

LAVORO ORIGINALE

Possibile ruolo di Hsp70 nel trasporto di autoantigeni alla giunzione dermo-epidermica nel lupus eritematoso sistemico

Possible role of Hsp70 in autoantigens shuttling to dermo-epidermal junction in systemic lupus erythematosus

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RIASSUNTO

La formazione di depositi di materiale immune lungo la giunzione dermo-epidermica nei pazienti affetti da lupus eritematoso sistemico (LES), è il risultato dall'interazione in situ degli anticorpi antinucleari con i loro rispettivi antigeni. L'esposizione al sole determina la liberazione di autoantigeni cutanei e simultaneamente la produzione di heat shock proteins (Hsp). In questo studio abbiamo valutato mediante immunofluorescenza la presenza di Hsp70 lungo la giunzione dermo-epidermica in 20 biopsie cutanee di pazienti affetti da LES ed in 20 soggetti di controllo. I depositi immuni cutanei erano principalmente composti da IgG, IgM e C3. Tali depositi sono stati evidenziati lungo la giunzione dermo-epidermica in tutte le biopsie dei pazienti affetti da LES. Erano inoltre evidenti depositi di materiale immune nei vasi cutanei ed in misura minore nei keratinociti epidermici. La presenza di Hsp70 è stata evidenziata nel 60% delle biopsie dei pazienti affetti da LES. Questa proteina era principalmente distribuita lungo la giunzione dermo-epidermica ed intorno ai vasi cutanei. E' stata inoltre dimostrata mediante immunofluorescenza doppia una co-localizzazione dei depositi di materiale immune con Hsp70. I nostri risultati suggeriscono che Hsp70 potrebbe trasportare gli autoantigeni coinvolti nella patogenesi del LES a livello della giunzione dermo-epidermica.

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INTRODUCTION

Under stress cells and tissues perform a series of physiologic adjustments, including the heat shock protein production (Hsp), these proteins play a role as molecular chaperons, they are divided in five main families: Hsp27, Hsp 60, Hsp70, Hsp90 and Hsp110 (1). Hsp are constitutively induced or expressed to support the correct folding of novel proteins or to refold damaged proteins (2). Hsp70 is the most representative member of Hsp family. It is largely distributed in the majority of eukaryotic cell compartments and interact with the cytoskeleton during translocation of proteins across membranes (3). The chap-

eroning activity of Hsp70 has been broadly demonstrated in the skin.

Systemic lupus erythematosus (SLE) is an autoimmune disease with a wide range of clinical and pathological manifestations and variable organ involvement and clinical course. From the immunological point of view, antinuclear antibodies production is the serologic hallmark of SLE. Autoantibodies induce generation of immune complexes, which in turn, upon deposition at the dermo-epidermal junction, may lead to a positive lupus band test (4). Granular deposition of immunoglobulin at the dermo-epidermal junction is found in clinically normal skin and lesional skin of systemic lupus patients, and in lesional skin of discoid lupus patients. Such immunopathologic abnormality is characteristic, but not pathognomonic, of lupus erythematosus. However the lupus band test is useful in differential diagnosis because other disorders have either a specific pattern of immunoglobulin deposition or no immunoglobulin deposition (5). In

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view that Hsp are induced in the skin after sun exposure, we theorized that Hsp would shuttle autoantigens to the dermo-epidermal junction. To assess our hypothesis the presence of Hsp70 concurrent with lupus band immunoreagents was studied in skin biopsies from lupus patients.

MATERIAL AND METHODS

Patients. Twenty patients (18 females and 2 males; mean age 23 years, range 16-35 years) with systemic lupus erythematosus diagnosed according to ACR classification criteria were studied (6). Skin biopsies were performed after informed consent had been obtained. Twenty biopsies taken from the skin of the back of healthy individuals during orthopaedic surgery were used as controls.

Skin Biopsies. Samples were obtained from non-lesional and unexposed skin using a 5 mm punch, under local anaesthesia with 1% xilocaine (Astra). The biopsies were rinsed in 0.15 M NaCl pH 7.5 buffer solution (PBS), and immediately included in Tissue-Tek (Ames), and frozen at -20°C. The specimens were then cryosectioned at 4 µm, and used for immunofluorescent assays.

Direct immunofluorescence (IF). Skin biopsies were examined by IF as follows: 4 µm skin sections were fixed in cold acetone, washed in PBS and incubated 30 minutes with monospecific goat anti human-IgG, IgA, IgM, C3, C4, C1q and fibrinogen antibodies (Sigma, St Louis MO). After washings with PBS, the slides were evaluated using an Olympus B-Max microscope equipped with epifluorescence. Controls were processed equally.

Double labelling Assay. Skin sections were incubated with FITC (green tagged) monospecific anti-IgG, IgA and IgM antibodies; after washing the slides were incubated with a monoclonal anti-

Hsp70 antibody (Sigma); next, specimens were washed and reincubated with a second antibody tagged in red (rabbit anti-mouse rodamin conjugated IgG) to disclose the Hsp70.

Fluorescein and rodamine filter combinations were used, with excitations of 488 and 546 nm and emissions of 520 and 568 nm respectively.

RESULTS

Patients. None of SLE patients were under treatment when biopsies were carried out, since the diagnosis was newly established. 18 patients had positive antinuclear antibodies by indirect immunofluorescence on HEp-2 cells; most common patterns were speckled in 10 patients and homogeneous in 8 patients. Four sera were also positive for anti nDNA (*Crithidia luciliae*).

Biopsies. All SLE biopsies had immunoreagents deposition along dermo-epidermal junction, into papillary vessels and, in a few cases, into epidermal keratinocytes. Deposition was mainly composed of IgM (90%), IgG (80%) and C3 (75%). Control biopsies showed a negative lupus band test and absence of deposition into papillary vessels (Tab. I).

Double labelling assays. Sixty percent of SLE biopsies had Hsp70 deposition simultaneously to immunoglobulins or complement. Hsp70 was broadly distributed along dermo-epidermal junction and into papillary vessels and, as shown by double fluorescence labelling assay, was co-localized with immune complexes chiefly composed of IgG and C3. Hsp70 deposition along epidermis was evident mainly in those biopsies with epidermal IgG deposition (Fig. 1).

As regard healthy controls, Hsp70 was weakly detected in the epidermis and was not present at all along the dermoepidermal junction, nor into dermal papillary vessels.

Table I - Immunoreagents deposited on SLE skin.

	IgG	IgA	IgM	C3	C4	C1q	Fibrinogen	Hsp70	Co-localization
SLE	16/20 DEJ, V, K	8/20 DEJ	18/20 DEJ, V	15/20 DEJ, V	6/20 DEJ, V	6/20 DEJ	10/20 V, DEJ	12/20 DEJ, V, K	16/20 DEJ, V
Control	0/20	0/20	1/20	0/20	0/20	0/20	1/20 SD	3/20 K	0/20

DEJ= Dermoepidermal junction; V= Papillary vessels; K= Keratinocytes; SD= Superficial dermis

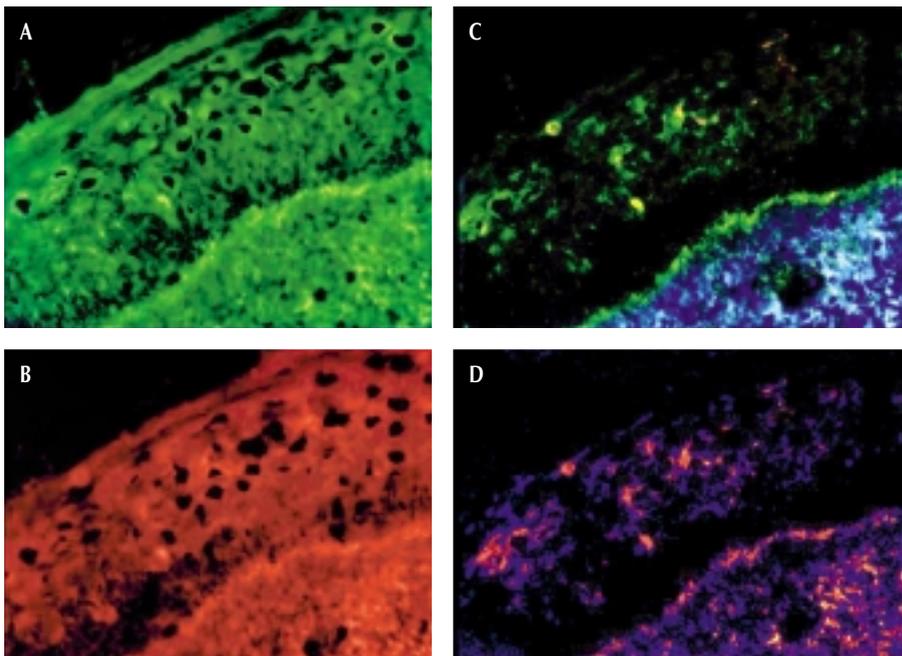


Figure 1 - Lupus skin studied by immunofluorescence. A) IgG deposition along dermoepidermal junction tagged in green (Lupus band). B) Hsp70 deposition along lupus band tagged in red. C) Double fluorescence labelling showing IgG and Hsp70 deposition, displaying yellow granules on a green surface of the dermoepidermal junction. D) Pseudocoloured double fluorescence, which display in red, co-localization sites of IgG with Hsp70 in epidermis, in dermoepidermal junction and in superficial dermis.

DISCUSSION

HSP70 is constitutively expressed in specific skin cells and fulfil essential roles in the protection and adaptation to a wide variety of environmental stimuli.

Previous studies have suggested that HSP may be important in translocation of sequestered antigens to cell surface in the course of autoimmunity. Furukawa et al. found that treating keratinocytes with prostaglandin J2 could induce HSP synthesis and, at the same time, the expression of Ro/SSA, La/SSB and U1RNP. Indeed it has been suggested that chaperons are involved in shuttling antigenic peptides in the cytosol and it was further postulated by Srivastava et al. (7) that HSP70, as well as other heat shock proteins, transfer peptides along the MHC class I processing pathway. Nevertheless it must be considered that, in addition to the chaperoning function for folding, transport and assembly of polypeptides, HSP70 protects cells from a number of apoptotic stimuli, including heat shock, tumour necrosis factor, growth factor withdrawal, oxidative stress, chemotherapeutic agents, ceramides, and radiation.

The aim of our study was to evaluate whether Hsp70 might contribute to autoantigen translocation to the dermo-epidermal junction in patients with SLE. Here we provide evidence that HSP70 molecules are deposited at dermo-epidermal junction in the

context of lupus band immune deposits.

Indeed the presence of Hsp has been demonstrated in normal skin, where the chaperone function of Hsp70 is related to keratinocytes growing (8). In cultured keratinocytes under stress conditions increased level of Hsp70 is paralleled by enhancing of autoantibody binding to autoantigens such as U1RNP, Ro and La (9).

However even if an association between HSP induction and the appearance of extractable nuclear antigens has been noted, the interaction between HSP70 and autoantigens has not yet been fully identified. Furthermore there is evidence that HSP70 itself may act as an autoantigen and that aberrant expression of HSP70 in skin of SLE patients might contribute to both skin lesions and antibody formation in SLE (10).

In addition it has been recently demonstrated that association of Hsp70 with autoantigens at the dermoepidermal junction can trigger off the production of autoantibodies against Hsp in Mrl/Mp-lpr/lpr mice and in SLE patients (11, 12). Such anti-Hsp production would result in a cross-reactivity to nuclear extractable autoantigens-complexed with Hsp, notwithstanding further investigations are necessary to clarify this hypothesis (13, 14).

Overall the pathogenic role of Hsp in the immunopathology of lupus skin is still under discussion (15-16). However the evidence provided by us that HSP70 are deposited along dermo-epidermal

junction from SLE patients is consistent with a role of HSP70 in autoantigens shuttling from the cell surface of base keratinocytes to the dermo-epidermal junction. In this contest we believe that Hsp70 could act as an innocent autoantigen transporter or as a chaperone helping in the refolding of au-

toantigens damaged by the UV-stress or by the inflammatory process.

While there is little direct evidence to support this attractive hypothesis, nevertheless our study may contribute to the recognition of the role of HSP70 in the course of autoimmunity.

SUMMARY

The lupus band is the result of immune complex deposition along the dermo-epidermal junction; such complexes are formed in situ by the interaction of antinuclear antibodies with their respective autoantigens. Dermal autoantigens are released after sun exposure, concurrently a heat shock protein production take place and would participate in autoantigen transfer to the dermo-epidermal junction. In this work the presence of Hsp70 along with the lupus band was investigated by immunofluorescence in twenty SLE skin biopsies.

Immune deposits were mainly composed by IgM, IgG and C3 and were found in all lupus biopsies at the dermo-epidermal junction. Immunoreagents were also present into papillary vessels and, with less extent, into epidermal keratinocytes. Hsp70 was present in 60% of lupus biopsies, and was mainly distributed along dermo-epidermal junction and around papillary vessels. Furthermore, by double fluorescence labelling assays, we found that immuno-reactants are co-localized with Hsp70. Our results suggest that Hsp70 would shuttle autoantigens to the dermo-epidermal junction.

Parole chiave - Lupus band test, Hsp70, lupus eritematoso, immunofluorescenza, giunzione dermo-epidermica.

Key words - *Lupus band, Hsp70, lupus erythematosus, immunofluorescence, dermo-epidermal junction.*

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