THE ROLE OF APOPTOSIS IN THE PATHOGENESIS OF DERMATITIS HERPETIFORMIS

M. CAPRONI, D. TORCHIA, E. ANTIGA, D. DEGL'INNOCENTI, E. BARLETTA[2], G. BARONI[1], M. SANTUCCI[1] and P. FABBRI

Department of Dermatological Sciences, University of Florence, Italy
[1]Department of Human Pathology and Oncology, University of Florence, Italy
[2]Department of Experimental Pathology and Oncology, University of Florence, Italy

Received November 8, 2004 – Accepted September 27, 2005

Apoptosis is a form of cell death that is claimed to be involved in a number of chronic inflammatory and malignant skin diseases. The aim of this study was to investigate whether apoptosis may contribute to the pathogenesis of epidermal changes in dermatitis herpetiformis (DH) and, in particular, whether certain apoptosis-related markers such as Bax, Bcl-2, Fas and Fas ligand (FasL) take part in this process. For the detection of apoptotic nuclei, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling technique (TUNEL) was employed on cryostat sections. Skin lesions from six and perilesional skin from four DH patients were stained with monoclonal antibodies to Bax, Bcl-2, Fas and FasL. The same evaluation was also performed on three patients affected by bullous pemphigoid (BP) and in two healthy donors. Using TUNEL technique, a remarkable increase in the apoptotic rate within the epidermal compartment was observed in DH and BP patients in comparison with normal controls. In our immunohistochemical analysis, Bax/Bcl-2 ratio was almost the same in the epidermis of perilesional/lesional DH, BP and healthy skin specimens. In DH and BP specimens both Bax and Bcl-2 proteins were increased in the dermal perivascular compartment. Fas showed a prevalently epidermal staining, both in DH and BP lesions, while FasL was distributed in perivascular and subjunctional dermis; some FasL+ cells infiltrated the DEJ and the basal layer of epidermis. This study allowed us to highlight conspicuous apoptotic phenomena in basal and suprabasal keratinocytes within lesional and perilesional skin of DH. We conclude that in DH, as well as in BP, apoptosis plays a role in the pathogenesis of cutaneous lesions in concert with other pathogenetic mechanisms.

Dermatitis herpetiformis (DH) is an autoimmune subepidermal blistering disease characterized by chronic and recurrent eruptions of erythematous, urticarial, papular, vesicular and bullous lesions. Granular IgA deposits at the dermal papillae represent the immunological marker of the disease, that is strictly associated with a gluten-sensitive enteropathy (GSE), indistinguishable from coeliac disease (CD) (1). To date, immunopathological mechanisms that lead to blister formation in DH are only partially known. In this respect, granular IgA deposition at the dermo-epidermal junction (DEJ), neutrophils and eosinophils together with activated CD4+ Th2 lymphocytes are supposed to represent the main immune mechanisms involved in the pathogenesis of the disease (1-2).

Apoptosis is a sequence of events based on cellular metabolism that lead to cell shrinkage,

Key words: dermatitis herpetiformis, apoptosis, TUNEL, bax, Bcl-2, Fas, Fas L

Mailing address:
Dr Paolo Fabbri
Clinica Dermatologica II
Via degli Alfani, 37
50121 Firenze, Italy
Phone: 0039-055-2758801
Fax: 0039-055-2758757
E-mail: fabbri@unifi.it

1721-727X (2005)

nuclear and cytoplasmic condensation, chromatin fragmentation and phagocytosis. Cell death with apoptotic morphology can be triggered by several stimuli, including intracellular stress and receptor-mediated signaling. These signals feed into evolutionary-conserved intracellular mechanisms, which have mainly been traced to the activity of the caspase family of cysteine-proteases. Three major pathways have been identified in mammalian cells: one involving death receptors, one the release of cytochrome *c* from mitochondria and one the disruption of calcium homeostasis and accumulation of excess proteins in endoplasmic reticulum (3-7).

The death receptor pathway is initiated by some cytokines of the TNF-α family such as TNF-α and Fas ligand (FasL). These molecules can act as extracellular activators of apoptosis upon binding to their respective receptors, which include TNF receptor 1 (TNFR1) and Fas, able to trigger activation of caspase-8 and finally of caspase-3.

The mitochondrial pathway is triggered both by external and internal cues, such as DNA damage. Pro- and anti-apoptotic members of the Bcl-2 family gather at the surface of mitochondria, where they compete to regulate cytochrome *c* release. Members of Bcl-2 family are characterized by one or more conserved homology Bcl-2 (BH) domains and have been classified in three functional groups. Members of group I, such as Bcl-2, possess anti-apoptotic activity, while members of group II, such as Bax, are pro-apoptotic. In the event of apoptosis, cytochrome *c* is released from mitochondria, leading to the activation of caspase-9.

Both death receptor and mitochondrial pathways converge at the level of effector caspase-3 activation. Activated caspases can be considered the central executioners of the apoptotic pathway by activation of the caspase-activated DNase (CAD), which enters the nucleus and cleaves DNA to produce DNA laddering. Such process is detectable by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling technique (TUNEL).

Apoptosis is a key mechanism allowing multicellular organisms to tightly regulate cell growth, preventing pathological processes such as cancer, immunodeficiency and autoreactivity (8). Furthermore, programmed cell death plays an important role during many inflammatory and malignant pathological processes (9), including skin diseases (10). In particular, Fas-induced keratinocyte apoptosis plays a recognized pathogenetic role in eczematous dermatitis (11), toxic epidermal necrolysis (12), acantholytic bullous dermatosis (13) and cutaneous lupus erythematosus (14).

No previous data are reported in literature concerning the putative participation of apoptosis in DH. Nevertheless, ultrastructural changes of DH basal keratinocytes, consisting of desmosomal alterations and a various degree of cytolysis, leading to the so-called "ghost cells", has been described since the sixties (15). Such cellular changes might now be interpreted as the morphological expression of an irreversible cellular damage. Moreover, apoptotic mechanisms, such as the activation of Fas/FasL pathway, play a major role in mucosal flattening and remodeling in CD, as demonstrated by several authors (16-17).

Thus, the aim of this study was to investigate if apoptotic alterations might also be present in the skin lesions of DH and to hypothesize which mechanisms could lead to the activation of keratinocyte death program.

The extent of apoptosis was also analyzed in DH patients by detection of apoptotic nuclei by TUNEL technique. Expression of apoptosis-associated molecules such as Bcl-2, Bax, Fas and FasL was then examined by immunohistochemical methods and compared with normal skin and with bullous pemphigoid (BP) specimens.

MATERIALS AND METHODS

Patients

Thirteen patients (eight males and five females; age range 12-49; mean age 31.2), presenting clinical and histological features typical of DH, were studied. All cases had granular deposits of IgA at the tips of dermal papillae and/or along the basement membrane of normal-appearing skin, as well as circulating IgA antibodies to endomysium (EMA), gliadin (AGA) and to tissue transglutaminase (anti-tTG antibodies).

Five patients affected by BP (age range 62-86, mean age 75.6) and three healthy donors (two females, one male) were also included in the study.

Biopsies

Upon informed consent, 4-mm punch biopsies were performed under local ring anaesthesia on the bullous

and/or peribullous skin of recent onset DH lesions, of not less than 48 hours. None of the patients were undergoing a gluten-free diet or taking dapsone or other immunosuppressive drugs.

Thirteen samples (eight from bullous skin, five from peri-bullous skin) were fixed with formalin for the immunohistochemical procedure; eleven peri-bullous specimens were immediately frozen in liquid nitrogen and stored at –80° C until used for the *in situ* study of apoptosis with the TUNEL procedure. Additional samples were obtained from five patients affected by BP; peri-bullous skin was processed for TUNEL procedure while bullous specimens for immunohistochemistry. Three more samples from healthy donors were used as further control.

In situ detection of apoptotic cells (TUNEL)

Skin cryostat sections were fixed with acetone for 15 min at room temperature, and washed with phosphate-buffered saline (PBS, pH 7.4) for 20 min. Endogenous peroxidase was inactivated by incubating slides with 0.3% H_2O_2 in methanol (v/v) for 30 min at room temperature. TUNEL staining was carried out by using an "*In situ* Apoptosis Detection kit" (Takara Bio Inc., Shiga, Japan). Briefly, sections were permeabilized for 5 min at 4°C and then incubated with terminal-deoxynucleotidyl-transferase (TdT) in the presence of fluorescein-conjugated dUTP (TdT incubation buffer) for 90 min at 37°C in a humified incubator; and as negative control reaction, one section from each patient was incubated in the presence of TdT incubation buffer without TdT. Sections were then washed three times with PBS for 5 min each wash and incubated with anti-FITC horse radish peroxidase conjugated antibody for 30 min at 37°C: sections were then washed three times with PBS and stained with 3,3'-diaminobenzidine as the chromogen (DAB Substrate System, Lab Vision Corp. Fremont, CA, USA) for 15 min at room temperature. DAB staining reaction was stopped by washing with distilled water, and slides were counterstained with 3% methyl green. Negative control was obtained for each sample by replacing the primary incubation with a fluorescein conjugated dUTP mixture without TdT. Sections were then analyzed using a Leica DM-R microscope (Leica Microsystems, Wetzlar, Germany).

Immunohistochemical procedure

The specimens were fixed in 10% formalin before being processed in paraffin. Hematoxylin-eosin stained sections from each histological specimen were reviewed by two pathologists to confirm the histological diagnosis. For immunohistochemical analysis, a representative section for each specimen was selected. All sections were deparaffined in Bio-Clear (Bio-Optica, Milan, Italy) and hydrated with grade ethanol concentration until distilled water; then they were placed in 3% hydrogen peroxide for blocking endogenous peroxidase. Antigen retrieval was routinely performed by microwave pre-treatment (Microwave Micro Med T/T Mega, Milestone, Bergamo, Italy) in citrate buffer 10mM pH 6.0 for 30 minutes. As primary antibodies we used commercial mouse monoclonal and rabbit polyclonal antibodies: anti-Bax (rabbit polyclonal, Dako, Carpinteria, CA), anti-Bcl-2 oncoprotein (clone bcl-2/100/D5, Ventana Medical Systems, Tucson, AZ), anti-Fas (clone GM30, Novocastra, U.K.) and anti-Fas ligand (clone 5D1, Novocastra). All tissue sections were placed on the Ventana Nexes automated stainer using as revelation system IVIEW DAB Detection Kit (Ventana Medical Systems). After the staining run was complete, tissue sections were removed from the stainer, counterstained with haematoxylin, dehydrated and mounted with Permount. Negative controls were included with each run by substituting the primary antibodies with no immune mouse sera. A positive control was used for each antibody: reactive lymph node (germinal center and interfollicular lymphocytes) for Bax, hyperplasia and follicular lymphoma for Bcl-2, small intestine for Fas and prostate for Fas ligand. The control sections were treated in parallel with the samples in the same run. Two indipendent "blind" observers evaluated serial sections. Stainings were quantitated using the following nomenclature to indicate the cell number per field: *weak* between 0 and 9, *moderate* between 10 and 19 and *strong* greater than 20.

For statistical analysis, the stained cells were counted in three consecutive microscopic fields (250X) and statistical significance ($p < 0.05$) was assessed by Student's *t* test.

RESULTS

In situ detection of apoptotic cells

Using the TUNEL method, apoptotic cells were found in basal and suprabasal layers of the epidermis in DH patients; such cells were even more numerous near the dermo-epidermal detaching sites. Few apoptotic cells were also present in the perivascular areas of the dermis (figs. 1-2). Similar findings were obtained in BP lesional skin, while neither epidermis nor dermis of the healthy subjects and negative control reactions showed TUNEL-positive cells.

Immunohistochemical findings

In DH lesional specimens, Bax protein was moderately expressed along the basal epidermis,

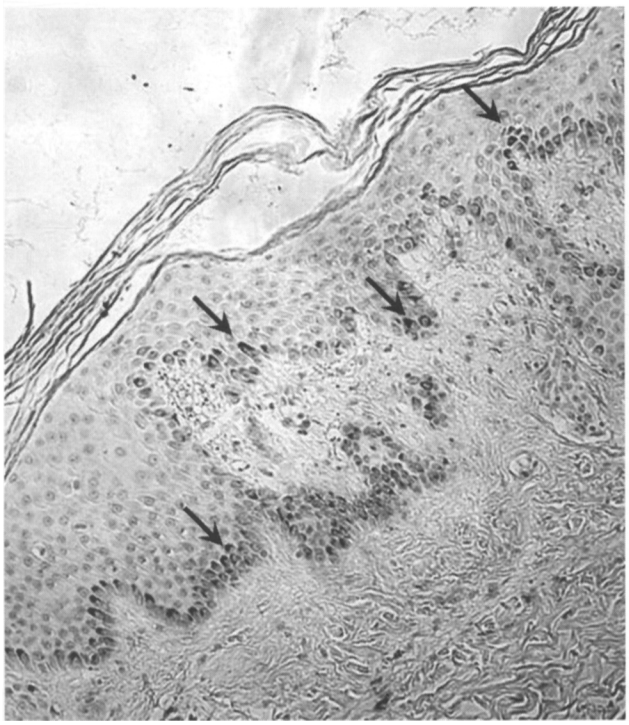

Fig. 1 *(TUNEL 200X, lesional DH): DNA fragmentation is detected within the nuclei giving a dark staining, evidences apoptotic cells in basal and suprabasal layers of the epidermis, and in the perivascular areas of the dermis (black arrows). Such finding suggests hyperactivation of apoptosis in DH lesions.*

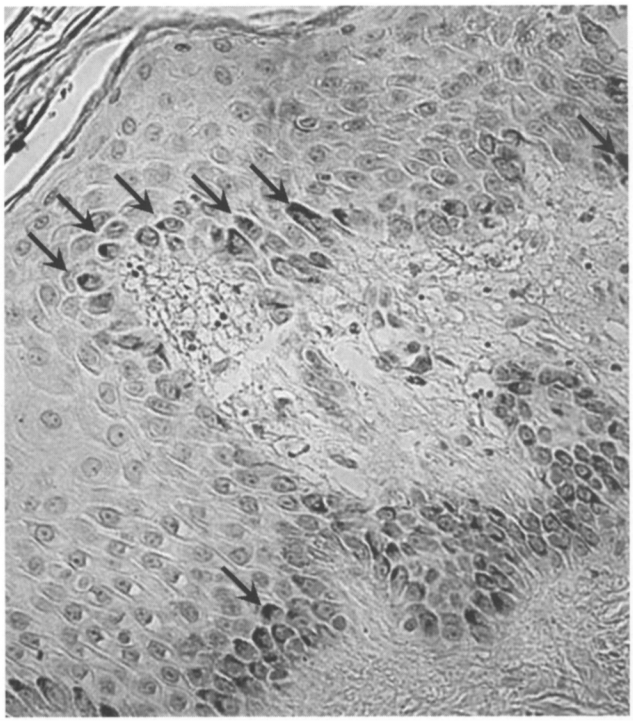

Fig. 2 *(TUNEL 250X, lesional DH): Detail of Fig. 10. It is shown that the dark staining is more evident at the detaching sites, with particular enhancement at the papillar tips (black arrows). The notable increase of apoptotic keratinocytes would determine a damage of the dermoepidermal junction, contributing to split formation.*

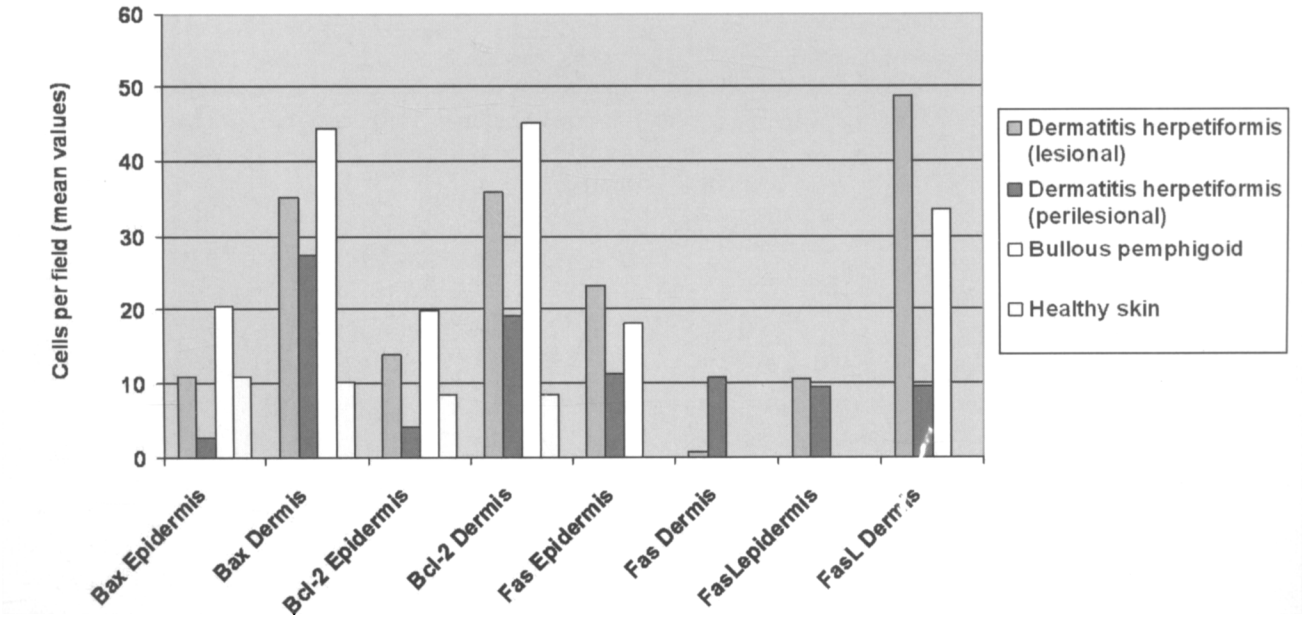

Fig. 3: *Diagram showing the quantitative expression of pro- and anti-apoptotic molecules investigated by immunohistochemistry. Bax and Bcl-2 levels almost overlap, suggesting that the mitochondrial pathway is not relevant in the induction of apoptosis. Instead, the high expression of epidermal Fas and dermal FasL indicates that the death receptor pathway may play a significant role in the development of cutaneous lesions of dermatitis herpetiformis and bullous pemphigoid.*

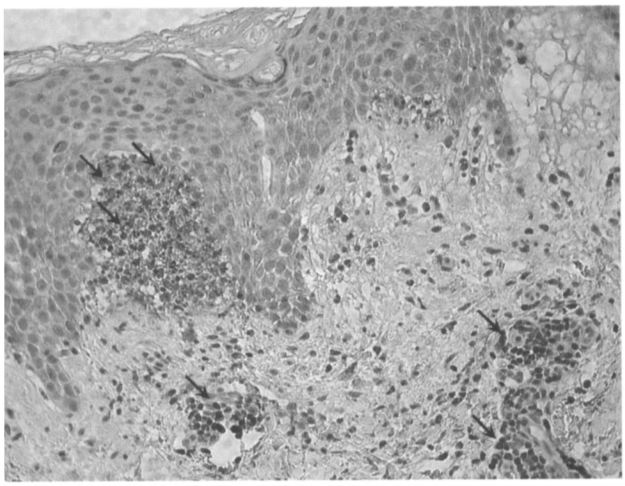

Fig. 4 *(peroxidase 250X, lesional DH): Expression of the pro-apoptotic protein Bax. Bax+ cells can be observed as dark elements scattered in the perivascular and papillary dermis (black arrows). In particular, the dermal papilla on the left side of the picture shows an intense Bax positivity given by the neutrophilic infiltration.*

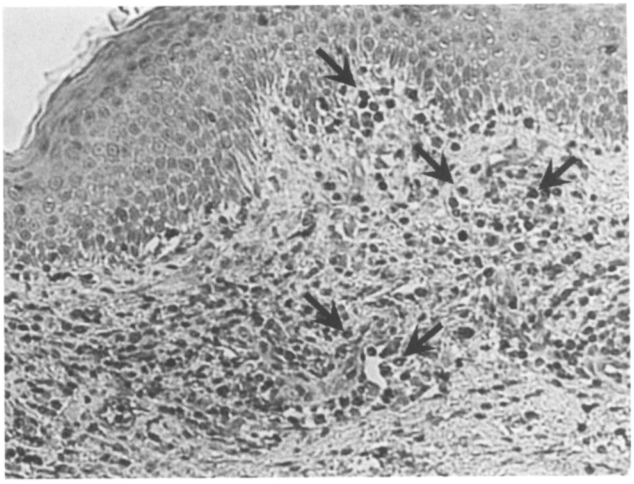

Fig. 5 *(peroxidase 250X, perilesional DH): Bcl-2+ cells infiltrating perivascular dermis (black arrows). Bcl-2 is expressed in the same sites and with similar intensity of Bax, indicating that the Bax/Bcl-2 ratio is balanced and the mitochondrial pathway of apoptosis is not so pathogenetically relevant in cutaneous lesions of DH.*

being significantly overexpressed ($p < 0.0001$) than in perilesional sites, while no difference was seen with respect to healthy controls (Fig. 3 and Table I). Both in lesional and perilesional skin, Bax showed a strong expression with a perivascular distribution in the upper dermis (Fig. 4); the number of positive cells was significantly higher than in healthy skin (p = 0.0037 and 0.0073, respectively).

Bcl-2, mainly detected in basal keratinocytes (Fig. 5), was more expressed in lesional than in perilesional epidermis (p = 0.0002), while no statistical difference could be highlighted in comparison to healthy controls. In contrast, Bcl-2 dermal staining was strong in DH specimens, showing a perivascular and interstitial pattern and

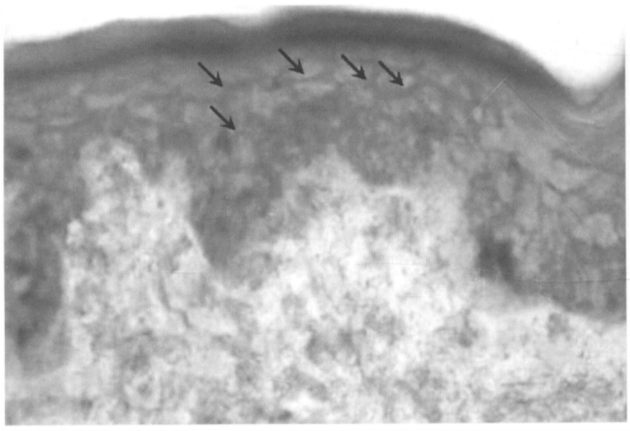

Fig. 6 *(peroxidase 250X, lesional DH): This image shows that the anti-Fas antibody diffusely stains the cell surface of the keratinocytes (black arrows). Since Fas receptor may trigger an intracellular cascade that results in the activation of apoptosis, this finding suggests that Fas-expressing keratinocytes are susceptible of programmed cell death.*

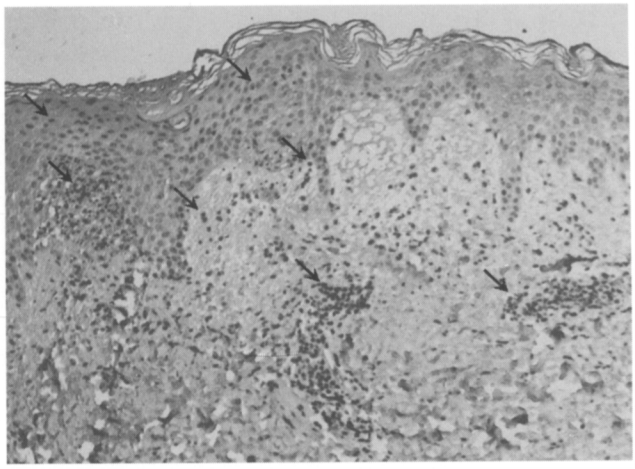

Fig. 7 *(peroxidase 250X, lesional DH): Many FasL+ cells, appearing as intensely dark elements, infiltrate the perivascular and subjunctional dermis, as well as the epidermis (black arrows). The topographic correspondence between Fas+ keratinocytes and FasL+ cells allows to hypothesize that ligation of FasL to Fas may activate the death receptor pathway of apoptosis*

Table I: ***Immunohistochemical results***

		DH lesional	*DH perilesional*	*BP*	*HS*	*p1*	*p2*	*p3*	*p4*	*p5*	*p6*
Bax	E	10.83 ± 5.23	2.66 ± 2.93	20.33 ± 9.89	11.0 ± 3.0	< 0.0001	0.0026	NS	< 0.0001	< 0.0001	0.0183
	D	35.23 ± 23.35	27.25 ± 16.33	44.4 ± 11.51	10.11 ± 5.69	NS	NS	0.0037	NS	0.0073	< 0.0001
Bcl-2	E	13.73 ± 6.65	4.13 ± 5.60	19.82 ± 9.59	8.44 ± 3.68	0.0002	0.048	NS	< 0.0001	NS	0.0035
	D	35.86 ± 28.89	19.0 ± 15.15	45.2 ± 16.38	8.44 ± 3.09	NS	NS	0.0088	0.0004	NS	< 0.0001
Fas	E	23.20 ± 14.62	11.25 ± 7.06	18.0 ± 14.38	Negative	NS	NS		NS		
	D	0.83 ± 1.61	10.66 ± 5.0	Negative	Negative	< 0.0001					
FasL	E	10.44 ± 6.88	9.33 ± 1.03	Negative	Negative	NS					
	D	48.73 ± 13.14	9.66 ± 2.25	33.55 ± 19.89	Negative	< 0.0001	NS		NS		

p1: Dermatitis herpetiformis (DH) lesional versus (vs) DH perilesional
p2: DH lesional vs Bullous pemphigoid (BP)
p3: DH lesional vs Healthy skin (HS)
p4: DH perilesional vs BP
p5: DH perilesional vs HS
p6: BP vs HS
E: number of positive epidermal cells (mean ± standard deviation from the mean)
D: number of positive dermal cells (mean ± standard deviation from the mean)
NS: not significant

being statistically over expressed in lesional than in healthy skin ($p = 0.0088$).

Expression of Fas in basal layers of epidermis resulted moderate both in lesional and perilesional DH (Fig. 6), while there was no staining in healthy skin specimens. Few Fas+ cells were detected in perilesional DH skin within perivascular superficial dermis, while no staining was evident in lesional DH and healthy skin dermis.

A moderate amount of infiltrating FasL+ cells was demonstrable within basal keratinocytes either in lesional or in perilesional DH epidermis, healthy controls resulting negative. Moreover, in lesional skin FasL expression was very strong in superficial perivascular and subjunctional dermis, with enhanced staining in correspondence to papillar tips (Fig. 7). In perilesional DH specimens FasL+ cell infiltration was significantly lower ($p < 0.0001$) than in lesional ones. Healthy controls always resulted negative.

With regard to BP findings, Bax was strongly expressed both in basal epidermis and papillar dermis; epidermal expression resulted significantly higher than DH lesional ($p = 0.0026$) and perilesional specimens ($p < 0.0001$). Bcl-2 protein was overexpressed if compared to DH specimens either in basal epidermis ($p = 0.0026$ for lesional and $p < 0.0001$ for perilesional ones) or in perivascular superficial dermis ($p = 0.0004$ for perilesional site).

Finally, Fas molecule was present in basal keratinocytes, while FasL+ cells were localized at the subjunctional level; no statistical differences could be highlighted in comparison with DH specimens. Fas dermal and FasL epidermal stainings resulted negative.

DISCUSSION

To date, DH is considered an immune-based dermatosis, although its pathogenesis is not completely understood. A genetic background (HLA-DQ2, -DQ8) seems to allow an immune hyperactivity that induces specific humoral and cell-mediated responses when DH patients are stimulated by environmental factors such as the ingestion of gluten (18). Polyclonal IgA_1 antibodies are detectable as junctional granular deposits in perilesional DH skin; they seem to be able to activate the complement cascade and subsequently to enhance neutrophil chemotaxis and cytokine elaboration. Some studies suggest that Th2 lymphocytes also play an important role in the DH immunoinflammation (19).

Bearing in mind that apoptosis plays an important role in immune-mediated dermatoses (11-14), we analyzed whether such process takes part in the development of DH cutaneous lesions. The first step of our study was the detection of DNA fragmentation (the final process of all apoptotic pathways) by TUNEL technique. We identified numerous apoptotic cells in the basal layer of epidermis in DH lesions, with enhancement in correspondence of detaching sites, while no TUNEL-positive cells were detectable in healthy specimens. Thus, we demonstrated hyperactivation

of apoptosis in DH and we speculated that such process may take a part in the development of cutaneous lesions. Such hypothesis is supported by the conspicuous presence of apoptosis in another autoimmune subepidermal blistering disease, i.e. BP.

Then, we investigated activation of the two major apoptotic pathways, i.e. mitochondrial (by Bax and Bcl-2 intracellular expression) and death receptor ones (by Fas and FasL expression on cell surface).

With regard to mitochondrial pathway, in DH and BP specimens both Bax and Bcl-2 proteins resulted increased in dermal perivascular compartment. Nevertheless, since TUNEL technique evidenced only a few apoptotic cells in the dermis, it could be speculated that balance of pro- and anti-apoptotic mechanisms is almost maintained and that this pattern would reflect an augmented turnover of infiltrating cells within inflammatory sites (20). In the epidermis, Bax/Bcl-2 ratio was almost the same in perilesional/lesional DH, BP and healthy skin specimens. The statistically significant differences between perilesional DH, lesional DH and BP specimens might be related to quantitative differences in cell activation induced by diverse amounts of inflammatory stimuli in the various considered sites. Thus, we suggest that the mitochondrial pathway of apoptosis would not be relevant in DH.

The most important findings of our study regarded the activation of apoptosis by death receptor pathway in cutaneous lesions of DH. In particular, we analyzed the expression of Fas and FasL; binding of FasL to Fas induces intracellular signals that eventually result in activation of caspases that cause nuclear fragmentation, beginning apoptotic cell death (21). As known, coexpression of Fas and FasL sets the conditions for spontaneous Fas-mediated cell death in the basal and suprabasal layer of normal epidermis, although keratinocyte apoptosis is very seldom observed under physiological conditions (22). By contrast, Fas-FasL system plays a major role in some skin diseases such as toxic epidermal necrolysis (12).

In our immunohistochemical analysis, Fas showed a prevalently epidermal staining both in DH and BP lesions, while FasL was mainly distributed in the dermis, some FasL+ cells also infiltrating the DEJ and the basal layer of the epidermis. Epidermal Fas expression can be significantly upregulated by different stimuli, including ultraviolet, cytokines (IL-1β, TNF-α, IL-15 and IFN-γ) (23) and viral infection (24). Nevertheless, since no previous studies were made on apoptosis in DH, trigger factors of Fas hyperexpression in DH lesional epidermis remain to be clarified.

Main infiltrating cell populations in DH lesional skin are constituted by T lymphocytes and neutrophils (19). Although FasL expression was initially confined to activated T cells, recent studies indicated that FasL can be expressed by neutrophils and other cell types (24). Thus, in DH lesions FasL might be prevalently expressed by neutrophils and by T lymphocytes; such cells would then interact with Fas+ basal keratinocytes, which would undergo programmed cell death.

Besides Fas/FasL-mediated apoptosis, we suppose that almost two other mechanisms, related to dermo-epidermal detachment, can contribute to apoptosis of basal keratinocytes: first, vesicle fluid would exert mechanic compression and then cause hypoxic damage (25-26), second, loss of connection between the basal layer and the dermis below would further enhance the apoptotic process (27-28). Furthermore, similar results were obtained in another subepidermal blistering disease such as BP, where analogous mechanisms can be supposed to be effective in the induction of programmed cell death.

To sum up, TUNEL technique and immunohistochemistry allowed us to highlight pro-apoptotic protein expression and occurring apoptotic phenomena in basal and suprabasal keratinocytes within lesional and perilesional skin of DH. In particular, quantitative and qualitative analysis of some major pro-apoptotic proteins did evidence early activation of programmed cell death, as demonstrated by TUNEL-positivity in perilesional DH skin with respect to healthy specimens. Significantly lower expression of such molecules in perilesional than in lesional DH skin could be attributed to pathological processes related to the later development of skin damage, i.e. cell infiltration and blister formation.

Thus, we conclude that in DH, as well as in BP, apoptosis plays a significant role in the pathogenesis of cutaneous lesions in concert with the over mentioned pathogenetic mechanisms.

REFERENCES

1. **Hall R.P.** 1992. Dermatitis herpetiformis. *J. Invest. Dermatol. 99:873.*
2. **Nicolas M.E., P.K. Krause, L.E. Gibson and J.A. Murray**. 2003. Dermatitis herpetiformis. *Int. J. Dermatol. 42:588.*
3. **Assunção Guimarães C. and R. Linden**. 2004. Programmed cell death – Apoptosis ans alternative deathstyles. *Eur. J. Biochem. 271:1638.*
4. **Orrenius S.** 2004. Mitochondrial regulation of apoptotic cell death. *Toxicol. Lett. 149:19.*
5. **Nakagawa T., H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner and J. Yuan.** 2000. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β. *Nature 403:98.*
6. **Scaringi L., P. Cornacchione, E. Ayroldi, L. Corazzi, E. Capodicasa, R. Rossi and P. Marconi.** 2004. Omeprazole induces apoptosis in Jurkat cells. *Int. J. Immunopathol. Pharmacol. 17:331.*
7. **C. Longhi, M. P. Conte, S. Ranaldi, M. Penta, P. Valenti, A. Tinari, F. Superti and L. Seganti.** Apoptotic death of *Listeria monocytogenes*-infected human macrophages induced by lactoferricin B, a bovine lactoferrin-derived peptide. *Int. J. Immunopathol. Pharmacol. 18:317.*
8. **Baetu T.M. and J. Hiscott.** 2002. On the TRAIL to apoptosis. *Cytokine Growth Factor Rev. 13:199.*
9. **Thompson C.B.** 1995. Apoptosis in the pathogenesis and treatment of disease. *Science 267:1456.*
10. **Paus R. and T. Rosenbach, N. Haas and B.M. Czarnetzki.** 1993. Patterns of cell death: the significance of apoptosis for dermatology. *Exp. Dermatol. 2:3.*
11. **Trautmann A.** 2000. T-cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. *J. Clin. Invest. 106:25.*
12. **Viard I., P. Wehrli, R. Bullani, P. Schneider, N. Holler, D. Salomon, T. Hunziker, J.H. Saurat, J. Tschopp and L.E. French.** 1998. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science 282:490.*
13. **Gniadecki R., G.B.E. Jemec, D.M. Thomsen and M. Hansen.** 1998. Relationship between keratinocyte adhesion death: anoikis in acantholytic disease. *Arch. Dermatol. Res. 290:528.*
14. **Baima B. and M. Sticherling.** 2001. Apoptosis in different cutaneous manifestations of lupus erythematosus. *Br. J. Dermatol. 144:958.*
15. **Mikalowski R.** 1964. Microscopie electronique de l'epiderme dans les bulles de la dermatite herpetiforme de Dühring-Brocq. *Ann. Dermatol. Syphilol. 91:255.*
16. **Giovannini C., P. Matarrese, B. Scazzocchio, R. Vari, M. D'Archivio, E. Straface, R. Masella, W. Malori and M. De Vincenzi.** 2003. Wheat gliadin induces apoptosis of intestinal cells via an autocrine mechanism involving Fas-Fas ligand pathway. *FEBS Lett. 540:117.*
17. **Ciccocioppo R., A. Di Sabatino, R. Parroni, P. Muzi, S. D'Alo, T. Ventura, M.A. Pistoia, M.G. Cifone and G.R. Corazza.** 2001. Increased enterocyte apoptosis and Fas-Fas ligand system in celiac disease. *Am. J. Clin. Pathol. 115:494.*
18. **Balas A., J.L. Vicario, A. Zambrano, B. Acuna and D. Garcia-Novo.** 1997. Absolute linkage of celiac disease and dermatitis herpetiformis to HLA-DQ. *Tissue Antigens 50:52.*
19. **Caproni M., C. Feliciani, A. Fuligni, E. Salvatore, L. Atani, B. Bianchi, S.M. Pour, G. Proietto, P. Toto, G. Coscione, P. Amerio and P. Fabbri.** 1998. Th2-like cytokine activity in dermatitis herpetiformis. *Br. J. Dermatol. 138:242.*
20. **Orteu C.H., L.W. Poulter and M.H. Rustin.** 1998. The role of apoptosis in the resolution of T cell-mediated cutaneous inflammation. *J. Immunol. 161:1619.*
21. **Nagata S. and P. Goldstein.** 1995. The Fas death factor. *Science 267:1449.*
22. **McCall C.A. and J.J. Cohen.** 1991. Programmed cell death in terminally differentiating keratinocytes: role of endogenous endonuclease. *J. Invest. Dermatol. 97:111.*
23. **Wehrli P., I. Viard, R. Bulloni, J. Tschopp and L.E. French.** 2000. Death receptors in cutaneous biology and disease. *J. Invest. Dermatol. 115:141.*
24. **Teraki Y. and T. Shiohara.** 1999. Apoptosis and the skin. *Eur. J. Dermatol. 9:413.*
25. **Khan S., R.P. Cleveland, C.J. Koch and J.R. Schelling.** 1999. Hypoxia induces renal tubular

epithelial cell apoptosis in chronic renal disease. *Lab. Invest. 79:1089.*

26. **Date T., S. Mochizuki, A.J. Belanger, M. Yamakawa, Z. Luo, K.A. Vincent, S.H. Cheng, R.J. Gregory and C. Jiang.** 2003. Differential effects of membrane and soluble Fas ligand on cardiomyocytes: role in ischemia/reperfusion injury. *J. Mol. Cell. Cardiol. 35:811.*
27. **Nievers M.G., R.Q. Schaapveld and A. Sonnenberg.** 1999. Biology and function of hemidesmosomes. *Matrix Biol. 18:5.*
28. **Cavalieri S., M. Rotoli, C. Feliciani and P. Amerio.** 2005. Expression of the high-affinity receptor (67 kDa) in normal human skin and appendages. *Int. J. Immunopathol. Pharmacol. 18:223.*