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Deposition of the lectin pathway of complement in renal biopsies of lupus nephritis patients

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ABSTRACT

Background/aims: Lupus nephritis (LN) is one of the most serious manifestations of SLE occurring in 66–90% of these patients. The complement system is part of the innate immunity and modulator of inflammation and the adaptative immune response. Mannan-binding lectin (MBL) and Ficolin-2 (FCN-2) are important members of the lectin pathway of complement activation. Despite the significant participation of complement in the pathogenesis of the LN, there are few reports demonstrating "*in situ*" deposition of complement components in renal biopsy specimens in this disorder. The present study investigated the deposition of complement components in kidney specimens of LN patients.

Methods: Renal biopsies of 11 patients with SLE and LN were evaluated for immunofluorescence staining for IgG, IgA, IgM, C3, and C1q. Additionally, MBL, FCN-2 and C5b-9 were researched using monoclonal antibodies.

Results: All the biopsies were positive for IgG, C3, and C1q, eight were positive IgM and five had IgA deposition in glomerular tissue. The terminal complex of complement C5b9 was positive in all cases, MBL in nine (82%) cases; seven (63.6%) of them presenting concomitantly FCN-2 deposition. Patients presenting MBL deposition had higher mean of urinary proteins (9.0 g/day) than patients with negative MBL deposition (mean of 2.3 g/day).

Conclusions: In this study, we demonstrated *in situ* the participation of complement in the renal injury, including MBL and FCN-2 of the lectin pathway; also the strong role of C5b-9 in the pathogenesis of LN.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystemic disease, which is associated with the production of autoantibodies and immune complexes formation, which predominantly target nuclear antigens. Lupus nephritis (LN) remains a major challenge and continues to be one the most severe manifestation of SLE, occurring in 50–80% of these patients [1]. The morphologic findings of LN in renal biopsy comprise a spectrum of vascular, glomerular and tubulointerstitial lesions [1]. LN is histologically evident in most patients with SLE, even those without clinical manifestations of renal disease. The progression of LN can be related to hypertension, proteinuria and renal failure. In the direct immunofluorescence, IgG deposition is found positive in more than 90% of cases; IgA and IgM staining in 60–70% of cases; C3 and C1q in around 80% of cases [2]. The presence of the three immunoglobulin (Igs) with C3 and C1q is denominated as "full house" pattern, characterizing LN and uncommon in other renal diseases. The presence of subendothelial deposits in glomerular capillaries is crucial in the induction of severe damage [3]. However, the clinical presentation does not correlate very well with the type and severity of renal biopsy histology findings [1]. Based on experimental models of autoimmune and immune complex disease in the kidney and on observations in human renal biopsies, it is well established that the glomerular patterns of immune complex-mediated injury are related to the site of accumulation of immunoglobulin, their antigen specificity, their capacity to bind and activate complement and other serine proteases as well as their ability to evoke a cellular inflammatory response [4]. Findings of hypocomplementemia in most of patients with active disease, and, at least in some, complement levels correlate with the activity of renal disease [5].

The complement system is part of the innate immunity and an important modulator of inflammation and the adaptative immune response. Mannan-binding lectin (MBL) and Ficolin-2 (FCN-2) are important members of the lectin pathway of complement activation. MBL is a C-type plasma lectin acting as an ante-antibody and/or as a disease modifier molecule [6]. MBL is able to eliminate

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potential pathogenic organisms by activating the complement cascade and by facilitating phagocytosis as an opsonin. In addition, it has been shown that MBL is able to promote direct opsonophagocytosis, clearance of apoptotic cells and to modulate inflammation [7]. High levels of MBL and related genotypes have been shown associated with disease severity in chronic inflammatory conditions [7,8]. Takahashi et al. [9] described that *MBL2* gene polymorphism influences susceptibility to SLE, but has no direct effect on disease characteristics and that serum MBL concentrations vary during the course of SLE. FCN-2 are pattern-recognition proteins capable to bind to specific pathogen-associated molecular patterns (PAMPs) on microorganism surfaces, triggering the innate immune response by either binding to collectin cellular receptors or activating the complement lectin pathway [10]. Watanabe et al. [11] related significant decreased FCN-2 levels in the serum of SLE patients, however, not associated with activity of disease or C3 and C4 levels.

Despite the significant participation of complement in the pathogenesis of the LN, there are few reports demonstrating "*in situ*" deposition of complement components in renal biopsy specimens in this disorder. Sato et al. [12] showed that complement pathway activation is a basis of LN pathogenesis and that alternative as well as lectin pathway are involved in the progression of glomerular injury of Japanese patients with SLE and LN.

Considering the central role of complement in renal injury in patients with LN, the present study investigated the deposition of complement components in kidney specimens of LN patients from South Brazil.

2. Materials and methods

The local Research Ethics Committee approved the study.

Eleven patients (eight female and three male, between 23 and 41 years old) diagnosis with SLE according to American College of Rheumatology [13] were included in the study. Renal biopsies that were used for routine diagnostic of LN and kept cryopreserved at −80 °C of until used. The diagnosis of LN was based on clinical and histological evaluation of renal biopsy tissue. For light microscopy, paraffin-embedded sections were routinely stained with hematoxylin and eosin, periodic acid–Schiff, AZAN or Masson trichrome and periodic acid–silver methenamine. Two experienced pathologists evaluated these sections. Histological evaluation was divided into groups according to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) WHO classification [14]. For routine immunofluorescence staining for IgG, IgA, IgM, C3, and C1q was performed on fresh frozen renal specimens was using corresponding fluorescein isothiocyanate (FITC) conjugated antibodies (polyclonal antibody, dilution 1/40, Dako, Copenhagen, Denmark). Kidney tissues of five autopsies from individuals without renal disease were used as negative controls.

All specimens were cut in cryostat in 4 μm thick sections and placed on albumin treated slides. For the detection of MBL, FCN-2, and C5b-9, the slides with the cryostatic skin sections were incubated overnight at 4 °C with the monoclonal antibody diluted in PBS buffer pH 7.4 (*anti-MBL*, *Mo Hyb 131-01*, diluted 1/50, Bio-Porto, Gentofte, Denmark; *anti Ficolin-2, Mo P-35*, diluted 1/20, Hycult, Uden, The Netherlands and *anti C5b-9, Mo w13-15*, diluted 1/20, kindly provided as a gift from Prof. Dr. RW), respectively. After incubation, the slides were washed for three times with PBS pH 7.4 and incubated with conjugated antibody (polyclonal goat anti-Mouse/FITC (Sigma,San Diego, USA) diluted 1/200 at room temperature for 60 min. For the detection of C3, IgG, IgA, IgM, and C1q, diluted polyclonal antibodies were applied to the skin sections and incubated for 60 min at room temperature. After washed for three times with buffer, the slides were mounted with glycerin alkaline and observed in fluorescence microscope (Olympus, Tokyo, Japan) using specific software to capture image. The intensity of all positive deposits was evaluated by two blinded independent observers and graded as follows: N = negative staining, L = minimal staining, M = moderate staining and S = strong staining.

On the same day which the patient has been biopsied, data from laboratory measurements of urinary proteins (g/day), creatinine clearance, serum creatinine, serum concentrations of C3 and C4, antinuclear and anti-DNA antibodies were collected.

3. Results

The findings of immunofluorescence deposition in patients with LN are demonstrated in Table 1 and in Fig. 1. All the biopsies ($n = 11$) were positive for IgG, C3, and C1q, with most of them showing intense pattern of deposition in capillaries. Eight (73%) patients showed deposition of IgM and five (45%) had IgA deposition in glomerular tissue. The "full-house" pattern was observed in 4 (36%) patients.

The terminal complex of complement C5b9 was positive in all cases, with different degree of intensity. MBL deposition was positive in nine (82%) cases; seven (63.6%) of them presenting concomitantly FCN-2 deposition. In two cases, both components of the lectin pathway were negative. MBL and C5b-9 were deposited in different areas in the renal tissue, including glomeruli, mesangial cells, blood vessels, tubular tissue and Bowman's capsule. Additionally, it was observed that patients presenting MBL deposition had higher mean of urinary proteins (9.0 g/day) than patients with negative MBL deposition (mean of 2.3 g/day). None of the other laboratory parameters were associated with different degree and/or pattern of deposition.

In the biopsies of controls, no positivity was observed for all investigated markers.

4. Discussion

LN carries significant morbidity, mortality and the renal involvement is a key determinant for the prognosis of SLE. The prognosis of LN depends on a large number of demographic, racial, genetic, histopathological, immunological and time-dependent factors [15].

The complement system plays a key role as a humoral component of the innate immune system and also starting the specific immune response. On the other hand, when inappropriately activated or regulated, complement can cause inflammation and tissue damage. Complement is involved in the pathogenesis of different

Table 1
Findings of immunofluorescence deposition in patients with lupus nephritis.

Patient number	Class	IgA	IgM	IgG	C3	C1q	C5b9	MBL	2-Ficolin
1	Class II	*Neg*	*L*	*S*	*S*	*S*	*S**	*S*	*M*
2	Class II	*M*	*M*	*S*	*S*	*S*	*S**	*S*	*M*
3	Class III	*L*	*M*	*S*	*S*	*S*	*M*	*M*	*M*
4	Class III	*L*	*L*	*M*	*M*	*M*	*L*	*L*	*L*
5	Class III	*L*	*Neg*	*M*	*M*	*M*	*M*	*Neg*	*Neg*
6	Class IV	*Neg*	*S*	*S*	*S*	*S*	*S*	*S*	*M*
7	Class IV	*Neg*	*M*	*S*	*S*	*S*	*M*	*M*	*M*
8	Class IV	*M*	*L*	*S*	*S*	*S*	*S#*	*S*	*M*
9	Class V	*Neg*	*Neg*	*S*	*L*	*L*	*L*	*Neg*	*Neg*
10	Class V	*M*	*L*	*S*	*S*	*S*	*M*	*M*	*Neg*
11	Class V	*Neg*	*S*	*S*	*S*	*S*	*M*	*M*	*Neg*

Neg = Negative; S = Severe; M = Moderate; L = Low.
* Moderate deposition of C5b9 also in blood vessels; #Deposition of C5b9 also in "Bowman's capsule".

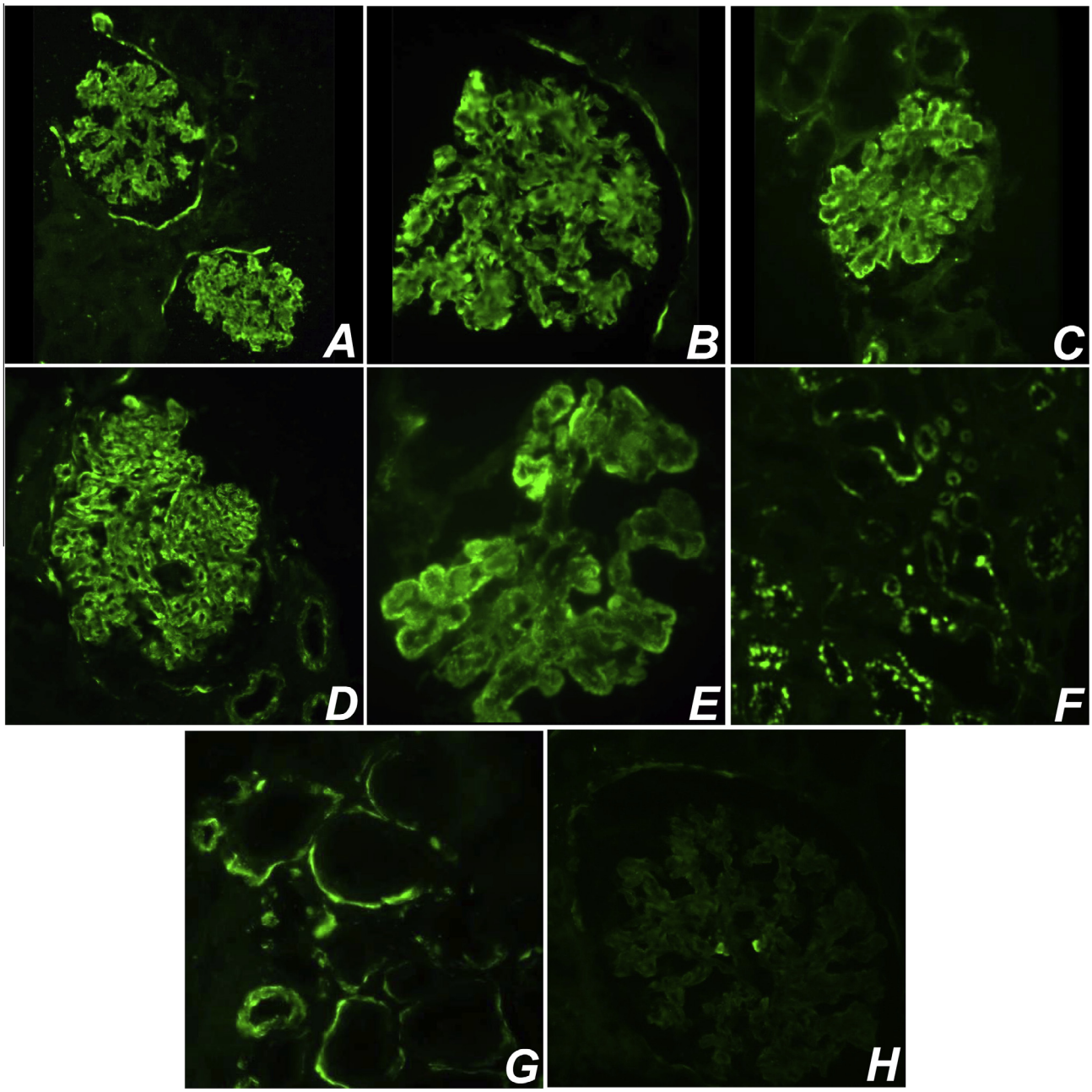

Fig. 1. Immunofluorescence findings in LN patients. Deposition of MBL (A and B), terminal complex of complement C5b-9 (C and E) and ficolin-2 (D) in glomerular capillaries, mesangial cells and Bowman's capsule (strong staining). In picture F and G deposition of MBL (200×) in tubular tissue. In picture H, minimal staining of ficolin-2 deposition.

inflammatory conditions, including SLE. In the present study, the authors investigated the deposition *in situ* of complement components, mainly MBL and FCN-2, members of the lectin pathway. MBL is known to mediate opsonization and complement activation via the lectin pathway; however, when MBL was shown to also bind to late apoptotic and necrotic cells, a role for MBL in body maintenance was highlighted [6]. Endo et al. [16] reported that the patients with IgA nephropathy presenting glomerular deposits of MBL were young and that the duration of the disease prior to renal biopsy was short compared with that of patients without MBL deposition. Also in IgA nephropathy, Roos et al. [17] reported that the most MBL-positive patients presented mesangial deposits of L-ficolin. Renal ischaemia/reperfusion (I/R) injury is a major issue in nephrology; both in renal transplantation and in other diseases characterized by acute renal failure and a large number of experimental studies have provided evidence that complement is involved in I/R injury [18]. In fact, MBL plays an adverse role in a number of renal inflammatory diseases, renal injury is largely due to the deposition of circulating MBL in the injured kidney, followed by activation of the complement cascade and production of pro-inflammatory molecules.

In autoimmune diseases such as SLE, the innate immune activation triggers the local release of cytokines and chemokines which attracts various subsets of leukocytes into the kidney including macrophages, T cells and B cells which occur in different subsets that contribute differently to the regulation of inflammation and renal immunopathology [19]. It's possible that in this process, new antigens are exposed, which are ligands for MBL including mannose and N-acetyl-glucosamine (GlcNAc) as well as ligands for ficolins such as GlcNAc. In our study, nine

(82%) cases presenting MBL deposition and seven (63.6%) FCN-2 deposition, demonstrated the activation of the lectin pathway in LN *in situ*. In previous studies, MBL, C3 and C5b-9 complex were found deposited in skin lesions of patients with pemphigus vulgaris indicating activation of complement through the lectin pathway with formation of terminal lytic complex [20]. The impact of lectin–glycan interactions in the control of immune tolerance, autoimmunity and chronic inflammation has been addressed recently [21]. Some authors have demonstrated that remodeling of cell surface glycans which occurs during inflammation were shown to modify the emigration and trafficking of immune cells to committed sites [21].

In fact, the generation of C3 convertase, independent of the activation pathway is a crucial point in the complement cascade. Once activated through the classical, alternative and the lectin pathways, split products such as C5a and C3a act as potent mediators of inflammation. The association of C3b and C3 convertase also results in the formation of C5 convertase, which activates the terminal complement complex pathway and the formation of the membrane-attack complex on cell surfaces, thereby resulting in cell lysis. In our study, the strong pattern deposition of C5b-9 was observed in the specimens only when MBL was also strongly positive, suggesting a role of MBL in the *in situ* complement activation. The terminal complex of complement was positive in all biopsies, confirming the participation of complement mediated lysis in the pathogenesis of glomerular, tubular and in two patients, vessels injury. In fact, soluble C5b-9 has been shown to be the most sensitive markers in assessing disease activity for SLE patients [22]. Moreover, the generation of the membrane attack complex C5b-9 appears to be the most nephritogenic effect of complement activation, which by inserting in sublytic quantities into the membranes of glomerular cells, leads to cell activation, proliferation of mesangial cells and the production of various disease mediators [23].

The participation of complement in renal injury is remarkable and a better understanding of the causes and pathogenesis of complement-mediated LN would to lead possible use of newer drugs, including anticomplement drugs, especially those that use complement regulatory proteins has been considered as potential therapeutic tool to prevent renal disease [23–25]. Until recently, the only treatment options for patients with LN were based on the use immunosuppressors. These treatments are aggressive and have side effects [24]. Experimental studies using recombinant protein inhibitors of complement with monoclonal antibodies that specifically inhibit terminal complement activation while preserving the critical functions of the early complement cascade have now been developed. In addition, humanized monoclonal anti-C5 antibody (eculizumab) is already used to treat atypical hemolytic, uremic syndrome, membranoproliferative glomerulonephritis and acute renal rejection. These antibodies target the C5 complement protein, blocking its cleavage and the subsequent generation of potent proinflammatory molecules. This antibody has been shown to inhibit complement safely and now is being investigated in a variety of clinical conditions [25]. Currently, anti-C5 monoclonal antibody and others (e.g. Rituximab) [26] offer expectation to be used therapeutically in LN and other immune-mediated glomerular disease, however, clinical trials must establish their efficacy.

Concluding, in this study, we demonstrated the participation of MBL and FCN-2 *In situ* LN tissue damage; reinforcing the role of lectin pathway of complement activation in the pathogenesis of this disease.

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