Immune regulation in IgA nephropathy

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Chapter 8

General discussion and summary

Primary IgA Nephropathy (IgAN) was first described by Berger and Hinglais in 1968 [1]. Nowadays IgAN is recognized as the most common primary glomerulonephritis worldwide [2;3]. Initially IgAN was thought to be a disease with a benign character, but later it appeared that up to 35% of patients eventually develop end stage renal failure. In western Europe and the United States of America about 10% of patients undergoing renal replacement therapy have IgAN as underlying disease. So the impact of IgAN on individual patients and more general on healthcare systems is significant.

Fourty years after the first description by Berger, the pathogenesis of this disease is still not resolved. Generally patients develop IgAN in their early twenties, however it may also become obvious later in life. Men are more often affected than women with a ratio of 2:1. It is important to realize that most persons present with microscopic hematuria, without further signs or symptoms. About 40% of patients have macroscopic hematuria, sometimes starting shortly after an upper respiratory tract infection. Unfavourable clinical outcome is associated with high levels of proteinuria and uncontrolled hypertension. Furthermore a decreased glomerular filtration rate (GFR), at the time the diagnosis IgAN is made, correlates with bad outcome [4]. The only way to confirm the diagnosis IgAN, is to perform a renal biopsy. Differences in incidence rates suggest that next to environmental factors, like viral infections, genetic factors are involved in the pathogenesis [5]. Although no exact information about incidence of IgAN is known, the prevalence of IgAN seems to be higher in certain countries, such as Japan, Australia and southern Europe. In the United Kingdom, United States and Canada the prevalence rates are lower [4]. It is important to realise that these incidence numbers are influenced by variance in referral rates and kidney biopsy criteria. Autopsy studies from Japanese unselected persons show a prevalence of mesangial IgA depositions of about 16% , whereas in autopsy series of unselected German persons the prevalence was about 5%. [6;7].

So far no reliable diagnostic test, other than a renal biopsy, is available to predict the presence of IgAN. The disease is characterized by depositions of IgA, mainly IgA1, together with complement factor C3 and sometimes IgG in the renal mesangium [3;8]. Recurrence of IgAN after renal transplantation

is frequently observed, whereas on the other hand it has incidentally been described that IgA depositions disappear from a kidney of an IgAN patient when it was transplanted to a non-IgAN patient [9;10]. These observations contribute to the idea that the so far unresolved pathogenesis of IgAN involves the immune system rather than specific renal factors.

Next to immunological factors, genetic factors are likely to be involved in the pathogenesis of IgAN. Racial differences in susceptability to IgAN are observed, for instance the incidence of IgAN among African Americans is lower in comparison with Caucasians [11;12]. The strongest argument that genetic factors play a role in the pathogenesis of IgAN is that familial clustering of IgAN has been described [13-17]. Genome-wide linkage analysis of 30 families with IgAN, 24 from Italy and 6 from the United States, showed a close association of IgAN to chromosome 6q22-23 [18]. This locus has been named *IGAN1*. Whether this locus is involved only in familial IgAN or also in the isolated form of IgAN is not clear. Recently two other candidate loci, 4q26-31 and 17q12-22, have been identified as suggestive to be linked to IgAN in 22 Italian families with IgAN [19]. So far no specific genes in these loci influencing the pathogenesis of IgAN have been identified. Although Schena et al. reported a poorer prognosis of familial IgAN, no clinical parameters are recognized that distinguish sporadic IgAN from familial IgAN [15].

The variability of IgAN, in clinical presentation and outcome and in the histological picture of the renal biopsies, might reflect the variability in genetic background or genetic factors involved in the pathogenesis of the disease. On the other hand differences in environmental factors or geographical factors might be involved in the onset of IgAN or might influence the clinical outcome and could be responsible for the wide variability of IgAN.

Recently we identified a homozygous twin with biopsy proven IgAN, both presenting with microscopic hematuria in combination with hypertension. Interestingly both patients participated in the study described in chapter 4 and both appeared to have DC that induced only very little IgA production by naïve B cells. So far homozygous twins with IgAN have not been described.

In the past 4 decades several abnormalities in the immune system of IgAN patients have been described. Both quantitative and qualitative aspects of IgA seem to differ in IgAN patients in comparison with controls. Overall the serum IgA titer is increased in 50% of patients as compared to controls [20]. However, other diseases with higher IgA levels in the circulation, like multiple myeloma or HIV infection, are not associated with depositions of IgA in the kidney. Several immunization studies have shown aberrant immune responses in IgAN patients [21-23]. Next to this, several qualitative aspects of IgA seem to be different in IgAN patients. The most constantly described abnormality concerns the disturbed glycosylation pattern of IgA in IgAN patients [24;25]. In IgAN patients there is a reduction of galactosyl residues in the hinge region of serum IgA1 [26]. The exact mechanism how the glycosylation of IgA is disturbed, is so far not known. Recently several glycosyltransferases have been described, but no differences in enzyme activity, nor in gene expression are present in IgAN patients.

Chapter one as a general introduction outlines the clinical features of IgAN. Further, the human IgA system is described and the different structural aspects of IgA are discussed. It is important to realize that the human IgA system is unique and contains both IgA1 and IgA2, both existing in monomeric and polymeric forms. For this reason it is difficult to develop a representative animal model for IgAN. There are two compartments, namely the mucosal and the systemic compartment, where IgA is produced. The mucosal compartment seems to be the most important one. At mucosal sites throughout the body, IgA is produced in large quantities and is secreted as secretory IgA (SIgA). SIgA exists of dimeric IgA bound to secretory component and is involved in mucosal defence against viral and bacterial pathogens. Small amounts of SIgA however are present in the circulation. So far it is not clear what the physiological role of SIgA in the circulation is. In IgAN in about 15% of cases SIgA is present in the renal biopsies [27]. The presence of SIgA correlates with the presence of mannose binding lectin (MBL), suggesting that SIgA is involved in inflammation in the kidney [28]. Next to mucosally produced IgA, IgA is produced in the systemic compartment, in the bone marrow by plasma cells. The role of IgA in the circulation is less clear.

To investigate the immune response in IgAN over the years several immunization studies, using different antigens and different routes of antigen administration have have been performed. These studies have shown different results with respect to the IgA response in IgAN patients [21;23;29-32].

Chapter two concerns a study in which the IgA response in IgAN patients upon simultaneous stimulation with two different antigens, administered via two different routes is investigated. IgAN patients and controls were immunized with cholera toxin subunit B (CTB) applied at the nasal mucosa and with keyhole limpet hemocyanin (KLH) which was administered subcutaneously. Both CTB and KLH are considered as neoantigens and induce a primary immune response. In this study we also performed bone marrow biopsies. We were able to detect CTB-specific immunoglobulin producing cells in the bone marrow, providing evidence for the so called mucosa-bone marrow axis. This further supports the idea that the two compartments, mucosal and systemic, in which IgA is produced are immunologically connected.

It appeared that after mucosal immunization IgAN patients had a significantly, lower IgA response both at the nasal mucosa, as well as in serum. Besides the number of CTB-specific IgA producing cells in the bone marrow was significantly lower in the patient group than in the controls. No differences were found between patients and controls with respect to antigen-specific IgG or IgM. After subcutaneous immunization with KLH no differences were found between the two groups, neither in the antigen-specific IgA response, nor in IgG or IgM. This is the first study in which persons are simultaneously immunized with two different neoantigens via two different routes. An IgA hyporesponse in IgAN patients after mucosal immunization was also observed after oral immunization with live typhoid vaccine [30]. It is important to realize that in both studies people were immunized with a neoantigen, so the described results represent a primary immune response. In general many immune responses after viral infections will be caused by recall antigens, leading to secondary immune responses, which are partly dependent on immunological memory. The contrast of mucosal IgA hyporesponse and higher IgA levels in the circulation could be explained by the hypothesis that decreased mucosal clearance of antigens can lead to prolonged immune

reactions leading to higher levels of immunological memory. No IgA hypo response was observed after the systemic immunization with KLH. Which factor or which factors are responsible for the observed IgA hypo response after mucosal immunization is not clear from this study.

Dentritic cells (DC) are professional antigen presenting cells and are involved in the initiation of immune responses. Besides DC can have a direct effect on B cells and are capable of skewing immunoglobulin production by naïve B cells towards IgA1 and IgA2 [33]. Therefore we postulated that DC could be responsible for the differences in IgA response between IgAN patients and controls. To test our hypothesis, nasal biopsies of IgAN patients and control persons were taken and stained for the presence of DC and subsets of DC. In chapter three we describe the result of this study showing that the number of DC in the nasal mucosa of IgAN patients is not decreased. As a matter of fact we even found higher numbers of CD1a positive DC in the epithelial layer and higher numbers of DC-SIGN positive cells in the lamina propria.

As the number of nasal mucosal DC in IgAN patients was not reduced as compared to controls, and therefore could not be responsible for the earlier described IgA hyporesponse, we postulated that the DC might be less effective in inducing IgA production by naïve B cells. In chapter four we investigated in an *in vitro* model the functional capacity of DC to induce IgA production in naïve B cells. The model used in this chapter is first described by Fayette et al. [33]. In this model naive B cells are cultured in the presence of CD40L-transfected cells, which mimic activated T cells. By making use of these transfected cells MHC restriction is bypassed. In these experiments we made use of monocyte-derived DC, CD40L-transfected cells and naïve B cells. The cells were cultured in the presence of different cytokines, like IL-10 and IL-2. The only variable in the system was the source of the DC, which was either from an IgAN patient or from a control person. In these experiments it appeared that DC derived from IgAN patients showed a reduced capacity to induce IgA production in the presence of IL-10. Although the mean IgA production induced by DC from IgAN patients was strongly reduced, DC from some individual patients induced a near normal IgA production. This

might reflect that IgAN is a heterogeneous disease. No differences in IgG or IgM production where observed, independent of the different cytokines that were used. An experiment using supernatant of CD40 stimulated DC showed that the increase in immunoglobulin production induced by this supernatant was less than the induction by whole DC and that in this case no differences between patients and controls were present. This suggests that a membrane bound factor is responsible for the reduced functional capacity of DC from IgAN patients to induce IgA production by naïve B cells.

From the results of chapter 2,3 and 4 it can be postulated that the IgA hyporesponse in IgAN patients observed after mucosal immunization can at least partially be explained by a functional defect of DC in the nasal mucosa, which is caused by a molecule present at the cell surface of DC. The number of sub-epithelial DC-SIGN positive DC and epithelial CD1a-positve DC in the nasal mucosa appeared to be increased in IgAN patients. This could be a compensatory increase for the described reduced capacity to induce IgA production.

IgA depositions in IgAN contain high levels of polymeric IgA. Therefore we were interested in the size distribution of antigen-specific IgA. In chapter 6 we describe that the antigen-specific IgA response contains high levels of polymeric IgA, both after mucosal and after systemic immunization. No differences in size distribution of antigen-specific IgA were present between IgAN patients and controls. Next to the size distribution of IgA we were interested in the presence of antigen-specific SIgA after mucosal immunization. A pathogenic role for SIgA is suggested by the fact that about 40% of IgAN patients have episodes of macroscopic hematuria, often preceded by upper respiratory tract infections [34]. Besides it was shown that SIgA is present in renal biopsies in 15% of the cases. The presence of SIgA in renal biopsies correlates with the presence of MBL depositions. Although SIgA is mainly present at mucosal surfaces, in various secretions, low concentrations of SIgA are present in the circulation. In purified IgA, the relative concentration of SIgA is higher in IgAN patients than in control persons [35]. In the study described in chapter 6 we were able to detect small amounts of antigen-specific SIgA in the

circulation of both IgAN patients and controls, after mucosal immunization. This is to our knowledge the first study showing antigen-specific SIgA in the circulation and supports the hypothesis that SIgA has a pathogenic role in a group of IgAN patients.

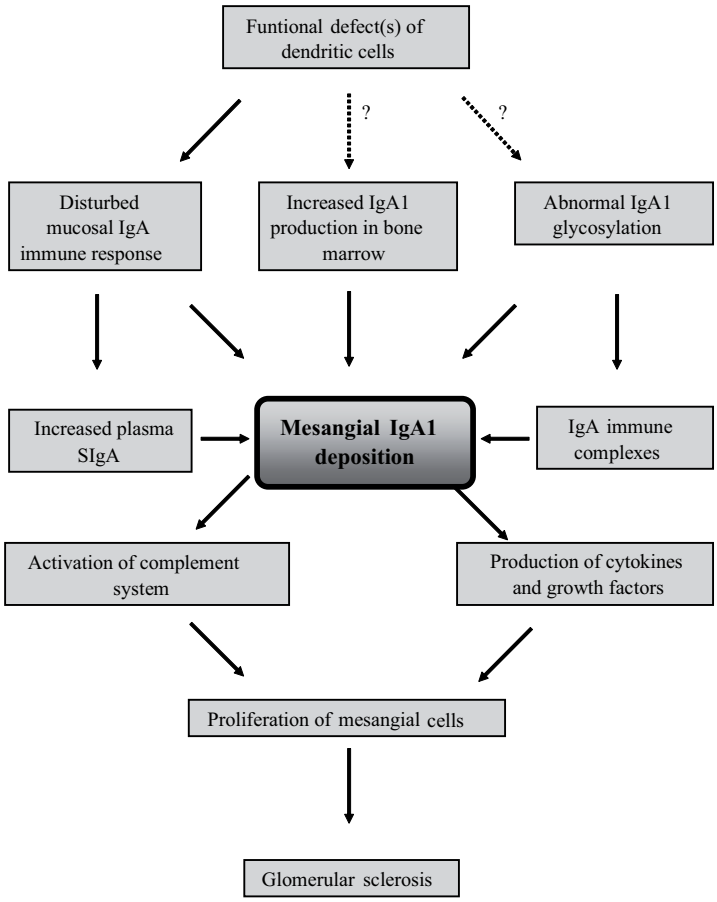

Figure 1. Overview of factors leading to mesangial IgA deposition and to progression of IgA nephropathy. A functional defect in DC might be responsible for the mucosal IgA hyporesponse and thereby to prolonged exposition to environmental antigens, eventually leading to higher levels of IgA memory cells and higher IgA plasma levels. Whether DC are directly involved in the glycosylation process of IgA and in the higher IgA production in the bonemarrow is speculative. The higher levels of SIgA in the circulation and the defective galactosylation of IgA will lead to mesangial deposition of IgA1. Once IgA is present in the kidney, this will lead to production of cytokines and growth factors and to activation of the complement system. Mesangial cells will proliferate, leading to glomerular sclerosis.

As IgA derived from IgAN patients is abnormally O-glycosylated , we discussed this issue in chapter seven. Importantly, this undergalactosylated form of the IgA1 hinge region was also over-represented in biopsies of patients with IgAN [36]. Therefore, it is likely that this abnormally glycosylated IgA is involved in the pathogenesis of IgAN. This abnormal glycosylated IgA has a higher tendency to self-aggregate and form complexes with IgG antibodies directed against epitopes in the hinge region of IgA1. Besides, in patients with Henoch-Schönlein purpura, a disease closely related to IgAN with a similar renal histological pattern, only those patients who had abnormally glycosylated IgA had renal involvement, whereas patients with normally glycosylated IgA did not have renal involvement [37]. SIgA has a different glycosylation pattern as compared to other forms of IgA [38]. Whether SIgA of IgAN patients is aberrantly glycosylated is at present not known.

IgAN is a heterogeneous disease, with a diverse and highly unpredictable outcome. The initial presentation can vary from microscopic hematuria with normal GFR, to gross macroscopic hematuria with end stage renal failure. Proteinuria can be absent, but can also be in the nephrotic range. Some patients remain stable for many years with a normal GFR, whereas others might develop end stage renal failure within a very short period of time. The histological hallmark of IgAN is the presence of IgA1 depositions in the renal mesangium, which is influenced by several factors (Figure 1). However, this picture can vary from slight mesangial hypertrophy with IgA1 depositions, to extracapillary proliferation with crescent formation. SIgA is present in renal biopsies in 15% of cases. Aberrant, undergalactosylated IgA1 seems to be involved in the pathogenesis. Since IgA1 glycosylation is determined within B lymphocytes, and only a fraction of the IgA1 seems to be affected, this suggests that alterations in glycosylation are only present in a subset of B cells. The question that arises is whether all these different clinical and histological pictures should be considered as one disease or that different characteristics represent a number of diseases with one common factor which is mesangial IgA deposition.

Further differentiation between IgAN patients, involving both immunological and histological investigations as well as clinical studies, is of importance to further unravel the pathogenesis of IgAN and eventually develop individual therapeutic strategies.

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