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Cathepsin D Expression in Chronic Plaque Psoriasis: An Immunohistochemical Study

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SUMMARY Cathepsins are lysosomal cysteine proteases, which are involved in a variety of physiologic processes such as proenzyme activation, antigen presentation, tissue remodeling, bone matrix resorption, and pathologic processes such as facilitating tumor invasion and modulating the process of programmed cell death. This study aimed to evaluate the pattern of cathepsin D (CD) expression in chronic plaque psoriasis in comparison to normal skin by means of immunohistochemistry. The study included 34 patients presenting with chronic plaque psoriasis and 10 age- and sex-matched normal subjects as control group. Sixty percent of normal skin showed granular positivity for CD confined to basal layer. CD is upregulated in psoaritic lesion with 94.1% positivity making a significant difference between psoriasis and normal skin as regards the percentage and distribution of CD expression, where the latter was predominantly diffuse in psoriatic lesion. The eight cases exposed to PUVA therapy showed reduction of CD positivity to 62.5% with a predominance of mild staining and focal expression compared to pretreatment biopsies. CD may have a role in the pathogenesis of psoriasis in view of its high percentage and diffuse expression in psoriatic epidermis. CD degradative capacity may be responsible for disordered differentiation and scale formation characteristic of psoriasis. Reduction of CD expression may be one of the pathways of PUVA mechanism of action.

KEY WORDS: psoriasis, cathepsin D, proteases, immunohistochemistry

INTRODUCTION

Psoriasis is a common, chronic, disfiguring, inflammatory and proliferative condition of the skin, in which both genetic and environmental influences have critical roles (1). It is currently thought to be a T cell mediated type I autoimmune disease (2).

Proteases are essential for cell and tissue homeostasis. Cathepsins, which are lysosomal cysteine proteases, are involved in a variety of physiologic processes such as proenzyme activation, antigen presentation, hormone maturation, tissue remodeling, and bone

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matrix resorption (3). Cathepsins also act as mediators of programmed cell death (4). Their implication in numerous vital processes and pathologies make them highly attractive targets for drug design (5).

Cathepsin D (CD) is a major intracellular aspartic protease of endosomes and lysosomes and is related to other aspartic proteases such as renin, pepsin and yeast protease A. All these enzymes are synthesized as inactive precursors, which are then processed either autocatalytically (e.g., pepsin) (6) or by other enzymes (e.g., renin) to remove an N-terminal propeptide (7). CD is involved in the proteolytic activation as well as proteolytic degradation of intracellular proteins (8). Increased levels of CD are correlated with tumor cell invasion and metastasis in malignant melanoma, squamous cell carcinoma and human breast cancer (9).

In skin, CD plays a role in both extracellular and intracellular catabolism. It may be involved in the control of cell differentiation during normal development and is thought to be associated with the final stage of desquamation (10).

The aim of this study was to evaluate the pattern of CD expression in chronic plaque psoriasis in comparison to normal skin by means of immunohistochemistry.

SUBJECTS AND METHODS

Patient group

The study was carried out in 34 patients presenting with chronic plaque psoriasis. The diagnosis was made on clinical and histopathological basis and all patients had received neither topical nor systemic treatment for at least 2 weeks before skin biopsy. Eight cases were followed after receiving PUVA therapy, where biopsies were taken post treatment and immunohistochemically stained for CD.

Control group

Ten age- and sex-matched apparently healthy volunteers were included in the study as a control group.

Both patients and control subjects were selected randomly from the Outpatient Dermatology Clinic, Menoufiya University Hospital during the period between July 2008 and June 2009.

The studied cases underwent complete history taking; general and dermatologic examinations; and skin biopsies. Biopsy samples were obtained under local anesthesia from all cases (lesions) and controls after obtaining a written consent. All biopsies were processed at Pathology Department, Faculty of Medicine, Menofiya University, where they were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethanol followed by immersion in xylene, then impregnated in paraffin. Several 5-micron (5-µm) thick sections from each block were taken: one to be stained with hematoxylin & eosin (H&E) for routine histopathological examination, while other sections were mounted on Super frost Plus slides and stored at room temperature, one to be stained immunohistochemically for CD and another one served as a negative control. Breast carcinoma tissue was used as a positive control. The primary antibody was mouse monoclonal antibody (cat.#MS-402-S0) raised against CD (Labvision, USA). It is received as 0.1 mL conc. and diluted by phosphate buffer saline (PBS) at 1:50 dilution. The sections underwent subsequent steps of deparaffinization, rehydration and boiling in citrate buffer solution (pH 6) for 20 minutes, followed by cooling. Incubation with primary antibody was done overnight at room temperature. Incubation with secondary antibody and product visualization was performed employing Envision (Dako Cytomation, Glostrup, Denmark) method with diaminobenzidine (DAB) substrate chromogen. Slides were finally counterstained with Mayer's hematoxylin.

Immunostained sections for CD were assessed as follows:

Cathepsin D immunoreactivity was evaluated as negative or positive; granular cytoplasmic expression in any number of cells was a prerequisite to assign CD positivity. The intensity of CD expression was assessed subjectively as mild, moderate and strong. The extent of CD expression was evaluated as diffuse or focal; diffuse expression indicated CD staining of all epidermal layers, while focal expression was assigned if not all epidermal layers stained for CD.

Statistical analysis

Results were collected, tabulated, statistically analyzed on IBM personal computer using SPSS version 11 statistical package. Fisher exact test was used to study association between two qualitative variables. Mann-Whitney test (nonparametric test) was used for comparison between two groups not normally distributed having quantitative variables. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Clinical and histopathologic features of examined psoriatic cases are presented in Table 1.

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Table 1. Clinical and pathologic characteristics of psoriatic cases

Clinical criteria	Cases (N=34)	
Age (yrs)		
X±SD	44.26±14.45	
Median	42	
Range	12-70	
Duration (yrs)		
X±SD	6.16±5.29	
Median	5	
Range	0.5-20	
Sex		
Male	20	58.82
Female	14	41.18
Pathologic criteria	n	%
Hyperkeratosis		
+	12	35.3
++	14	41.2
+++	8	23.5
Parakeratosis		
+	22	64.7
++	9	26.5
+++	3	8.8
Acanthosis		
+	9	26.5
++	19	55.9
+++	6	17.6
Granular cell layer		
Absent	5	14.7
Partially preserved	29	85.3
Spongiosis		
+	19	55.9
++	13	38.2
+++	2	5.9
Angiogenesis		
+	17	50.0
++	14	41.2
+++	3	8.8
Inflammation		
+	21	61.8
++	11	32.4
+++	2	5.9

CD status in normal skin (control cases)

Granular staining pattern characteristic of CD was seen in 6/10 (60%) cases, which was focally expressed mainly in the basal layer of the epidermis (Fig. 1), while the remaining four cases were completely negative for CD. The intensity of staining ranged between mild degree in four cases to moderate in two cases.

CD status in chronic plaque psoriasis

Cathepsin D was expressed in 32/34 (94.1%) cases as cytoplasmic granules, with diffuse (Fig. 2) expression in 25/34 (73.5%) and focal staining (Fig. 3) in 7/34 (20.6%) cases. The intensity of CD expression varied among cases and showed mild, moderate and strong expression. Mild intensity was seen in 11/34 (32.4%), moderate in 15⁄34 (44.1%) (Fig. 3) and strong in 6/34 (17.6%) cases (Fig. 2). Only two cases 2/34 (5.9%) showed complete absence of CD expression.

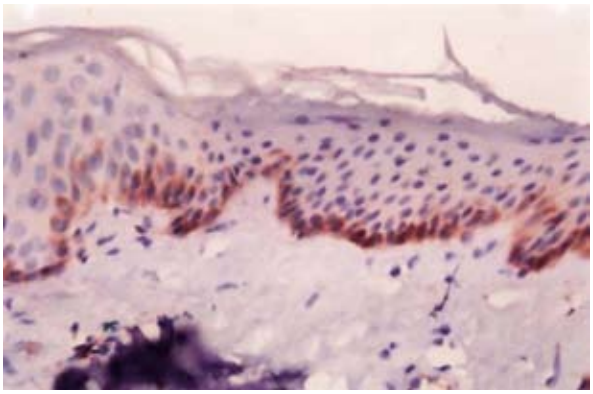

Figure 1. Cathepsin D expression in the basal layer of normal skin (immunohistochemical staining, X400).

Comparison of CD expression between control and psoriatic cases

There was a significant difference between control and psoriatic cases regarding CD expression, since 6/10 (60%) of control cases showed CD expression in comparison to high positive expression in 32/34 (94.1%) psoriatic cases.

As regards CD distribution, there was a highly significant difference between control and psoriatic cases, since all positive control cases had focal CD

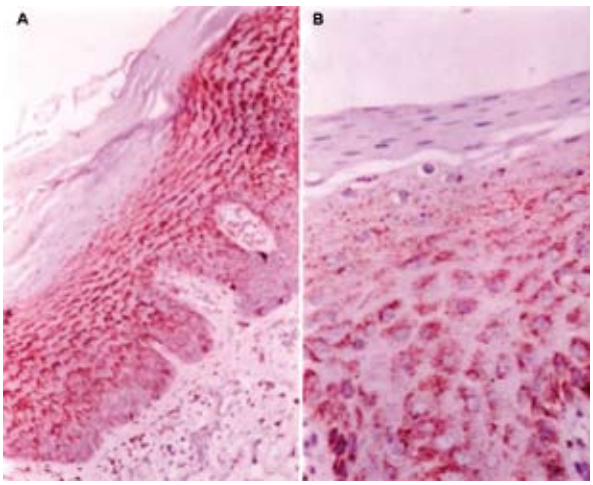

Figure 2. Strong diffuse granular expression of cathepsin D in psoriatic lesion (immunohistochemical staining; (A) X200 and (B) X400).

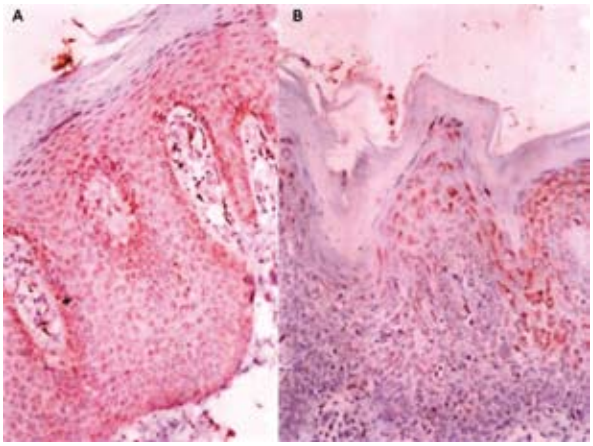

Figure 3. Moderate diffuse (A) and focal (B) expression of cathepsin D in psoriatic lesion (immunohistochemical staining, X200).

expression localized mainly to basal epidermal layer, whereas most (73.5%) of psoriatic cases had diffuse distribution (Table 2).

Cathepsin D expression and clinicopathologic parameters in psoriatic group

CD positivity as well as intensity and distribution of expression did not differ statistically as regards the studied clinical and pathologic parameters (data not shown).

Eight cases were followed after receiving PUVA therapy where biopsies were obtained post-treatment and immunohistochemically stained for CD revealing reduction in the positivity to reach 62.5%. Positive cases showed mild staining in most cases (4/5) and only one case showed moderate staining. All positive cases showed focal expression, which was significantly different from CD distribution in specimens before treatment, since 75% of pretreatment biopsies showed diffuse expression (Table 3, Fig. 4).

DISCUSSION

The epidermis is a multilayered squamous epithelium composed mainly of keratinocytes that migrate from the basal layer to the skin surface. During migration, they undergo epidermal differentiation, i.e. keratinocytes are transformed into corneocytes. These cornified cells form the uppermost layer of the skin, the stratum corneum, which provides a protective barrier between our body and the environment. Corneocytes are eventually eliminated by a process called desquamation. Proteases are important players in the process of keratinocyte differentiation, particularly during the desquamation process. Desquamation requires degradation of corneodesmosomes, modified desmosomal structures that account for the adhesion of corneocytes in the stratum corneum (11). Desmosomes comprise the junctions holding together the outer squames of the stratum corneum. Desmoglein 1 is a component of the desmosomal protein complex whose postulated roles in late differentiation include squame adhesion. Desmoglein 1 must be cleaved in order to attain final squame separation; because the stratum corneum is a nonviable tissue, it can only be achieved by enzymatic degradation (12).

Table 2. Comparison of cathepsin D expression between psoriatic cases and control group

Parameter	Group				Fisher's exact test	P value
	Case (N=34)		Control (N=10)			
	n	%	No	%		
Positivity					7.63	
Positive	32	84.2	6	60.0		<0.05
Negative	2	33.3	4	40.0		S
Intensity					0.188	
Mild	11	73.3	4	40.0		>0.05
Moderate/strong	21	88.2	2	20.0		NS
Distribution					13.7	
Focal	7	53.8	6	60.0		<0.01
Diffuse	25	100	0	0.0		HS

NS = nonsignificant; S = significant; HS = highly significant

Table 3. Evaluation of treatment effect (PUVA) on CD immunostaining in eight psoriatic cases

Parameter	Pretreatment		Post-treatment		Fisher's exact test	P value
	n	%	n	%		
Positivity						
Positive	8	100	5	62.5	3.69	>0.05
Negative	0	0.0	3	37.5		NS
Intensity						
Mild	5	62.5	4	50.0	1.00	>0.05
Moderate& strong	3	37.5	1	12.5		NS
Distribution						
Focal	2	25.0	5	100	0.02	<0.05
Diffuse	6	75.0	0	0		S

NS = nonsignificant, S = significant

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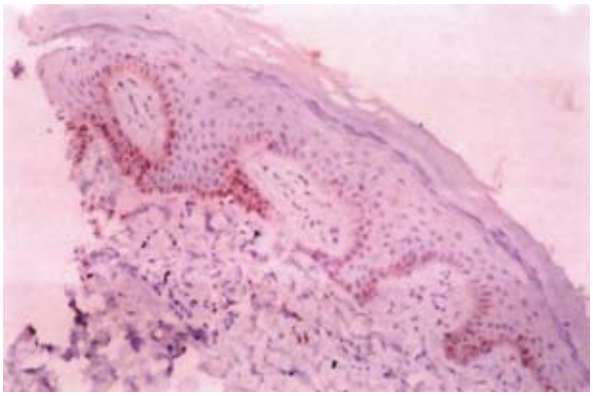

Figure 4. Post PUVA therapy showing faint basal expression of cathepsin D (immunohistochemical staining, X200).

In our study, CD was expressed in the basal layer of normal epidermis of control cases, which may suggest its role in the degradation system of newly generated epidermal cells similar to the basal expression of cathepsin B and D in rat skin (8). In addition, according to Lazarus and Poole (13), CD is immunolocalized to the basal layer of rabbit epidermis and its detection was moderate to weak in lower spinous and basal layer of the skin. It has been shown biochemically that CD is present in the stratum corneum of normal skin and participates in its desquamation (10,14). Demonstration of CD expression in normal epidermis may assume its role in degradation of unneeded proteins resulting from drastic structural alterations in epidermal cells. Cathepsin D was demonstrated to be crucially involved in the activation of TGase 1 and in the regulation of cornified cell envelop (CE) protein expression during epidermal differentiation (15). CD-deficient mice revealed reduced TGase 1 activity and reduced protein levels of the CE proteins involucrin and loricrin. It is suggested that epidermal hyperkeratosis is the result of a diminished ability of corneocytes to bind intercellular lipids, caused by the reduced expression of involucrin and loricrin in the stratum corneum.

It is suggested that CD may be involved in the control of cell differentiation during normal development. Horikoshi *et al*. (14) found increased expression and increased activity of CD isoforms in the skin depending on the stage of epidermal differentiation. Igarashi *et al*. (12) found that this aspartic proteinase CD with a peak activity at pH 4 may play an essential role in the process of desquamation of normal epidermis. They also demonstrated CD to be abundantly present in the granular and stratum corneum layers (fully activated in the stratum corneum). In viable epidermis, it is predominantly confined to lysosomes of the spinous and granular layers. Apparently, CD is activated in lysosomes of the granular layer, where it can participate in the massive degradative processes that accompany cornification, including the activation of transglutaminase. In the upper granular and transition layers of the normal epidermis, CD is spewed out into the intercellular spaces between the adjacent corneocytes, where it becomes associated with desmosomes to function in further degradative processes unique to the stratum corneum, the last of which is desquamation.

Vashishta *et al*. (16) demonstrated human keratinocytes to secrete significant amount of procathepsin D. The secreted procathepsin D affects the proliferation of HaCaT cells and also induces secretion of cytokines that help in normal physiologic functions such as overcoming stresses and enhancing the regeneration process of wound healing as an example of keratinocyte growth.

Premature activation of CD in the spinous layer would interrupt the orderly process of epidermal differentiation. This interruption is confirmed in psoriasis, a disease in which the cohesive forces holding adjacent squames together are altered, leading to premature desquamation. The psoriatic plaque is abnormal in that the granular and transitional cell layers are attenuated. Organelles, including the nuclei, are normally degraded in these layers; nuclei are retained in the psoriatic stratum corneum. Involucrin is cross-linked by transglutaminase in the granular cells into the cornified envelope (17) and has been localized to the upper spinous and granular layers of the normal epidermis (18). It is expressed precociously in the psoriatic epidermis (18) and this premature expression has been associated with hyperproliferation (19). As CD has been implicated in the activation of transglutaminase (20), it may contribute to catalyzing enzymatic processes during cornification. The cohesive forces holding together adjacent squames of the stratum corneum are altered in psoriasis; this leads to premature desquamation. The active forms of CD immunolocalize to the transition layer and stratum corneum in normal epidermis, but they do not label the stratum corneum of the psoriatic plaque. CD, like involucrin, is precociously expressed in psoriatic cells.

In our study, CD was expressed in 94.1% of psoriatic lesions and was seen staining the whole epidermal layer of the lesions, compared to Kawada *et al*. (9) and Chen *et al*. (21) who found all psoriatic cases to show positive expression. In psoriatic epidermis, it has been found that the CD antibody labels all the viable layers but not the stratum corneum (21) and its

expression in spinous layer was strong and may be responsible for desquamation and scale formation in psoriasis depending on its degenerative capacity by stimulation of transgltaminase. Granular staining of CD seen in our study and in others refers to its lysosomal location.

In our study, we found that CD expression in psoriatic epidermis was significantly higher compared to control group ($P<0.05$), confirming its role in the pathogenesis of chronic plaque psoriasis and consistent with Kawada *et al*. (9) who found cathepsins L, B and D to play a role in the pathogenesis of psoriasis; they suggest that CD in psoriasis is mainly activated and partially proteolysed. Egberts *et al*. (15) also found a significant increase in epidermal expression of the active intermediate as well as the mature form of CD in psoriasis.

Depending on the environment, CD can induce or inhibit apoptosis, acting through different mechanisms. Minarowska *et al*. (22) found CD to play an important role in apoptosis, and the mature form of CD to regulate intrinsic apoptosis pathway through stimulation of cytochrome C (CytC) release from mitochondrion. Berchem *et al*. (23) observed that 3Y1-Ad12 cell lines transfected with CD gene showed overexpression of CD, which can prevent apoptosis independently of the catalytic function of CD. Suppression of apoptosis is one of the pathogenic mechanisms of induction of psoriasis (24).

Several authors have suggested that CD is involved in the regulation of blood vessel formation, especially in solid tumors. Significant association between CD expression of host stromal cells and vascular density was described in breast cancer tumors (25) and ovarian tumors (26). Angiogenesis is one of the hallmarks of the pathogenesis of psoriasis, which could be enhanced by the overexpression of CD, since it may influence the production and degradation of both activators and inhibitors *in vitro*. Briozzo *et al*. (26) observed CD to facilitate the release of pro-angiogenic bFGF from ECM. Moreover, CD is able to inactivate the anti-angiogenic factors including angiostatin, 16K prolactin (PRL) and endostatin *in vitro* (28).

According to Vallat *et al*. (29), PUVA therapy leads to complete reversal of pathological epidermal changes and lymphocytic activation, where they showed an overall reduction of epidermal acanthosis in lesional psoriatic skin by 58%. PUVA treatment of lesional psoriatic skin also led to thinning of both the stratum Malpighi and stratum corneum, returning of the granular layer, restoration of keratinocyte maturation with nuclear elimination (orthokeratotic maturation), and elimination of neutrophils from the stratum corneum. Our results showed either complete absence of CD expression in the epidermis exposed to PUVA, or just its faint and focal expression in the basal layer, which is consistent with Kawada *et al*. (9), who found the cathepsins L, B and D expression to have recovered to normal in specimens from patients after PUVA treatment, which may also support the concept of cathepsins involvement in the pathogenesis of psoriasis.

CONCLUSION

According to our study results, CD may have a role in the pathogenesis of psoriasis in view of its high percentage and diffuse expression in psoriatic epidermis. CD degradative capacity may be responsible for disordered differentiation and scale formation characteristic of psoriasis. Reduction of CD expression may be one of the pathways of the PUVA mechanism of action.

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