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On anti-lamin B1 autoantibodies as major determinant of thromboprotection in SLE patients displaying antiphospholipid antibodies

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ABSTRACT. Autoantibodies directed against components of the nuclear lamina have been initially described using the Western-blot technique. IgG autoantibodies against lamin B1 have been properly identified using an enzymatic immunoassay, mainly in systemic lupus erythematosus (SLE) patients' sera. It is clear from these works that a subgroup of SLE patients display high titers of autoantibodies against lamin B1 (aLB1), and that those aLB1 were not observed in other rheumatic diseases. In addition, these aLB1 were strongly associated with the lupus anticoagulant. A subsequent exciting finding in a group of 259 SLE patients was the frequency of thrombosis displayed depending on aLB1 and lupus anticoagulant (LAC) status as follows: presence of LAC and abscence of aLB1 50%, presence of both LAC and aLB1 22.7%, abscence of both LAC and aLB1 25.5%, abscence of LAC and presence of aLB1 20.8%. This effect associated with the presence of aLB1 was proposed as a «thromboprotection» and was independent of other antiphospholipid-related antibodies tested such as anticardiolipin and anti-beta(2)GPI. The mechanisms involved in this phenomenon remain to be elucidated, although no evidence was found that supports a direct role of lamin B1 in apoptotic blebs.

Key words: Autoantibodies, anti-lamin B1, nuclear envelope, antinuclear antibodies, antiphospholipid, lupus anticoagulant.

RESUMEN. *Los autoanticuerpos dirigidos contra componentes de la lámina nuclear se describieron detectándolos con técnica de electroinmunotransferencia. Los anticuerpos IgG contra la lámina B1 se han identificado mediante ensayo inmunoenzimático, principalmente en el suero de pacientes con lupus eritematoso sistémico (LES). Se sabe que un subgrupo de pacientes con LES exhiben títulos altos de anticuerpos contra lámina B1 (aLB1), y que estos aLB1 no se observan en otras enfermedades reumáticas. Adicionalmente, estos aLB1 se asocian fuertemente con el anticoagulante lúpico (AL). En otro trabajo, la frecuencia de trombosis en un grupo de 259 pacientes con LES según la positividad a aLB1 y a AL fue así: AL positivo, aLB1 negativo 50%; ambos AL y aLB1 positivos 22.7%, ambos AL y aLB1 negativos 25.5%, AL negativo con aLB1 positivo 20.8%. Este fenómeno de baja frecuencia de trombosis asociado con la presencia de aLB1 se ha propuesto como trombo-*

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protección. Este efecto es independiente de pruebas como anticuerpos anticardiolipina y anticuerpos contra B2 glucoproteína I. El mecanismo involucrado debe ser identificado.

Palabras clave: *Autoanticuerpos, antilámina B1, envoltura nuclear, anticuerpos antinucleares, antifosfolípido, anticoagulante lúpico.*

INTRODUCTION

Autoantibodies to lamins, the major polypeptide components of the nuclear lamina, have been reported in selected sera from patients with autoimmune diseases, including anti-lamin B in systemic lupus erythematosus (SLE) and anti-lamins AC in autoimmune chronic active hepatitis (CAH). In a carefully performed study, Senecal et al[1] studied the frequency, specificity, and isotypy of autoantibodies to major and minor lamins by immunoblotting on purified rat liver lamins in 190 sera from normal controls (n = 62), rheumatic disease controls (n = 42), and autoimmune disease patients (n = 86). The frequency of anti-lamin in normal controls was 85.5%, and ranged from 77 to 100% in the other groups. In particular, anti-lamin B were not more common in SLE than in normal sera. Anti-lamin isotypes were IgG and/or IgM. The highest end point titers (greater than or equal to 1:3,200) were observed with chronic autoimmune hepatitis (CAH), SLE, and rheumatoid arthritis (RA) sera with IgG anti-lamins AC, B, or ABC, or with IgM anti-lamins ABC. None of these SLE and RA patients had evidence of liver disease. Reactivity with minor lamins was more frequent in CAH. We conclude from those initial works that anti-lamin autoantibodies were present in sera from most individuals, that the highest titers are found in sera from patients with autoimmune diseases, and probably those patients displaying a highest titer anti-lamin antibodies could represent an special subgroup. However, the non-specificity of these Western-blot detected anti-lamin antibodies was evident, and this notion was enforced by the findings of other groups[2] that found this non-specific IgG isotype anti-lamin reactivity using immunofluorescence and Western-blot techniques in sera of 60 patients with chronic fatigue syndrome. These authors speculate that occurrence of autoantibodies to a conserved intracellular protein-like lamin B1 provides new laboratory evidence for an autoimmune component in chronic fatigue syndrome; however, the anti-lamin reactivity in this patients was at low titers, and it was already known that clinically healthy subjects could display this reactivity.

aLB1 QUANTITATIVE DETECTION

Research on aLB1 reached an important advance once an specific solid-phase enzyme-linked immunoassay using recombinant human LB1 was developed,[3] solving the discrimination step between unespecific low-titer reactivity and high-titer aLB1, as evidenced in the work by Senecal et al,[3] determining the frequency and clinical significance of high titers of IgG autoantibodies to nuclear lamin B1 in a large number of unselected and well-characterized systemic lupus erythematosus (SLE) patients, disease controls, and normal healthy controls.

aLB1 AND FREQUENCY OF THROMBOTIC EVENTS

In this cross-sectional study, detection of anti-lamin B1 autoantibodies with the new immunoassay was performed on serum samples obtained at first evaluation of 238 consecutive French Canadian adults: 61 healthy control subjects, 20 patients with osteoarthritis, 22 with ankylosing spondylitis, 11 with autoimmune hepatitis, 30 with rheumatoid arthritis, and 94 with SLE. SLE patients were studied for 57 disease manifestations. A case-control study was performed to analyze the relationship between anti-lamin B1 status and thrombotic

manifestations between SLE onset and last follow-up. High titers of anti-lamin B1 were strikingly restricted to a subset of 8 SLE patients (8.5%). The mean anti-lamin B1 titer was higher in this subset than in the other SLE patients or any control group ($p < 0.001$). By univariate analysis and stepwise multiple logistic regression, the most striking association of anti-lamin B1 was with lupus anticoagulant (LAC) antibodies ($p = 0.00001$). Although LAC was significantly associated with thrombosis in our SLE patients, anti-lamin B1 was not. The frequency of thrombosis in SLE patients expressing both LAC and anti-lamin B1 was similar to that in patients without LAC ($p = 1.0$). However, patients expressing LAC without anti-lamin B1 had a greater frequency of thrombosis ($p = 0.018$). High titers of IgG anti-lamin B1 autoantibodies are highly specific for a subset of SLE patients whose clinical characteristics include the presence of LAC and other laboratory manifestations of the antiphospholipid syndrome. The presence of LAC without anti-lamin B1 may define a subset of SLE patients at greater risk for thrombosis.

aLB1 AND THROMBOPROTECTION

The forementioned findings introduced the concept that the expression of clinical features associated to antiphospholipid antibodies may be co-modulated, at least from the statistical approach. This hypothesis was of clinical relevance because if confirmed a new clinical subset of patients displaying antiphospholipid antibodies may arise, and with the presence of high-titer aLB1, be the major determinant for thromboprotection.

The same group of researchers performed other work in order to demonstrate the association between autoantibodies to nuclear lamin B1 (aLB1) and protection against thrombosis (thromboprotection) in patients with systemic lupus erythematosus (SLE), and to elucidate the mechanism by which aLB1 cause thromboprotection *in vivo*.[4] Since a number of autoantigens in SLE have been localized specifically to the external surface of apoptotic blebs, it was hypothesized that circulating aLB1 may block the procoagulant effect of apoptotic blebs by binding to LB1 displayed at the external bleb surface. A cross-sectional study was performed using serum samples obtained at first evaluation of 259 English Canadian and French Canadian patients from SLE registries at 3 hospitals. A case-control study was performed to analyze the relationship between aLB1 and lupus anticoagulant (LAC) status and thrombotic manifestations between onset of disease and last follow-up. Reactivity of aLB1 with Jurkat or endothelial cells which had been induced to undergo apoptosis was determined by indirect immunofluorescence. Localization of LB1 in apoptotic cells and blebs was analyzed by confocal microscopy and surface labeling of cell membrane proteins. High-titer aLB1 was restricted to a subset of SLE patients (46 patients), with an overall frequency of 17.8% (range 11.6-24.3% in the three centers). LB1 antibodies were significantly associated with LAC but not with antibodies to cardiolipin (aCL) or beta(2)-glycoprotein I (anti-beta(2)GPI). The frequency of thrombosis differed markedly depending on aLB1 and LAC status, as follows: presence of LAC and absence of aLB1 50%, presence of both LAC and aLB1 22.7%, absence of both LAC and aLB1 25.5%, absence of LAC and presence of aLB1, 20.8%. Further subclassification of patients based on aCL and anti-beta(2)GPI status revealed that in the presence of LAC but in the absence of aCL, anti-beta(2)GPI and aLB1, the frequency of thrombosis was 40%, whereas in the presence of aLB1 it decreased strikingly, to 9.1%. LB1 was found to be translocated into surface membrane blebs during apoptosis and to be entirely enclosed within the apoptotic bleb plasma membrane of Jurkat and endothelial cells. The presence of aLB1 in SLE patients with LAC essentially nullifies the strong prothrombotic risk associated with LAC. Hence, aLB1 is associated with thromboprotection. Reactivity of aLB1 with apoptotic blebs does not seem to play a direct role in

mediating this protection, since LB1 is buried within apoptotic blebs and inaccessible to circulating aLB1.

Several works describe involvement of intermediate filament proteins, including laming B1 in functions such as nucleotide excision repair,[5] or as the target of new antibodies used as marker tools in cancer,[6] even as a fundamental determinant of RNA synthesis in human cells,[7] or the coexpression with vimentin in spontaneous apoptotic human neutrophil model.

Despite these efforts, the mechanism by which aLB1 confers thromboprotection in SLE remains to be elucidated.

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