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Rapid Communication

Mannose binding lectin deposition in skin of lupus erythematosus patients: A case series

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ABSTRACT

Introduction: Mannose binding lectin (MBL) has been linked to predisposition to systemic lupus erythematosus (SLE) and to disease activity. Some studies found deposits of MBL in glomerular tissue of patients with lupus nephritis. There is no research about the deposition of MBL in skin.
Materials and methods: Skin biopsies from lesional and non lesional skin of 4 discoid lupus erythematosus (DLE) and 10 SLE patients were submitted to immunofluorescence staining for IgG, IgA, IgM, C3, C4, C1q, C5b-9 and MBL. Charts were reviewed for demographic, clinical and serological data. Patients with SLE had disease activity measured by SLEDAI.
Results: MBL was found only in SLE lesional skin and its presence showed an association trend towards higher disease activity. Deposition of C5b-9 occurred in vessels only in patients with SLE (70%) and in the two patients with kidney involvement.
Conclusions: MBL deposition was found in the lesional skin of SLE patients but not in SLE non lesional skin nor in DLE patients, and it seems to be less frequent and less strong than observed in the kidneys biopsies, suggesting that the complement participation in the pathophysiology of SLE process may not be the same in these two clinical manifestations.

1. Introduction

Complement is one of the most important mechanisms of natural resistance preventing infections in humans and animals [1]. It comprises more than 30 serum and membrane-bound proteins that can be activated through three different pathways: the classical (CP), the alternative (AP) and the lectin (LP) pathway. Activation of complement leads to a sequential cascade of enzymatic reactions resulting in the formation of the potent anaphylatoxins C3a and C5a and the membrane attack complex C5b-9 [1]. The CP is initiated when C1q binds to the Fc portion of IgG or IgM on immune complexes, becoming activated and subsequently cleaving C4 and C2 to form the CP C3 convertase, C4bC2a [1]. The AP is triggered when C3b binds to non self structures and subsequently to factor B forming a complex that is cleaved by factor D to form the C3 convertase C3bBb [1]. Finally, the LP is activated when mannose binding lectin (MBL) or ficolins bind to carbohydrate on the surface of foreign antigens which induces conformational changes in the associated proteases called MASPs, leading to the assembly of the LP C3 convertase C4bC2a [1].

Changes in the function or deficiency of individual components of the complement system have been described, mainly in those of the classical pathway. Congenital deficiencies of C1q, C2 and C4 components have been linked with genetic predisposition to both systemic lupus erythematosus (SLE) and in discoid lupus (DLE) [2]. In addition, the terminal complex C5b-9 or membrane attack complex (MAC), the final product of complement activation, is considered responsible for cell destruction seen in target tissues and a sensitive marker in assessing disease activity in SLE [3]. The role of the LP in lupus has attracted attention recently although its participation in the disease is not clear [4,5].

MBL seems to have a dual role in autoimmune diseases. While increased MBL levels are linked to enhanced complement activation and tissue damage, its deficiency has been associated with defective clearance of cellular debris providing a stimulus for autoantibody formation [5]. Furthermore, MBL deficiency is linked to

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susceptibility to infections which have been considered a contributory factor in the development of SLE [4].

Complement components have been found in the dermoepidermal junction of lupus patients with skin manifestations. C1q, C3 and C4 are detected in lupus band test (LBT), with C3 being the most commonly found [6]. Deposition of LP components have not yet been looked for in skin lesions of lupus although its deposition in glomerular tissue is well documented [7,8].

In this study, we aimed to evaluate the participation of MBL in the immune mechanism of skin lesion in skin biopsies of lupus patients and the association with lupus manifestation.

2. Materials and methods

This study was approved by the local Committee of Ethics in Research and all participants signed a consent. We included 14 patients, one male and 13 females; four with DLE and 10 with SLE. All SLE patients fulfilled at least 4 classification criteria according to the American College of Rheumatology [2]; all DLE patients had biopsy proven disease. As control, skin from four necropsy patients without skin or rheumatic diseases were investigated.

Patients were submitted to a punch biopsy with a 4 mm puncher of lesional and sun-protected skin of inner arm or posterior cervical region, and the material was kept cryopreserved at −80 °C until used. Light microscopy, paraffin-embedded sections were routinely stained with hematoxylin and eosin and periodic acid–Schiff. All specimens were cut in cryostat in 4 μm thick sections and placed on albumin treated slides. Immunofluorescence staining for IgG, IgA, IgM, C3, C1q was performed on fresh frozen skin specimens using corresponding fluorescein isothiocyanate (FITC) conjugated antibodies (polyclonal antibody, dilution 1/40, Dako, Copenhagen, Denmark).

For the detection of MBL 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 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 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 washing three times with buffer, the slides were mounted with alkaline glycerin and observed under fluorescent microscopy (Olympus, Tokyo, Japan) using specific software for image capture. The intensity of positivity deposits was evaluated by two blinded independent observers and graded as follows: negative staining (−), minimal staining (+), moderate staining (++) and strong staining (+++).

On the same day of the biopsy, patient́s disease activity was evaluated through SLEDAI index [2] and the charts were reviewed for clinical and laboratory profile.

Data was collected in frequency tables. Central tendency was expressed in mean ± SD and median values. To evaluate the association of SLEDAI with the presence of MBL deposition, Mann Whitney test was used. Calculation was done with the software Graph Pad Prism 4.1.

3. Results

3.1. Casuistic and samples

Among the 10 SLE patients investigated, nine were female and one male, with age between 22 and 58 years (mean of 43.71 ± 11.28 years) and disease duration from one to 20 years (median of 5 years); 2/14 (16.6%) were Afrodescendant and 12/14 (85.71%) were European descendants.

In relation to SLE patients, 6/10 (60%) had arthritis, 5/10 (50%) leucopenia and 2/10 presented glomerulonephritis. Central nervous system involvement and pleuritis were seen in one patient each one. All SLE patients were ANA (antinuclear antibody) positive with titers from 1/160 to 1/2560; 6/10 (60%) anti-Ro, 3/10 each were anti-La or anti-dsDNA. One patient each was positive for anti-RNP and anti-Sm. SLEDAI varied from 0–10 (median of 2), serum C3 ranged from 26–136 mg/dl (median of 80 mg/dl) and serum C4 from 7–155 mg/dl (median of 19 mg/dl). Only one LED patients had positive ANA at low titer (1/80).

Concerning treatment, 10/14 (71.4%) of the patients used antimalarials; 9/14 (64.3%) oral glucocorticoid; 5/14 (35.7%) methotrexate; 3/14 (26.06%) azathioprine; and one patient each was treated with tacrolimus, cyclophosphamide and thalidomide.

3.2. Immunofluorescence in skin biopsies

Analysis of immunoglobulin deposition showed that in lesional skin, IgM was present in 1/4 (25%) of DLE and in 4/10 (40%) of SLE patients. IgG was present in lesional and no lesional skin of all patients (SLE and DLE) and there was no IgA deposition. Non lesional skin had IgM in 1/4 (25%) of DLE patients and 3/10 (30%) of SLE patients and IgG was present in all patients although DLE patients had a weak deposition (Table 1).

The results for C3, C1q, MBL and C5b-9 in lesional and non lesional skin can be seen in Table 1. Deposition of C5b-9 with a granular pattern in basal membrane was seen in 12/14 (85,71%) of all patients, but occurred in vessels only in patients with systemic disease, in 7/10 (70%) (Fig. 1). The two patients with kidney involvement had vascular deposition of C5b-9. There was no C1q deposition in DLE patients, and was seen in 4/10 (40%) lesional skins and in only one (10%) non-lesional skin of patients with SLE. Deposits of C3 were seen in all biopsies of both SLE and DLE. A total of 4/10 (40%) of LES patients presented MBL deposits in lesional skin, and only one of them had MBL in non lesional skin. No LED patient had MBL deposition in the lesional or non lesional skin.

SLEDAI varied from 2 to 10 (median of 3.0) in patients with MBL deposition and from 0 to 2 (median of 2.0) in those negative for MBL ($p = 0.071$). No differences could be noted in the clinical and autoantibody profile in those patients with and without MBL deposition in the skin.

4. Discussion

The role of MBL has been studied in lupus under several contexts. The first aspect is related to the association of *MBL-2* gene with predisposition to the disease. Results in this aspect have been contradictory. While Tsai et al. [9] could not link *MBL-2* gene polymorphism to LES, Sandrini-Garcia et al. [10] found association of MBL polymorphism with lupus nephritis and antiphospholipid syndrome.

The second aspect is about associations of MBL serum levels in lupus. Panda et al. [11] have shown that high MBL levels were associated with major organ involvement and activity disease in untreated SLE patients, particularly in those with proteinuria and anti-ds-DNA positivity. They questioned if MBL could behave as acute phase reactant. As MBL deposition is found in renal tissue of lupus glomerulonephritis patients, these authors highlight that nephritis may not directly correlate with plasma but tissue MBL levels. Interesting enough in this same study, patients with musculoskeletal and cutaneous manifestations had low serum MBL [11].