Glomerular endothelial activation, C4d deposits and microangiopathy in immunoglobulin A nephropathy

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ABSTRACT

Immunoglobulin A nephropathy (IgAN) is considered as mesangiopathy since it initiates in the mesangium; however, other glomerular components are involved and the glomerular capillary wall offers the first contact to circulating macromolecular IgA1. Acute and active forms of IgAN are associated with endocapillary hypercellularity and vascular damage of various degrees, in severe cases with microangiopathy (MA) without or with thrombosis [thrombotic microangiopathy (TMA)]. Vascular damage activates complement and coagulation cascades. A defective complement regulation has recently been detected in active and progressive cases of IgAN. C4d deposits in renal biopsies have been found to be an early risk factor. These observations have raised interest in manifestation of MA and TMA in progressive cases of IgAN. MA–TMA lesions have been found in various percentages (2–53%) of patients with IgAN according to patients' selection and pathology definition of TMA. The association with hypertension (HTN) was so strong that it led to the hypothesis that MA/TMA in IgAN was a mere consequence of severe HTN. Old and new clinical and experimental data indicate that in IgAN the interaction of the glomerular capillary wall with immune reactants and complement uncontrolled activation leading to C4b deposits favours the development of MA–TMA, which plays a role in progression and renal function decline. The central role of complement activation is relevant also for the new therapeutic interventions offered by the pharma.

Keywords: C4d, coagulation, complement, IgA nephropathy, thrombotic microangiopathy

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is a glomerular disease mostly affecting the mesangium, as prevalent IgA deposition associated with mesangial proliferation is the characteristic hallmark of this entity. However, other glomerular components are involved and, when activated, they play an independent role in the progression [1]. The Oxford classification of IgAN and further observations proved the value as a risk factor not only of mesangial hypercellularity (M), but also endocapillary hypercellularity (E), segmental glomerular sclerosis (S), tubular atrophy and interstitial fibrosis (T), and crescents (C) [2, 3]. IgAN is frequently associated with hypertension (HTN) and elevated mean arterial blood pressure, which are among the most powerful clinical risk factors for progression in both adults and children with IgAN [4–6]. However, vascular lesions related to HTN, such as arterial intimal fibrosis and hyaline arteriolosclerosis, have not been found to be significantly associated with poor renal outcomes in the Oxford IgAN [2], and in the long-term follow-up VALIGA studies [6].

The value of endothelial activation and endocapillary hypercellularity (E lesion) due to increased number of cells within glomerular capillary lumina, causing narrowing of vessel lumina, is associated in the retrospective studies with corticosteroid/immunosuppressive treatment, which blunt the negative effects of this lesion on progression [6–8]. Only sophisticated analysis of the interaction of E with steroids/immunosuppression allowed the rescue of this lesion as a true risk factor for progression, as demonstrated in adult cohorts never treated with immunosuppressive therapy [9]. Recent data have shown in uncontrolled studies that E lesions can regress under appropriate immunosuppression therapy, with similar beneficial effects after mycophenolate and reduced doses of steroids [10].

Acute and active forms of IgAN are associated with E and vascular alterations including severe cases of fibrinoid necrosis. This suggests glomerular capillary wall activation and reaction to the immune challenge triggering local inflammation involving the coagulation cascade with local complement activation, mimicking the mechanism active in vasculitis [11]. Renal vascular lesions in IgAN may include hyalinosis or thickening of arteriolar wall and microangiopathy (MA) with or without thrombosis and full expression of thrombotic MA (TMA) [12].

The frequency of MA–TMA lesions in IgAN has been a matter of debate, and the analysis was limited by the differing criteria employed to define these histological lesions and the clinical features. HTN and malignant HTN are so frequently associated that it was hypothesized that MA and TMA in IgAN are a mere

consequence of severe HTN. However, the reconsideration of old *in vitro* observations, recent experimental data and clinical observations indicates a primary role of the activation of the inflammation–complement–coagulation cascades and glomerular capillary wall injury in patients with IgAN and MA–TMA features. This process in IgAN may be relevant for progression, and it deserves consideration for new therapeutic approaches.

MA AND TMA IN IgAN

Acute MA has been recently re-defined according to the renal area of interest as: (i) arterial MA with thrombi or myxoid intimal swelling; (ii) arteriolar MA with fibrin, endothelial swelling or denudation, intramural fibrin or intimal swelling; and (iii) glomerular MA with the presence of fibrin, endothelial swelling or denudation, mesangiolysis or glomerular microaneurysms [13]. Chronic MA is defined by fibrous intimal thickening and concentric lamination and/or recanalization in the arterioles and arteries, which may have at glomerular level a double contour in glomerular capillary wall [13]. Arterial intimal sclerosis and arteriolar hyalinosis are detected and thrombi may be present or not in the full-blown picture of TMA [14, 15]. The hallmark of MA under electron microscopy (EM) is subendothelial space widening [16]. TMA is a non-specific morphologic finding that occurs in a number of clinical settings, including but not limited to thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome/atypical haemolytic uraemic syndrome, malignant HTN, preeclampsia, anti-phospholipid antibody syndrome, scleroderma and auto-immune diseases, radiation nephropathy, transplantation, sepsis or toxic injuries from various pharmacologic agents [17]. In addition, MA/TMA have been detected in lupus nephritis [18], membranous nephropathy [19], membranoproliferative patterns [20], anti-glomerular basement membrane [21], ANCA-associated, pauci-immune crescentic [22] and post-infectious glomerulonephritis [23].

Injury to the microvascular endothelium is pivotal to the development of TMA, as it leads to exposure of thrombogenic components of the glomerular basal membrane to coagulation proteins and platelets, complement activation, recruitment of neutrophils, thrombus formation, interstitial oedema and permanent damage [24–26].

Glomerular endothelial damage in IgAN

Signs of glomerular endothelial damage were detected in renal biopsies of patients with IgAN, including separation of endothelial cells from the GBM with dilatation of the subendothelial space, mesangial interposition and matrix accumulation [27]. The separation from the GBM is supposed to induce loss of glomerular endothelial cells, the obliteration of capillaries and inflammatory cell infiltration, fibrin exudation, rupture of the glomerular basement membrane and frequent crescent formation (in the scenario of a necrotizing pattern). All these lesions contribute to acute and chronic damage and the progression of IgAN. The most frequent clinical manifestation of endothelial damage in the presence of these alterations is proteinuria.

Experimental and renal pathology evidence of endothelial glomerular activation in IgAN

Renal endothelial cells play an important role in preserving the integrity of the vascular bed, including the glomeruli [28, 29]. Endothelial cells are the first cells to be exposed to damage induced by haemodynamic, immunologic or metabolic insults as they are the most abundant cells inside the capillaries. Endothelial cells are therefore likely to be involved in the pathogenesis and progression of IgAN.

Circulating IgA has some favoured attraction for endothelial cells as suggested by the detection of IgA reacting with endothelial cells (defined as anti-endothelial cell antibodies) reported by Kincaid-Smith and colleagues 30 years ago in 32% of patients with IgAN [30]. This biomarker positivity was associated with younger age, proportion of crescents >10%, fibrin crescents, and focal and segmental necrotizing lesions. A more recent study found anti-endothelial cell antibodies in 45% of patients with IgAN, mostly in cases with malignant HTN [31]. The test was designed to detect in ELISA the binding of plasma IgA to endothelial cell membrane lysates. Some data from our studies in these years suggested that the binding to cultured mesangial and endothelial cell lysate of IgA glycophorms isolated from patients with IgAN, or *in vitro*-prepared galactose deficient IgA (Gd-IgA), was modulated by the addition of sugars [32]. This observation suggests that internal sugar residue exposure of Gd-IgA1 in patients with IgAN favours their binding to cells by a lectinic (intersugar) interaction, more than by true antigen–antibody nature. We reported an increase in lectin-binding circulating IgA in patients with IgAN, and this binding was reversed *in vitro* by the addition of competitive sugars including mannose [32, 33]. In an elegant experimental model, Davin *et al.* [34] showed that the perfusion of aggregated IgA formed by the Mannose-binding Lectin Concanavalin A (ConA) and polymeric IgA (pIgA) into the aorta of rats led to: (i) a mannose-dependent binding of both IgA and lectin to the glomerular capillary wall; (ii) a focal and segmental proliferative glomerulonephritis with IgA, C3 deposits and fibrin/fibrinogen in most glomeruli; and (iii) focal thrombosis and small areas of capillary tuft necrosis in 10–15% of glomeruli, and segmental infiltration of these glomeruli by polymorphonuclear leucocytes and platelets. These lesions were inhibited by the competing sugar mannose and were not reproduced by aggregates of IgA with or without other lectins [34].

The attraction of macromolecular IgA for the endothelium was shown in an experimental model of nude mice, which were injected with purified pIgA obtained from gddY mice (a strain of spontaneous IgAN-prone mouse) labelled with a fluorescein tag [35]. Renal samples were evaluated by confocal microscopy by real-time imaging system. Molecular tomography and real-time 3D *in vivo* imaging showed that injected IgA from the susceptible mice began depositing along the glomerular capillary wall from 1 min and accumulated for 2 h, and then disappeared, followed by mesangial deposit formation [36]. Of interest, IgA in gddY mice is polymeric and has a high capacity for activating complement cascades, including the lectin pathway [36].

Even considering the differences in IgA molecular structure and immune clearance in mice and in humans, these *in vitro*

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observations and experimental models indicate that macromolecular IgA circulating in IgAN—provided with aberrant glycosylation and lectin-binding activity—is likely to be attracted by glomerular endothelium and possibly in conjunction with high permeability and increased pressure, displays a predisposition for deposition in the mesangial area. Of particular interest is the induction of IgAN with lectin pathway activation and signs of TMA in the experimental model of IgA-ConA injection [34].

It is of interest that this so-called 'fatal attraction of IgA and the glomerular mesangium' has recently been suggested by another experimental model of IgAN using transgenic mice overexpressing human IgA1, produced by innate-like B cells rather than high-affinity maturated IgA [37]. Mice overexpressing human IgA1 only had endocapillary IgA1 deposits without mesangial damage, and this was achieved, together with development of haematuria and proteinuria, only in mice transgenic for sCD89, the soluble receptor for IgA of myeloid cells and complexes and overexpression of the mesangial IgA1 receptor and transferrin receptor 1 [1]. But the interest of these observations, from the point of view of our review, is that endothelial cells are exposed to circulating macromolecular IgA1, and this contact is likely to directly activate, under favourable conditions, the endothelium itself, with the clinical and histological above-mentioned consequences.

Apart from the direct activation from circulating macromolecular IgA, endothelial cells may receive back messages from the mesangial area where IgA and complement are deposited. We previously demonstrated that *in vitro* interaction of mesangial cells with Gd-IgA1 isolated from patients with IgAN induced an up-regulated nitric oxide synthase (NOS) activity with enhancement of inducible NOS (iNOS) mRNA transcription [38]. Meanwhile, Gd-IgA1 glycoforms isolated from patients with IgAN induced down-regulation of vascular endothelial growth factor (VEGF)-A mRNA transcription and protein synthesis. The depression of the VEGF-A production was mediated by nitric oxide, since it was blunted by NOS-specific inhibitors. The induction of iNOS represents a key moment in inflammation, and release of iNOS after reaction of macroaggregated IgA and Gd-IgA1 with mesangial cells may be crucial in activation of related mediators [38]. The pro-inflammatory milieu triggered by the response of mesangial cells to macromolecular IgA1 may provide a backward activation of endothelial cells and damage, favouring endocapillary hypercellularity, apoptosis, local coagulation and complement cascade activation [38, 39].

Coagulation and complement cascade activation at endothelial cell level

Activated glomerular endothelium layer leads to the release of cell adhesion molecules (e.g. P-selectin, E-selectin, ICAM-1 and VCAM-1) and cytokines (e.g. IL-6, IL-8 and MCP-1) with neutrophil recruitment and adhesion [26]. Leucocytes as well as injured endothelium are the source for tissue factor (TF), the initiator of the extrinsic pathway of coagulation [40]. The activation of endothelial cells triggers two major pathways of innate defense, the complement and the coagulation systems [17, 41]. Interactions between the two cascades are mostly regulated by the serine protease activity of various components of both systems, which can interact. The coagulation cascade activates complement, as thrombin can cleave C3 with generation of C3a and then C5a formation, both crucially involved in inflammatory cell recruitment. C3a and C5a may, in turn, intensify the coagulation pathway by induced release of TF, Factor X activation, and platelet activation and aggregation with thrombi formation. On the other hand, thrombin regulates the decay-accelerating factor with the aim to limit inflammation and reduce the positive loop of activation leading to thrombosis. Another regulating factor acting on complement and coagulation cascades is thrombomodulin, which controls thrombin, but also binds to C3b and complement factor H (CFH) and accelerates the inactivation of C3b. Exposure of the endothelium to C5b-9 resulted in von Willebrand factor (VWF) secretion [42] leading to platelet activation and aggregation [43], all contributing to a prothrombotic state with thrombus formation, endothelial cell detachment, oedema and arteriolar occlusion [25, 26, 44, 45]. The presence of large VWF multimers has been detected in circulation in IgAN patients as well as in idiopathic TMA. Upon activation, endothelial cells shredded microparticles that injure endothelial cells and lead to further complement activation [46].

Several data indicate complement activation in IgAN is mostly acting at mesangial levels, where co-deposition of C3 with IgA is supposed to confer particular nephrotoxicity to the IgA1-containing immune material [1, 47]. Indeed, in the absence of C3 deposits, the renal damage is generally mild without clinical manifestation as in the 'lanthanic form of IgAN'. Increased levels of complement breakdown products in circulation and other signs of slight consumption of CFs in plasma suggest a continuous subtle complement activation in IgAN [47]. Recent data indicate a possible role of a defective control of the alternative and lectinic complement pathways. Increased levels of CFH-related proteins (CFHR1–5) detected in IgAN may compete with factor H activity leading to increased production of C3bBb convertase [47, 48]. The regulation of complement activation at cellular membrane level is controlled by a series of factors including CD46 (also called MCP), which prevents the formation of the convertases C3bBb (alternative pathway) and also the convertase C4bC2a (lectinic pathway). We reported defective CD46 expression in circulating cells of patients with progressive forms of IgAN in comparison with non-progressive cases [49]. High intrarenal transcript of complement regulatory proteins, including CD46, have been detected in recurrent IgAN as well as in chronic allograft nephropathy [50].

C4d deposits at glomerular levels have also been reported to be associated with progression and even predict unfavourable courses at an early stage of IgAN [51]. In the absence of C1q activation, C4d deposits would theoretically be produced by the lectin pathway activation [52]. However, the mechanism leading to C4d production and deposition are not completely clear, as Gd-IgA1 selectively activates the alternative complement pathway, lacking activity on the lectin pathway mostly driven by sugar residues containing mannose or *N*-acetyl glucosamine [53]. A possibility of activation of lectinic pathway in IgAN via

Table 1. Studies addressing TMA lesions in patients with IgAN

References	Method	Biopsies (n)	TMA (%)	HTN (%)	Proteinuria	ESRD (%)	Predominant Oxford scores	Type of study
Chang et al. [56]	LM/EM	435	23	100	Yes	70	M1, S1, T2, C2	R
El Karoui et al. [14]	LM	128	53	82	Yes	>80	M1, T1, T2	R
Nasri [58]	LM	102	2	100	Yes	100	M1, S1, T1, T2	R
Cai et al. [57]	LM/EM	944	20	67	Yes	50	S1, T1, T2, C1-C2	R
Haas and Mirocha [59]	LM/EM	2290	49	>90	Yes	?	M1, T1, T2	R
Chua et al. [13]	LM/IHC	128	20	77	Yes	55	M1, S1, T1, T2	R

IHC, immunohistochemistry; M1, mesangial proliferation >50%; S1, presence of glomerulosclerosis; T1, tubulo-interstitial compromise >25%; T2, tubulo-interstitial compromise >50%; C1, presence of crescents <25%; C2, presence of crescents >25%; R, retrospective.

the glycans of IgG anti-IgA1 glycans has recently been proposed [54]. In both recurrent IgAN and chronic rejection renal biopsies, an increased expression of CD46 has been detected [50]. It could then be speculated that this phenomenon may be the consequence of an attempt to downregulate this process. It is of interest that C4d was reported to be commonly found in MA, and a recent study suggests that MA in IgAN develops following complement activation [55].

MA and TMA in IgAN

The main studies about TMA in patients with IgAN are depicted in Table 1. The first extensive investigation is a retrospective study by Chang et al., which detected TMA by light microscopy (LM) and/or EM in 10 (2.3%) of 435 biopsies performed from 1998 to 2004 showing IgAN [56]. Seven biopsies had arteriolar thrombi and glomerular changes by EM, while three had glomerular changes by EM only. All 10 patients were hypertensive and 6 had malignant HTN; 5 had nephrotic-range proteinuria. The outcome was poor, with end-stage renal disease (ESRD) in 70% of the cases. The spectrum of glomerular injury patterns ranged from normal glomeruli to mesangial proliferative glomerulonephritis. The most common lesion was extensive global glomerular sclerosis involving >50% of glomeruli, focal segmental sclerosis with collapsing aspects in some cases. Crescents were detected in 40% of the cases, mostly focal, of which only one patient showed segmental necrosis. Two cases revealed focal collapsing glomerulopathy, of which one also had cellular crescents. Seven cases had advanced and extensive global glomerulosclerosis (>50% of glomeruli). Histologic features of TMA, such as arteriolar thrombi (50%), glomerular capillary double contours (20%), mesangiolysis (10%) and glomerular capillary thrombi (10%), were observed. All cases showed moderate-to-severe interstitial fibrosis and tubular atrophy, with interstitial inflammation and interstitial oedema. This study concluded in providing evidence that a TMA injury in the setting of IgAN was not an uncommon finding and, when present, is usually found in advanced stages and associated with proteinuria and severe HTN [55].

Different findings were reported in a study from France by El Karoui et al. [14]. In 128 patients diagnosed with IgAN, 68 (53%) were found to have changes in acute or organized TMA in arterioles and/or arteries. The diagnosis of TMA was made on the basis of fuchsinophilic staining in involved vessels on the Masson trichrome stain; EM was not performed and CD61 stain for platelet glycoprotein GPIIIa was performed in 17% of the cases. Only 8 of 68 patients with histologic TMA changes on the renal biopsy had laboratory evidence of TMA. The disease presentation was proteinuric and survival poor, and this was worst in patients with laboratory evidence of TMA. A very high percentage of patients were either frankly hypertensive (48%) or normotensive on antihypertensive treatment (34%), and 14% had malignant HTN. The frequency of HTN was higher than that in other series [57, 58], probably due to the fact that several patients were diagnosed in a very active HTN clinic in the same institution. This biased recruitment of patients is likely to account for the much poorer survival (80% of malignant hypertensive patients ended up in ESRD). Their data revealed that IgAN-associated TMA increased markedly in frequency with increasing HTN. However, IgAN-associated TMA did not necessarily develop in a setting of advanced parenchymal lesions, with 19% occurring in patients with normal glomerular filtration rate (GFR), and 23.9% occurring in patients with minimal to mild (Oxford Class T0) interstitial fibrosis/tubular atrophy. It thus appears clear that TMA preceded the development of glomerulosclerosis and interstitial fibrosis rather than being a consequence of it, and the study concluded that neither HTN nor advanced parenchymal lesions are necessary prerequisites to the development of TMA [14].

In contrast to El Karoui et al., Nasri detected in 102 cases of IgAN, lesions of TMA in 2% patients, all of them with malignant HTN [58]. In a more recent study from China, Cai et al. examined biopsies from 944 IgAN patients by LM and EM who were followed for a median of 4 years [57]. Acute or chronic TMA changes were detected in 20% of the cases. Patients with TMA had a higher mean systolic blood pressure, lower GFR, greater proteinuria at the time of biopsy and more malignant HTN (10% versus 1%). Patients with TMA were also more likely to progress to ESRD (39% versus 11%), and TMA was an independent predictor of progression.

The largest study was recently reported by Haas and Mirocha in 2290 native renal biopsies of IgAN from 2010 to 2017, investigated by EM in 80% of the cases [59, 60]. Of these biopsies, 49 (2.2%) showed changes of TMA by LM, EM or both; only 2 of these patients had systemic changes of TMA (haemolytic anaemia and thrombocytopenia). Lesions of TMA involved glomeruli, arterioles and arteries in equal percentages

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and in only 10% of them overall. The single most common histologic lesion was arteriolar fibrin thrombi, which was present on 24 of the 49 biopsies. Glomerular changes of TMA by EM were seen in 67% of the cases. Patients with TMA were younger, and consistent with previous studies were proteinuric and more likely to be hypertensive and to have had malignant HTN. They also had more advanced chronic kidney disease. The Oxford MEST-C scores were detected as the only difference between patients with and without TMA T scores, particularly when arterial involvement was present [59].

As an important contribution to the issue of MA, IgAN was recently produced by Chua et al. [13]. They started from the reports of detection of between C4d deposits in cases of IgAN with progressive course [13] and their own previous observation of C4d detection in renal biopsy tissue of patients with MA from various clinical conditions [55]. In their latest study, Chua et al. [13] reported 128 renal biopsies from adult and paediatric patients who presented with IgAN or IgA vasculitis (IgAV) with nephritis followed for a mean of 50 months. MA lesions were present in 20% of all biopsies (23 and 9% of patients with IgAN and IgAV, respectively). When MA was present, it was focal, localized to glomeruli (14%), arterioles (81%) or both (4%). Active MA was detected in 35% of the cases and chronic MA in 65%. MA was associated with C4d and C5b-9 deposits, greater chronic changes and HTN. However, the poor survival observed in patients with MA was independent of HTN. The authors concluded that patients with IgAN or IgAV showing C4d positivity and MA represent a clinical subgroup at risk of poorer renal survival, even when corrected for HTN, suggesting the role of complement-mediated MA in disease progression.

CONCLUSIONS AND COMMENTS

The reported fraction of IgAN patients with morphologic lesions of MA–TMA varies greatly in different studies, from ~2% to >50%. These differences can be largely accounted for by the morphologic criteria used to define such lesions. However, findings reported to date, including those from Haas and Mirocha [59, 60], agree that the presence of MA in IgAN is associated with more frequent HTN and malignant HTN, as well as more severe proteinuria and abnormal GFR, all features associated with worse clinical outcomes in IgAN [61]. Consideration should therefore be given to the presence of microvascular damage in a subgroup of patients IgAN accounting in median for 20% of the cases. The relationships between these lesions and lectin complement pathway activation and C4d deposits suggest the hypothesis that the interaction of endothelial cells with macromolecular IgA1 provided with peculiar glycan exposure activates local inflammation, and the innate immunity of complement and coagulation pathways [13, 40, 49]. The process may be favoured in a context of defective control of complement activation at cellular surfaces. This chain of events suggests the potential benefits of new drugs targeting the complement cascade [62].

CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part.

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