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TRAIL Expression in Lesional and Non-lesional Skin of Patients with Atopic Dermatitis

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Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor superfamily, and has been implicated in the regulation of various physiological and pathological immune responses. The present study aims to study TRAIL expression in skin lesions and non-lesional skin of patients with atopic dermatitis (AD) as well as in lichen simplex chronicus and in normal control skin. The study has been conducted on 49 patients with atopic dermatitis, 3 cases were acute, 15 cases were subacute, 28 cases were chronic and 3 cases were lichen simplex chronicus. Two skin biopsies were obtained from all patients; one from lesional skin and the other from the apparently normal non-lesional skin. Skin biopsies were also obtained from 3 normal volunteers. Immunohistochemical staining technique was performed to demonstrate TRAIL expression in skin samples of patients and controls. In the cells of the dermal infiltrate the expression of TRAIL was the higher in acute lesions (80 cells ± 4/H.P.F.) than subacute (25cells±5/H.P.F.) and chronic lesions (20 cells ± 2/H.P.F.) and the differences were highly significant (p=<0.0001 for both). The expression in non-lesional skin (50 cells ± 5/H.P.F.) was significantly lower than acute lesions and lichen simplex ((p=<0.0001 and =0.045 respectively), significantly higher than subacute and chronic lesions (p=0.037 and =0.014 respectively) but was not statistically different from normal controls. In conclusion, the expression of TRAIL in skin of patients with AD differs according to the stage of the disease. To better understand its functions, we suggest that TRAIL expression studies should be performed in other diseases and controlled following pharmacological treatment.

Introduction

Atopic dermatitis (AD) is characterized by chronic or relapsing pruritic eczematous lesions with a typical morphology and distribution.[1] The pathogenesis

of AD is not completely understood and involves a complex series of interactions between resident and infiltrating cells orchestrated by proinflammatory cytokines and chemokines.[2] In the dermis of AD lesions, there is a marked perivascular T cell infiltrate,[3,4] in which both CD4+ve and CD8+ve T cells are present.[5] The infiltrating T cells express pro-inflammatory cytokines, in particular, interleukin (IL)-5 and IL-13.[6,7] In chronic lesions, IFN-γ-producing cells have also been described.[8,9]

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) superfamily, and has been implicated in the regulation of various physiological and pathological immune responses.[10] This might, at least partially, be because of its wide expression among cells of the immune system, including activated T cells,[11,12,13,14] B cells,[11,15] monocytes,[16,17,18] dendritic cells,[19] natural killer cells,[14,20,21] and neutrophils.[18,22,23]

The TRAIL system consists of the ligand (TRAIL) and five different cellular receptors (TRAIL-R1 to TRAIL-R5).[24] TRAIL-R1 and TRAIL-R2 are death receptors that have the ability to initiate the apoptosis- signalling cascade after ligation, whereas TRAIL-R3 and TRAIL-R4 are decoy receptors lack this ability and are actually reported to prevent extensive apoptosis in cells and tissues expressing both TRAIL and the death receptors, TRAIL-R1 and -R2. Osteoprotegerin is a soluble receptor for TRAIL and may also act as a soluble decoy receptor. The balance of the expression levels between the death receptors and decoy receptors is an important factor determining the apoptotic effect of TRAIL.[25,26]

TRAIL originally received considerable attention following the observation that it selectively induced apoptosis in cancer but not in normal cells.[27,28] In addition to its therapeutic potential, TRAIL might act as a natural guardian eliminating transformed cells at an early stage.[29] However, recent work suggested that TRAIL also has several physiological functions that are not limited to the killing of transformed cells. For instance, TRAIL has been shown to induce apoptosis in several primary cells, such as hepatocytes, [30,31] HIV-activated T cells, [32] plasma cells, [33] immature dendritic cells, [34] and neutrophils.[22,23] Regulators of TRAIL expression have been reported such TNF-α, which is a down-regulator of TRAIL expression, whereas IFN-γ up-regulates the expression of TRAIL. [35]

The present study aims to study tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression in acute, subacute and chronic skin lesions as well as in non-lesional skin of patients with atopic dermatitis as well as in normal control skin samples for more understanding of the pathogenesis of this disease.

Materials and Methods

The present study has been conducted on 49 patients with atopic dermatitis, attending the Dermatology outpatient clinic of Al-Minya University Hospital. Twenty-four patients (49%) were males and 25 (51%) were females. The age of patients ranged from 1 year to 35 years with a mean age and Standard Deviation (SD) of 9.1 ± 7.3 years. All patients satisfied the UK Working Party's refinement of the diagnostic criteria of the Hanifin and Rajka for atopic dermatitis. [36] Of all patients

included three cases (6.1%) were acute, 15 cases (30.6%) were subacute, 28 cases (57.1%) were chronic and 3 cases (6.1%) were lichen simplex chronicus. Patients received no topical or systemic treatment for at least 1 month before biopsy taking.

Skin Biopsies

Skin biopsies were obtained from all patients using sterile 4 mm punches after taking informed consents. Two biopsies were taken; one from lesional skin and the other from the apparently normal non-lesional skin. Each biopsy was immediately fixed in 10% formalin, embedded in a paraffin block and sectioned into 5 µm thick sections. These sections were utilized for routine haematoxylin and eosin (H&E) as well as for immunohistochemical staining. Skin biopsies were also obtained from 3 normal control persons and have been subjected to the same embedding and staining procedures as for patient biopsies.

Immunohistochemistry

Immunohistochemical staining technique was performed to demonstrate TRAIL expression in skin samples of patients and controls. A ready-to-use detection system (lab EnVision + System, Peroxidase (DAB), lab vision®, Cat# TP-015-HD) was used. The primary antibody was the monoclonal mouse anti-human TRAIL /TNFSF10 antibody (R&D system Corporation®, Cat# 375). It was used in a dilution of 25µg/ml. The expression of TRAIL in the cells of the dermal infiltrate was evaluated according to the number of positively stained cells/H.P.F. **(Table 1)**.

Score	*Number of positive cells/ H.P.F.*
0	No positively stained cells
1	1- 25 cells
2	26 – 50 cells
3	51 – 75 cells
4	75-100 cells
5	> 100 cells

Table 1: Scoring system for quantifying TRAIL expression in the cells of the dermal infiltrate.

Statistical analysis

Data were collected and tabulated using Excel Software. Statistical analysis was done using SPSS (version 11.0). The numerical data was expressed as mean ± standard deviation (SD). T-student test was used to compare numerical values. The (t-test) values were expressed in terms of p-value. P value was considered significant when it is < 0.05 and highly significant when it is < 0.001.

Results

TRAIL was noticed to be expressed mainly in the cells of the dermal infiltrate. In addition, some degree of expression was also noticed in vessel wall, endothelial cells, fibroblasts as well as periappendageal.

TRAIL expression in dermal infiltrate

Lesional skin **(Table2; Fig.2-4)**

The expression of TRAIL was found to be much higher in the cells of the dermal infiltrate in acute than subacute or chronic cases. In acute cases, the mean TRAIL expression was found to be 80 cells ± 4/H.P.F. (score 4) **(Fig.2)**. This expression was noticed to decline to 25 cells ± 5/H.P.F (score 1-2) in subacute cases **(Fig.3)**. This decline is statistically highly significant (p=<0.0001). The mean TRAIL expression also declined to reach 20 cells ± 2/H.P.F (score 1) in chronic cases **(Fig.4)**. This decline is also statistically highly significant (p=<0.0001).

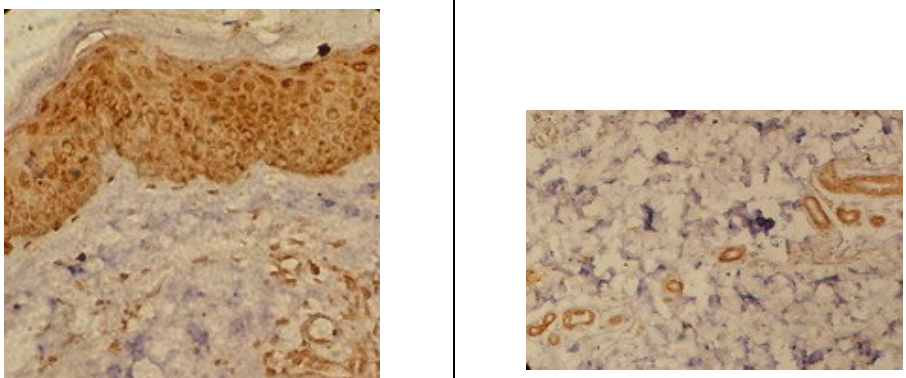

Fig 3: Subacute atopic skin lesion showing moderate TRAIL expression in the dermal infiltrate as well as in epidermal cells, periappandageal, perivascular and endothelial cells (Immunoperoxidase; X200).

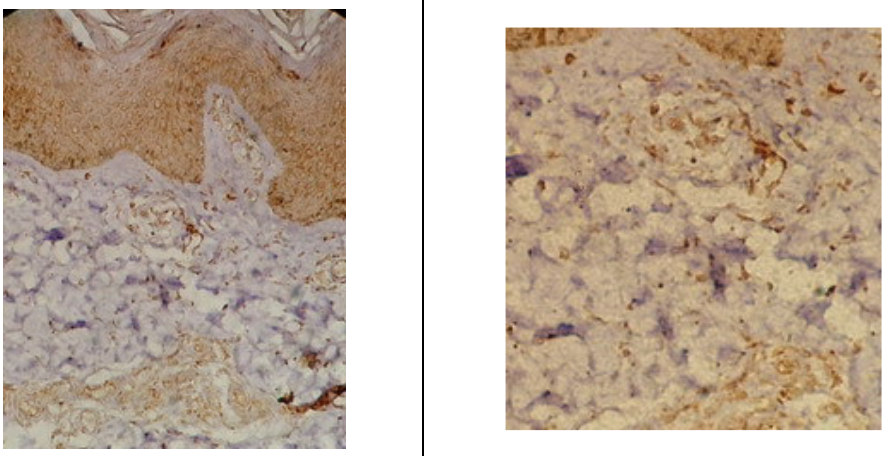

Fig 4: Chronic atopic skin lesion showing mild TRAIL expression in the dermal infiltrate as well as in epidermal cells, periappandageal, perivascular and endothelial cells (Immunoperoxidase; X200).

Lichen simplex **(Table2; Fig.5)**

Biopsies obtained from patients with lichen simplex showed high expression of TRAIL (100 cells ± 24/H.P.F; score 4-5) in the dermal infiltrate **(Fig.5)**. Although this expression was not statistically different from that observed in acute lesions (p=0.2), it was much higher than that noticed in subacute and chronic skin lesions as well as non-lesional skin and this difference is statistically highly significant (p=<0.0001).

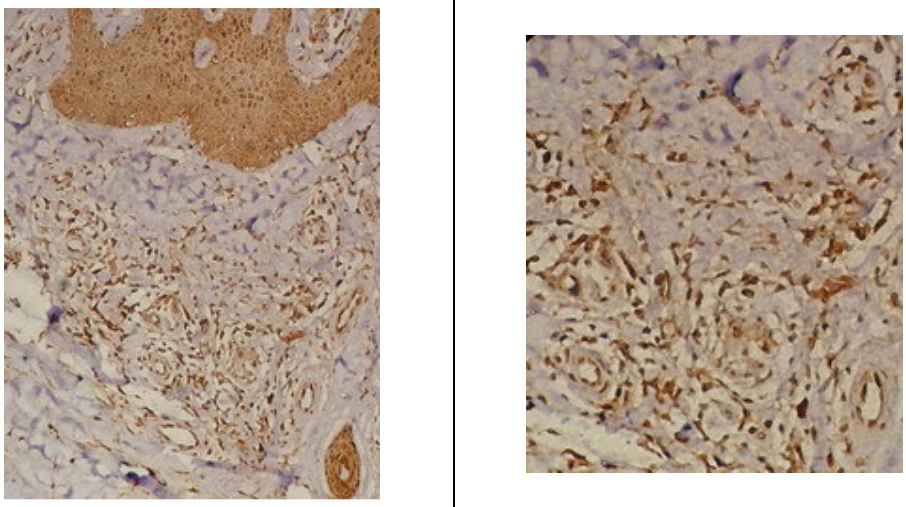

Fig 5: Lichen simplex lesion showing TRAIL expression in the dermal infiltrate as well as in epidermal cells, periappandageal, perivascular, endothelial cells and fibroblasts (Immunoperoxidase; X100).

Non-lesional skin (Table2; Fig.6)

Biopsies obtained from non-lesional skin of patients with AD showed moderate level (50 cells ± 5/ H.P.F; score 3) of TRAIL expression in the dermal infiltrate **(Fig.6)**. The level of expression was lower than that observed in the acute lesions of AD and this difference was statistically highly significant (p=<0.0001).

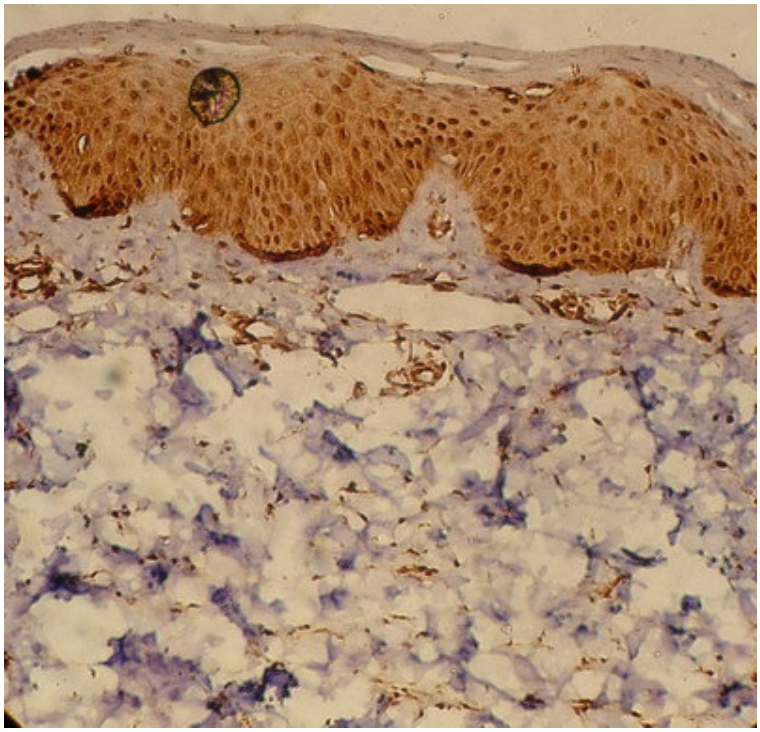

Fig 6: Non-lesional skin of an atopic patient showing moderate TRAIL expression in the epidermis with mild expression in the dermal inflammatory infiltrate (Immunoperoxidase; X200).

Normal control **(Table2; Fig.7)**

Biopsies obtained from normal control subjects also showed moderate level (30 cells ± 5/ H.P.F.; score 3) of the TRAIL expression in dermal infiltrate **(Fig.7)**. The expression was lower than that observed in acute lesions of AD as well as in non-lesional skin and this difference was statistically highly significant (p=0.0001 for both). To the contrary, TRAIL expression in the skin samples of normal controls was higher than that observed in chronic skin lesions of AD and this difference was statistically highly significant (p=0.0001). However, when compared with that observed in subacute lesions, the expression was not statistically different (p=0.12).

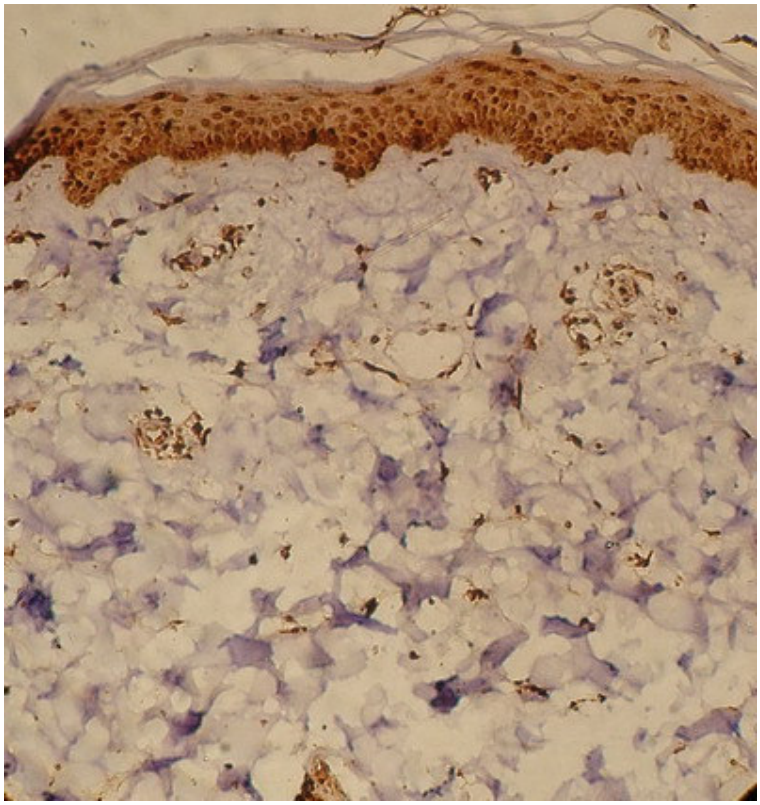

Fig 7: Skin from a normal control subject showing marked TRAIL expression in the epidermis with mild expression in the dermal cells (Immunoperoxidase; X200).

	Acute (N=3)	Subacute (N=15)	Chronic (N=31)	Lichen Simplex (N=3)	Non-lesional skin (N=10)	Normal skin (N=3)
Mean number of TRAIL +ve cells/H.P.F. in dermal infiltrate	80±4	25±5	20±2	100±24	50±5	30±4

Table 2: TRAIL expression in dermal infiltrate of lesional and non-lesional skin of patients with AD, lichen simplex chronicus and in normal controls.

Discussion

In spite of the recent progress in the field, the physiological role of TRAIL remains to be determined. [39] TRAIL has been shown to be critical in the regulation of the immune response. It induces apoptosis in dendritic cells, regulating adaptive immune response,[19] and it mediates apoptosis in neutrophils, [18,22,23] suggesting that it also controls innate immune responses. In addition, TRAIL also exerts anti-inflammatory activities. [22,23,30,31,32,33,34,37,38,39,40]. An understanding of the immunologic basis of AD may have important implications for clinical management of the disease. [41] The present study aims to study TRAIL expression in lesional as well as in non-lesional skin of patients with atopic dermatitis and comparing it with normal control samples for more understanding of the pathogenesis and immunologic basis of this disease.

In the present study, the expression of TRAIL in the cells of the dermal infiltrate was observed to be significantly higher in acute than in subacute (p=<0.0001) or chronic skin lesion (p=<0.0001) of patients with AD. In addition, the level of expression in acute lesions was also higher than that observed in non-lesional skin (p=<0.0001) of patients with AD as well as in normal control skin (p=0.0001). These findings are consistent with previous reports. TRAIL-positive mononuclear cells were present in greater numbers in lesional skin of patients with AD compared with non-lesional skin and normal controls.[42]

Our results show that TRAIL is expressed in a high level at an early stage of inflammation in acute skin lesions of AD. After subsidence of the acute phase, the level of TRAIL expression starts to decline to be almost close to the expression level found in normal skin. This suggests that TRAIL, with its immune regulatory and anti-inflammatory effects, is expressed in a high level at an early stage of inflammation in an attempt to control inflammation and minimize tissue damage. Once the acute phase resolved and the acute inflammation started to subside, the level of TRAIL gradually declines to its normal level found in normal skin. This view may be supported by the finding that the proportion of TRAIL-expressing CD8 cells, with their cytotoxic/ suppressive role, are two folds higher than CD4 cells in lesional skin of AD patients.[39] It was suggested that TRAIL exerts its anti-inflammatory activities through the induction of apoptosis in inflammatory cells,[22,23,30,31,32,33,34] blocking of the cell cycle, [37] activation of inhibitory phosphatases,[38] and increasing the expression of IL-1Ra.[39] The latter has a crucial role in the prevention of massive tissue damage during inflammatory responses. [40]

The results of the present study also show that the non-lesional skin of patients with AD expresses a moderate level of TRAIL in cells of the dermal inflammatory infiltrate. TRAIL expression in cells of the dermal inflammatory infiltrate in non-lesional skin was significantly lower that observed in acute lesions (p==<0.0001) but significantly more than that observed in subacute and chronic skin lesions as well as in normal control skin. This observation suggests that the apparently normal skin of patients with AD harbour some immunohistochemical abnormalities regarding TRAIL expression with more than normal expression in dermal inflammatory

infiltrate. Thus we suggest adding a new finding to the list of abnormalities previously reported [41] in the apparently normal skin of patients with AD.

In patients with lichen simplex, skin biopsies showed expression of TRAIL in the cells of the dermal infiltrate, which was not significantly different from that observed in acute skin lesions of AD (p=0.2). However, the level of expression was significantly higher than that seen in subacute lesions (p=<0.0001), chronic lesions (p=<0.0001), non-lesional skin (p=<0.0001) as well as normal control subjects (p=0.007). To the best of our knowledge, TRAIL expression was not previously studied in the lesions of lichen simplex chronicus. We suggest that the persistence of inflammation in such persistent and chronic lesions may be responsible for the continued expression of TRAIL by the infiltrating cells as well as by the local tissue in an attempt to terminate such chronic inflammatory process. In addition, we suggest considering this highly significant difference (p=<0.0001) in TRAIL expression in lesions of lichen simplex chronicus as a point of immunohistochemical differentiation between this type of eczema and the ordinary chronic skin lesions of AD.

In conclusion, the expression of TRAIL in skin of patients with AD differs according to the stage of the disease, being the highest in the acute stage then declines thereafter. Non-lesional skin of patients with AD is abnormal regarding TRAIL expression with more than normal expression in dermal inflammatory infiltrate. The lower than normal TRAIL expression in the ordinary chronic skin lesions of AD helps to differentiate them from those of lichen simplex chronicus. To better understand its functions, we suggest that TRAIL expression studies should be performed in other diseases and controlled following pharmacological treatment.

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