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New insights in the pathogenesis of IgA nephropathy

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SUMMARY

IgA nephropathy (N) or Berger's disease is the most common form of primary glomerulonephritis worldwide and one of the first cause of end-stage renal failure. The disease is characterized by the accumulation in mesangial areas of complexes containing polymeric IgA1. The mechanisms involved in the pathogenesis of IgAN is only now emerging. We discussed here three essential points: i) the generation of abnormal IgA1 and formation of IgA1 complexes; ii) the generation of mesangial injury mediated by interaction of IgA1 complexes with mesangial IgA1 receptors, and iii) the progression of IgA-mediated mesangial injury towards renal failure. In summary, our data reveal that quantitative and structural changes of IgA1 play a key role on the onset of the disease due to functional abnormalities of two IgA receptors: the FcαRI (CD89) expressed by blood myeloid cells and the transferrin receptor (CD71) on mesangial cells. Abnormal IgA induces release of soluble CD89 soluble leading to the formation of circulating IgA complexes, which in turn may be trapped by CD71 that is overexpressed on mesangial cells in IgAN patients allowing formation of IgA1 deposits. The elucidation of IgA-receptor interactions may open new avenues for drug design and treatment of IgAN.

Key words: ***IgA nephropathy. Pathogenesis. IgA receptors.***

NUEVAS OBSERVACIONES EN LA PATOGENIA DE LA NEFROPATÍA IgA

RESUMEN

La nefropatía (N) IgA o enfermedad de Berger es la más común de las glomerulonefritis primarias en el mundo y una de las primeras causas de enfermedad renal crónica terminal. La enfermedad se caracteriza por el acumulo en áreas del mesangio de complejos que contienen IgA1 polimérica. Los mecanismos patogénicos empiezan a ser conocidos. Discutimos aquí tres puntos esenciales: i) la generación anormal de IgA1 y la formación de complejos IgA1; ii) la aparición de daño mesangial mediado por la interacción de complejos IgA1 y de receptores IgA1, y iii) la progresión hacia la insuficiencia renal causada por el daño mesangial mediado por la IgA. En resumen nuestros datos sugieren que los cambios cuantitativos y estructurales de la IgA juegan un papel importante en el inicio de

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la enfermedad debida a anomalías funcionales de dos receptores de la IgA: el FcαRI (CD89) expresado en las células mieloides sanguíneas y el receptor de la transferrina (CD71) en las células mesangiales. La IgA anormal provoca la liberación de CD89 soluble, la formación de complejos circulantes IgA, que pueden ser atrapados por CD71 sobre expresado en las células mesangiales de pacientes con NIgA, dando lugar a la formación de depósitos IgA1. El conocer las interacciones entre los receptores de IgA permite pensar en el diseño de nuevos medicamentos para el tratamiento de la NIgA.

Palabras clave: ***Nefropatía IgA. Patogenia. Receptores para la IgA.***

INTRODUCTION

IgA nephropathy (IgAN), the most common primary glomerulonephritis worldwide, is defined by the deposition of IgA1 in the glomerular mesangium and less frequently of C3, IgG and IgM, together with mesangial cell proliferation, matrix expansion and clinical features of renal injury, including hematuria and proteinuria[1]. There is no effective treatment and 20-40% of patients progress to end-stage renal failure after 20-25 years of disease activity. The mechanisms involved in the pathogenesis of IgAN is only now emerging. They can be divided in three essential steps: i) the generation of abnormal IgA1 and formation of IgA1 complexes; ii) the generation of mesangial injury mediated by interaction of IgA1 complexes with mesangial IgA receptors, and iii) the progression of IgA-mediated mesangial injury towards renal failure.

*Step 1: Generation of abnormal glycosylated IgA1 and formation of IgA1 complexes***.** The first strong evidence of a nephritogenic circulating factor in the pathogenesis of IgAN came from the recurrence of mesangial IgA deposits in patients after transplantation of a normal kidney[2]. Observations from several studies revealed that the level of serum IgA is two- to three-fold enhanced in approximately half of the IgAN patients[3]. The ratio of polymeric to monomeric IgA in the circulation is also increased in comparison with normal individuals. Circulating macromolecular IgA contain polymeric IgA (pIgA) which have the ability to bind secretory component, the extracellular domain of the polymeric Ig receptor. However, secretory IgA, the main Ig in secretions, is not found in the renal deposits of IgAN. Instead, the mesangial IgA apparently originates in the bone marrow and no evidence is found for pIgR dysfunction in IgAN[3].

Abnormal IgA has been noted in patients with IgAN two decades ago[4]. Indeed, our studies of renal eluates demonstrate the highly anionic nature of mesangial IgA which were under complexed forms[4]. This indication of aberrant structure has been confirmed since by several authors[5]. Because the deposited mesangial IgA is exclusively of the IgA1 subclass, several authors have proposed that the unique structure of the IgA1 hinge region may be involved in the IgAN pathological process. The hinge region located between the CH2 and CH3 domains of IgA1 usually contains 3 to 5 O-linked side chains. The O-glycans are based on N-acetylgalactosamine (GalNAc) O-linked to serine or threonine residues. Galactose (Gal) may be linked to the GalNAc by a specific enzyme, β1,3 galactosyltransferase, to form the disaccharide Galβ1,3 GalNAc. This disaccharide may be extended with one or two sialic acid (NeuAc) units that are added by α2,3 sialyltransferases. Sialic acid units may also be added to GalNAc by the α2,6 sialyltransferases[5]. Studies from the last decade have revealed that IgAN patients may have undergalactosylated IgA1 in the circulation and in mesangium areas[6]. These O-truncated glycans have been identified through the use of lectins, such as *Jacalin*, *Helix aspersa* and *Vicia villosa,* that recognize either β1,3 glycosidic residues between galactose and GalNac residues or terminal GalNac residues. Increased sialylation of IgA1 has also been described and could contribute to its negative charge. Although abnormally glycosylated IgA1 has been clearly demonstrated in IgAN patients, the origin of this molecular alteration remains unknown. Reduced activity of β1,3 galactosyltransferase has been shown in B cells from IgAN patients, and it has been proposed that the abnormally glycosylated IgA may reflect a defect in the O-glycosylation pathway in these patients[6]. However, molecular mechanisms for such deficiency is unknown and it has not been ascertained whether the glycosylation defect is constitutive or acquired.

Besides the intrinsic structural alterations of IgA described above, another feature of IgAN is the presence of large amounts of IgA immune complexes

in the circulation and in the mesangium of IgAN patients[4,7,8]. Formation of IgA1 complexes may involve at least three distinct events: i) the self-aggregation of abnormal glycosylated IgA1; ii) the formation of complexes through binding to soluble IgA Fc receptor I (FcαRI), and iii) the amplification of the size of these complexes by IgA interaction with others components. While the first event is directly linked to an abnormal structure of IgA1 possibly at their site of generation[5,6,9], the second one is dependent on the interaction with the FcαRI (CD89) that is expressed on cells of the myeloid lineage[10]. This receptor represents a heterogeneously glycosylated transmembrane protein that binds both IgA subclasses with low affinity. The FcαRI α chain lacks canonical signal transduction domains, but associates with the FcR γ-chain that bears activation motifs (ITAM) in the cytoplasmic domain. In normal individuals, the FcαRI/FcR γ chain complex can mediate both cellular activation and inhibition of heterologous receptors depending on the type of interaction with multivalent or monovalent ligand, respectively[10,11]. In patients with IgAN, a reduced expression of FcαRI was observed on the surface of their circulating monocytes in spite of normal FcαRI transcription levels[12]. The presence of plasma IgA seems essential for the reduction in FcαRI expression on monocytes. This was shown by incubating IgAN patients' cells without or with homologous plasma and by incubating purified polymeric IgA with monocytes from normal individuals[12]. The mechanism proposed to explain this phenomena involves the shedding of the extracellular domain of the FcαRI[3]. Support for this hypothesis was provided by the demonstration of soluble FcαRI in the serum of IgAN patients and not in serum from normal individuals. This protein was identified as a glycosylated FcαRI of 50-70 kDa with a 24 kDa protein core[13]. Production of this soluble FcαRI could be produced by incubating polymeric IgA with transfected cells expressing FcαRI. These results indicate that interaction of pathogenic IgA with FcαRI promotes cleavage of the extracellular domain of FcαRI releasing IgA/FcαRI complexes into the circulation. Such cleavage may involve yet uncharacterized protease, thereby explaining the decreased FcαRI membrane expression.

The detection of circulating complexes containing IgA and soluble FcαRI in IgAN patients serum raises the possibility that these complexes may be involved in the development of this disease. To demonstrate this we have generated human FcαRI transgenic mice that serve as a novel animal model for the spontaneous IgAN[13]. Although with very low affinity, human FcαRI interacts with mouse polymeric IgA to form complexes that are deposited in the mesangium of the FcαRI Tg mice. Human FcαRI transgenic mice develop mesangial IgA deposition, hematuria, mild proteinuria and macrophage infiltration around the renal glomeruli[13]. The disease can be transferred to wild-type recipents by infusion of serum IgA/soluble FcαRI complexes from these Tg mice. To examine the contribution of patient IgA, a model of SCID-FcαRI Tg mice was created. These mice do not develop IgAN spontaneously, but they develop the manifestations of IgAN when IgA from IgAN patients is injected. Interestingly, IgA from healthy subjects did not result in IgAN in the SCID-FcαRI Tg mice, thereby inferring that abnormally glycosylated IgA coupled with FcαRI participates in the pathogenesis of IgAN.

The third event seems dependent on interactions of abnormally glycosylated IgA1 with other proteins. Indeed it has been shown that hypogalactosylation generates antigenic determinants that can be recognized by naturally occurring IgG and IgA1 antibodies, thereby leading to the formation of circulating immune complexes[5]. However, IgG is not always found to be co-deposited in the mesangium of IgAN patients. In some patients, IgA has also been found complexed to other proteins such as fibronectin and collagen. In all of these situations (self-aggregation, interaction with soluble FcαRI and/or immune complex formation), complexes may impede the recognition of IgA by the asialoglycoprotein receptor (ASGPR)[9]. The ASGPR serves as an IgA receptor on hepatocytes by its ability to bind terminal galactose or N-acetylgalactosamine on O-linked and some N-linked glycans[9]. However, ASGPR seems to play an important role in the clearance of IgA2 rather than of IgA1. Furthermore, no dysfunction of ASGPR has been demonstrated in IgAN[3].

Step 2: Mesangial injury is mediated by interaction of IgA1 complexes with mesangial IgA receptors. Mesangial IgA deposits have been shown to be primarily of IgA1 isotype and to be composed largely of polymeric IgA, although monomeric IgA can also be found in the deposits[3]. As expected from the above these deposits contain abnormal glycosylated IgA1[6]. Although several candidate proteins have been identified as putative mesangial IgA receptors, known of them were found in the mesangium[14]. We have recently shown that the transferrin receptor, TfR or CD71, can serve as an IgA1 receptor[15]. The TfR binds only polymeric IgA1, but not monomeric IgA1[16]. In contrast to the FcαRI, this IgA receptor is not expressed on mature blood leukocytes and is only weakly expressed on quiescent mesangial cells[17]. Interestingly, in patients with IgAN TfR expression is strongly induced in the mesangium and

correlates with severity of the disease[17]. Because overexpression of this IgA1 receptor (TfR/CD71) is found in mesangium of patients with IgAN[17], it may participate in the selective deposition of IgA1 complexes. Similar observations have now been made in renal biopsies of patients with Henoch-Schoenlein purpura GN with mesangial IgA deposition[17]. Interestingly, overexpression of TfR was also seen in patients with lupus, but only in those with IgA deposition. Our recent studies[16] indicate that abnormal glycosylated IgA1 and IgA1 complexes may favor the interaction with TfR as observed on cultured normal human mesangial cells. Furthermore, IgA1 polymers can induce TfR expression, cytokine release and cell proliferation which could in part be responsible for the observed injury and recurrence of deposits after transplantation (Moura et al, unpublished data).

Another receptor for IgA and IgM, Fcα/μR, has also been described (reviewed in ref. 14). Transcripts for this receptor may also be expressed by human mesangial cells. This receptor has been found to be upregulated by IL-1 thereby implying another basis for IgA deposition. However, no Fcα/μR protein has been found on cultured human mesangial cells[16]. We cannot exclude that Fcα/μR can be expressed in some cases of IgAN with IgA and IgM deposits especially those involving a very aggressive inflammatory process.

Step 3: Progression of IgA-mediated mesangial injury towards renal failure. One the remarkable feature of IgAN is the heterogeneity of the disease concerning its evolution towards end-stage renal disease (ESRD). Between 20 to 40% of IgAN patients progress towards ESRD after 20-25 years of disease activity. The mechanisms involved in disease progression remains mostly unknown. It is interesting that severity of IgAN is often associated with leukocyte infiltration in the kidneys. Indeed, the presence of monocyte/macrophages and T cells correlates with the progression of IgAN[18,19]. Interestingly, although transmembrane FcαRI expression is decreased on blood phagocytes of patients, increased IgA bound to these cells are linked to the appearance of glomerulosclerosis and mesangial proliferation[12]. Defective FcαRI endocytosis rates and increased IgA recycling towards the cell surface have been demonstrated on blood phagocytes from patients with IgAN[10]. As a consequence, cells from these patients express high IgA levels. Whether FcαRI is mediating an activating process following patients' IgA binding remains to be determined. Another factor that may also contribute to evolution towards ESRD is the overexpression of TfR by mesangial cells and its capacity to mediate mesangial IgA1 complex deposition which seems to correlate to disease severity[17]. This interaction could thus lead to an inflammatory response by promoting the release of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α[9], with consequential proliferation and progression to fibrosis and renal impairment[3]. This hypothetical cycle of events could thus explain the progression and chronicity of the disease. Genectic factors may be involved in controlling disease heterogeneity. Although some progress has been made in localizing these genetic factors and a possible chromosomal locus involved in this disease[20], the candidate genes remain, however, unknown.

CONCLUSIONS

We proposed that pathogenic mechanisms in IgAN are initiated by alterations in IgA1 structure followed by a polymerization process of IgA1 involving soluble FcαRI and other components. An *in situ* secondary event would take place with the induction of a mesangial IgA receptor, the TfR, on mesangial cells. This represents a crucial step to mediate the formation of IgA deposits and eventually contributes to generate of the third event concerning progression of IgAN towards ESRD. The elucidation of the mesangial TfR-IgA1 interaction as a mechanism for selective mesangial IgA1 deposition suggests new avenues for drug design and treatment of IgAN.

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