyten.

Wij denken dat deze proteolytische enzymen geactiveerd C3 afbreken waardoor versterking van de amplificatie route van complement wordt onderbroken. Verder onderzoek is nodig om deze hypothese nader te onderbouwen.

Het tweede gedeelte van dit proefschrift richt zich meer op de directe effecten van IgA op het mesangium.

Eerdere bevindingen van ons laboratorium hebben laten zien dat IgA kan binden aan mesangium cellen en deze cellen aanzet tot verhoogde productie van groeifactoren. Deze studies zijn ook door anderen bevestigd. De wijze waarop IgA bindt aan mesangium cellen is nog steeds een open vraag. Er zijn 3 membraan moleculen bekend waarmee IgA kan reageren n.l.: de pIgA receptor of secretoir component (SC). Dit molecuul komt voor op epitheel cellen waar het functioneert als een transporteur van dimeem IgA van het weefsel compartiment naar bijvoorbeeld het lumen van de darm. Tot nu toe zijn er geen bevindingen die het voorkomen van SC op mesangium cellen ondersteunen. Het tweede molecuul waar IgA aan kan binden is de zgn. asialoglycoproteïne receptor dat voorkomt op o.a. lever epitheel. Ook voor dit molecuul zijn er bij de mens geen sterke aanwijzingen op mesangium cellen van de mens. Tenslotte is er een derde receptor voor IgA beschreven, n.l. CD89. Deze receptor komt voor op monocyt/macrofagen, granulocyten en eosinofielen. Eerdere studies hebben gesuggereerd dat mesangium cellen van de mens een hoge mate van mRNA expressie voor CD 89 hebben. In hoofdstuk 4 hebben wij uitgebreide studies beschreven waarin gebruik is gemaakt van specifieke polyclonale- en monoclonale anti-CD89 antistoffen voor het aantonen van CD89 in vitro op gekweekte mesangium cellen, en in coupes van nieren en milt van patiënten met IgAN of controles.

Terwijl de binding van IgA aan mesangium cellen duidelijk optrad, vonden wij geen enkele aanwijzing, noch in vitro noch in weefsel biopten, voor het voorkomen van CD89 op nier mesangium cellen. Ook met zeer gevoelige PCR-methoden konden wij geen mRNA voor CD89 in mesangium cellen bevestigen. Onze studies worden gesteund door tenminste 3 andere onderzoeksgroepen en aangenomen mag worden dat de eerder gepubliceerde gegevens van de groep van Egido op een technische fout moet berusten.

Zoals eerder vermeld treedt ondanks de afwezigheid van CD89 en de andere receptoren voor IgA toch binding van IgA op aan mesangium cellen. Verder onderzoek beschreven in hoofdstuk 5 van het proefschrift levert bewijzen dat IgA bindt aan een mesangium celmembraan eiwit van ongeveer 83 Kd. Wij zijn er nog niet in geslaagd om de identiteit van dit molecuul te bepalen. In het hoofdstuk 6 wordt beschreven dat oplosbaar CD89 (S CD89) kan vrijkomen van cellen die

CD89 tot expressie brengen, wanneer deze cellen worden geactiveerd. Wij vonden dat SCD89 alléén vrijkomt van cellen als het CD89 in de membraan gassocieerd is met een andere peptide keten, nl de J. Chain die ook gebruikt wordt door andere membraanmoleculen zoals FC-gammareceptoren. Het SCD89 is net als CD89 in de membraan in staat om IgA te binden. Wij hypothetiseren dat SCD89 een regulerende rol speelt op de klaring en functie van IgA en daardoor mogelijk een rol zou kunnen spelen bij IgAN. Verdere studies op dit onderdeel zijn van essentieel belang voor het nader definiëren van de depositie van IgA in het mesangium. Indien dit lukt kan een deel van de puzzel worden opgelost, van hoe en waarom IgA neerslaat in het mesangium van de nier bij patiënten met IgAN.

CURRICULUM VITAE

De schrijver van dit proefschrift, Ralf Westerhuis, werd geboren op 26 mei 1966 te Hildesheim. In 1986 werd het Atheneum diploma behaald aan het Scharnhorst Gymnasium te Hildesheim. In het volgende jaar begon hij aan zijn medische studie aan de Medizinische Hochschule Hannover, Duitsland. Naar het behalen van het eindexamen tot arts begon hij 1994 met zijn interne opleiding in de afdeling nierziekten, Medizinische Hochschule Hannover (hoofd Prof. Dr. K.M. Koch). 1997 tot 1999 werkte hij als onderzoeker bij de afdeling Nierziekten van het Leids Universitair medisch Centrum (hoofd Prof. Dr. L.E. van Es, sinds 1 juli 1998 Prof. Dr. L.C. Paul). Het onderzoek werd gedaan onder leiding van Prof. Dr. M.R. Daha en Dr. C. van Kooten. Van maart to juni 1999 vervolgde hij zijn opleiding tot internist in Hannover en daarna sinds juni 1999 aan de universiteitskliniek Aken, afdeling Nierziekten (hoofd Prof. Dr. J. Floege).

ACKNOWLEDGEMENTS

The two years of working in Leiden has been a great experience and a wonderful time in my life. I want to thank all the people working at the laboratory of the department of nephrology for their help and kindness. Especially, I thank Arnout for helping me during my first steps at the lab. I also thank Nicole for being a great and enthusiastic coworker and her contribution to a lot of the experiments presented in this thesis. I also thank Ngaisah and Evert for their help and their ability to create wonderful working conditions. Furthermore I thank Ger and Barbara for a really great time in Leiden. Greatest thanks to my wife Anette and our loving children Peer, Nils and Mia. Finally I thank my parents, my family, Chris and my brother Harald for their help and support for reaching the various goals in my life.