Increased expression and potential role of tumor necrosis factor-related apoptosis-inducing ligand TRAIL in atopic dermatitis

Sheren B. El Sayed* Neveen S.I. Seif Eldin** Alla A. Ally*,

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* Department of Medical Microbiology and Immunology*,
Department Dermatology, Venereology and Andrology**, Faculty of Medicine, Ain Shams University, Cairo Egypt

mailto:sherin_bendary@yahoo.com

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Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis of many transformed but also of non-transformed cells. In addition, TRAIL receptor activation has been reported to activate non-apoptotic signaling pathways. In this study, 30 patients with atopic dermatitis, and 20, sex and age matched healthy controls were enrolled. According to their response to topical hydrocortisone cream, class 7 and betamethasone valerate 0.1% cream, class 3 steroid therapy for 2 weeks, patients were divided into 2 groups, Group I(good steroid responders) and Group II (poor steroid responders). For every patient and control complete blood count using Coulter Counter, serum total IgE quantitative measurement by a commercially available ELISA kit, peripheral blood lymphocytes and monocytes TRAIL expression by direct immunofluorescence flow cytometry were performed. We report an increased expression of TRAIL in peripheral blood T cells and monocytes from patients with atopic dermatitis (AD), group I &II, compared with control individuals. The average absolute eosinophil count and IgE levels in group II atopic dermatitis patients showed significant correlation with severity of the disease and showed a non homogeneous distribution reflected by significant association with family history of atopy, when both parents were atopic. TRAIL expression in both CD4+ and CD8+ T cells as well as CD14+ monocytes was significantly higher in group I AD patients compared with group II.

Introduction

Atopic dermatitis (AD) is a chronic, relapsing, highly pruritic, inflammatory skin disease that frequently predates the development of allergic rhinitis or asthma [1]. It is caused by complex interplay of the expression of many different genes & multiple environmental factors affecting their
expression [2]. In the dermis of AD lesions, there is a marked perivascular infiltrate in which both CD4+ and CD8+ T cells are present. The majority of these cells are of the CD45 Ro+ memory/effector phenotype and express the selective skin-homing receptor, cutaneous lymphocyte-associated antigen (CLA) [3,4]. These T cells contain and release high amounts of preformed IL-5 and IL-13 [5]. In chronic lesions, IFN-γ-producing cells have also been described [6]. Although Th2 type cytokines have been reported to be important for the development of allergic diseases according to the so-called Th1/Th2 paradigm, this concept is still controversial. As assessed by skin biopsy, both Th1 and Th2 types of inflammation are observed at the site of AD [7].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family with pro-apoptotic activity [8]. Similar to other members of the TNF family such as CD40L, FasL and TNF, TRAIL can be released in soluble form and low amounts can be measured in sera of normal controls [9]. Crystal structures have shown that, it occurs in trimer and it can be cleaved by cysteine proteases to generate a soluble form of the ligand [10]. TRAIL is expressed on the surface of activated T lymphocytes, IFNα-stimulated monocytes, dendritic cells and IFNγ-stimulated NK cells serving an anti-metastatic and anti-tumorogenicity activity [11,12,13,14] not only on malignant transformed cells but also of HIV-infected lymphocytes, normal monocytes, neutrophils [15], and macrophages [16].

Five TRAIL receptors have been identified: death receptor 4 (DR4/TRAIL-R1) and death receptor 5 (DR5/TRAIL-R2) have the ability to initiate the apoptosis-signalling cascade after ligation, whereas decoy receptor 1 (DcR1/TRID/TRAIL-R3), death receptor 2 DcR2/TRAIL-R4/TRUNDD and the soluble receptor osteoprotegrin lack this ability. They can afford protection from TRAIL-mediated killing by being concomitantly expressed with the death receptors and competing for binding to TRAIL, this may explain that TRAIL can selectively kill cancer cells but not normal cells [17,18]. The decoy receptors, TRAIL-R3 and R4, 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 [19].

TRAIL also has several physiological functions that are not limited to the killing of transformed cells. TRAIL has been shown to induce apoptosis in several primary cells, such as hepatocytes [20], HIV-activated T cells [21], plasma cells [22], immature dendritic cells [23], and neutrophils [15]. Moreover, TRAIL has been shown to activate a caspase-independent signaling pathway leading to the activation of nuclear factor-kB (NF-kB) [24]. TRAIL also exerts anti-inflammatory activities, which may include the induction of apoptosis in inflammatory cells [25], blocking the cell cycle [26], increasing the expression of interleukin 1 receptor antagonist (IL-1Ra), and activation of inhibitory phosphatases [27]. The ratio between IL-1 and IL-1Ra seems to be crucial for the intensity of the inflammatory response in many diseases. Most studies involving the physiological and pathological role of TRAIL were done in-vitro and little is known about TRAIL expression in-vivo conditions [28].

Although the death of certain cells can lead to functional deficiencies, prolonged survival of some effector cells can cause tissue injury and play a role in the pathogenesis of diseases [29]. Recently, apoptosis of epidermal keratinocytes was highlighted as a mechanism underlying the pathology of eczema in atopic dermatitis [4] through three major mechanisms that have been postulated: increased IL5 expression by CLA+ T cells which extends the life span of eosinophils, upregulated expression of Fas receptors and Fas L on peripheral blood CD4+ and CD8+ T cells of AD patients, lastly it was demonstrated that keratinocytes apoptosis can be mediated by skin...
infiltrating T cells and is mediated by Fas L expressed on the surface of T cells invading the epidermis or by soluble Fas L released from peripheral blood lymphocytes of AD patients [30]. Also the damaged keratinocytes decrease the effectiveness of the epidermis as a barrier against allergens and infectious agents and may contribute to the development of chronic eczema [31]. Due to the structural and functional similarity between TRAIL and Fas L and the fact that increased TRAIL expression on peripheral blood leukocytes of AD patients has been reported [20], TRAIL was assumed to play a role in the dysregulated apoptosis that contributes to the pathogenesis of AD [32].

**Aim of the work**

The aim of this work was to examine the expression of TRAIL on peripheral blood lymphocytes & monocytes of atopic dermatitis patients, to find its correlation with disease severity before starting treatment with topical steroids, and to assess the difference in the level of TRAIL expression between those receiving steroids whether good or poor responders and those who are not receiving steroids.

**Patients and methods**

Thirty patients with atopic dermatitis were enrolled in this study. These patients were selected from the outpatient clinic of the dermatology, venereology and andrology department, Ain Shams University, Cairo, Egypt.

Atopic dermatitis was diagnosed according to the criteria defined by Hanifin J.M [33].

For every patient, detailed history was taken, including the age of onset, duration of present illness, personal and/or family history of atopy. Exclusion criteria included those with other allergic diseases such as asthma, allergic rhinitis, or allergic conjunctivitis; also those with any other systemic disease were excluded. Patients were asked to stop any systemic or topical treatment for at least 4 weeks before enrollment in the study. A written consent was obtained from every patient before the study was conducted.

Atopic dermatitis severity was assessed according to the objective SCORAD scoring system, which was recommended by European Task Force on Atopic Dermatitis (ETFAD )[35]. It is a modification of the SCORAD index that excludes the subjective symptoms as pruritus and sleep loss, to minimize the errors caused by variability in patients’ ages and backgrounds. The objective SCORAD consists of the extent and intensity items, the formula being $A \times 5 + 7B \times 2$. In this formula $A$ is defined as the extent (0-100), and $B$ is defined as the intensity (0-18).

The maximum objective SCORAD score is 83 (plus an additional 10 bonus points). Bonus points are given for severe disfiguring eczema (on face and hands). Based on its results, AD has been classified into mild (<15), moderate (15-40) and severe (>40). The SCORAD index is influenced by subjective ratings that may be affected by social and cultural factors. Therefore ETFAD [35] recommends the objective SCORAD as it is representative and well evaluated.

Topical mild steroid therapy (hydrocortisone cream, class 7) [36] was given for patients with mild atopic dermatitis whose objective SCORAD was < 15. In addition, moderate steroid therapy (Betamethasone Valerate 0.1% cream, class 3) [36] was given for patients with moderate and severe AD according to the objective SCORAD index score. Topical steroid therapy was given for every patient twice daily for for 10 days according to the improvement.. According to
their response to topical steroid therapy, patients were divided into 2 groups. Group I (good responders): included 20 patients (8 males and 12 females, with age range 10.5-19 years; mean ± SD 15.46 ± 4.3) 3(15%) patients had mild AD, 11 (55%) had moderate AD and 6 (30%) had severe AD. These patients showed clinical improvement with a shift in their objective SCORAD to a lower score. Group II (poor responders): included 10 patients (5 males and 5 females, with age range 10-18 years; mean ±SD 15.41 ± 4.6) 1(10%) patients had mild AD, 6(60%) had moderate AD and 3 (30%) had severe AD. These patients did not respond to topical steroid therapy for 2 weeks, or showed no shift in their objective SCORAD to a lower score). The control group: composed of 20 sex and age matched healthy controls (11 males and 9 females, age range 11-19 years; mean ± SD 16±4.1), who had no personal or family history of atopy or any other systemic disorder.

Venous blood was collected from all patients and controls included in this study into tubes containing K- EDTA and analyzed before starting steroid therapy.

**Laboratory evaluation**

For every patient and control the following laboratory tests were done: Complete blood count using Coulter Counter (Coulter Microdiff 18, Fullerton, CA, USA), Serum total IgE quantiative measurement by a commercially available ELISA kit (Med' Biotech, Inc., Agenzyme Company, Industrial Road, San Carlos, CA, USA),and Peripheral blood lymphocytes and monocytes analysis of the patients and control groups was also performed by direct immunofluorescence flow cytometry (Coulter EPICS XL),as follows: Venous blood was collected into tubes containing K-EDTA (1.2mg/ml) and analyzed within 6 hours .One hundred µl of each sample was stained using 10µl of each of FITC (fluorescin isothiocyanate) conjugated mouse monoclonal antihuman CD14 antibodies (Caltag Laboratories, CA, Burlingame), FITC conjugated mouse monoclonal antihuman CD8 antibodies (Caltag Laboratories, CA, Burlingame), PC5 (phycoerythrycin) conjugated mouse monoclonal antihuman CD4 antibodies (Caltag Laboratories, CA, Burlingame), and PE (phycoerythrin) conjugated mouse monoclonal antihuman TRAIL antibodies (R&D systems, Minneapolis, MN,USA ).The tubes were then incubated in dark at room temperature for 15 minutes . Erythrocytes were lysed using ammonium chloride lysing solution (Al -Gomhoreya CA, Egypt ).After two washes with phosphate buffered saline (PBS ),the cells were resuspended in PBS for flow cytometric analysis ( fig 1).Negative isotype matched controls ( IgG mAB ) were included with each sample to determine the non specific binding of the monoclonal antibodies. The results were expressed as the percentage of the positive cells relative to the isotytic control (%), and mean fluorescence intensity (MFI) which is defined as the ratio between the mean fluorescence intensity of the cells incubated with the tested monoclonal antibodies and mean fluorescence intensity of the cells incubated with isotypic matched controls.

**Statistical methodology**

Analysis of data was done by IBM computer using SPSS ,version 12 as follows: description of quantitative variables as mean, SD and range, and description of qualitative variables as number and percentage, Chi-square test ,One-way ANOVA test , Kruskall Wallis test , Unpaired t-test, Correlation co-efficient test ,and ROC was used to find out the overall predictivity ,and the best cut of value with detection of sensitivity, specificity at this cut off value [36].

1 Sensitivity = true ve+/true +ve + false -ve
2 = ability of the test to detect +ve cases
Results

By comparing group I (steroid responders), and group II (non steroid responders) as regards age of patients, sex, paternal and maternal family history of atopy, our results show that in group II, 100% of patients had positive (+) both paternal and maternal family history of atopy, while in group I, all patients had + ve maternal family history of atopy and 50% had +ve paternal family history of atopy. On the other hand there was no statistically significant difference between group I and II as regards other variables.

Also by comparing between age of disease onset and family history of atopy, we found that by the presence of + F.H of atopy in both parents, the age of disease onset was smaller (inverse correlation), with a statistically significant difference between them (P<0.05).

According to the objective SCORAD results 4 (13%) patients had mild AD, 16 (53%) had moderate AD and 10 (33%) had severe AD. Comparison between group I and II AD patients as regards different laboratory data (Hb, WBCs, IgE, neutrophils and eosinophils), we found that group II had statistically higher eosinophils and IgE levels than group I with a highly significant difference between them (P<0.01). Also the average absolute eosinophil count and IgE levels in group II patients of atopic dermatitis showed significant correlation with severity of the disease and showed a non homogeneous distribution reflected by significant association with family history of atopy, when both parents were atopic. Both groups (I and II) had higher eosinophils and IgE levels than the control group with also a highly statistically significant difference between them (P<0.01).

Comparison between group I and II AD patients as regards the objective SCORAD revealed no statistically significant difference between them.

Comparison between studied groups (I, II, and controls) as regards different TRAILs, revealed that TRAIL expression in both CD4+ and CD8+ T lymphocytes as well as CD14+ monocytes was significantly higher in AD patients (group I and II) compared with normal controls, which usually demonstrated little TRAIL expression (P<0.01). In particular, CD8+ T cells expressed large amounts of TRAILs in AD. By comparing between group I and II, it was found that group I had statistically higher levels of all TRAILs than group II (Table 1, Fig. 1, 2).

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<table>
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<th>Variables</th>
<th>Group I N=20</th>
<th>Group II N=10</th>
<th>Control N=20</th>
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<td>CD8-MFI</td>
<td>12.9±2.8 (b,c)</td>
<td>3.7±0.7 (a,c)</td>
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<td>CD8 %</td>
<td>69.9±10 (c)</td>
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<td>&lt;0.01**</td>
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<td>CD4 MFI</td>
<td>8.5±1.6 (b,c)</td>
<td>2.6±0.4 (a,c)</td>
<td>1±0.06 (a,b)</td>
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<td>CD4 %</td>
<td>49.5±11 (c)</td>
<td>50.9±10 (c)</td>
<td>18.4±10 (a,b)</td>
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<td>CD14 MFI</td>
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<td>CD14 %</td>
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<td>52.7±14 (c)</td>
<td>11.9±5.2 (a,b)</td>
<td>73</td>
<td>&lt;0.01**</td>
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Group I =a, Group II =b, Group III controls = c

**Table (1):** Comparison between studied groups as regards different TRAILs.

**Fig 1:** Comparison between studied groups as regards different TRAILs

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Fig 2: Percentage of the positive cells relative to the isotypic control: Expression of TRAIL in different subsets of blood cells from AD patients and normal controls. Monocytes, CD8+ cells, and CD4+ cells were analyzed within PBMC and identified using anti-CD14, anti-CD8, and anti-CD4 monoclonal antibody (mAb), respectively. TRAIL expression was measured by flow cytometry using mouse anti-TRAIL mAb. Isotypic Control IgG1 mAb staining is shown in gray.

By studying the correlation between TRAIL expression on CD4+ and CD8+ Lymphocytes and CD14+ monocytes with the objective SCORAD index score in AD patients, it was found that all TRAILs expression were significantly inversely correlated to objective SCORAD index score (i.e. increased TRAIL expression was associated with a decrease in the objective SCORAD score, P<0.01)(Fig 3).

Fig 3: Correlation between TRAIL expression (e.g. CD 14 MFI) versus objective SCORAD index (Showing an inverse correlation).

Discussion

TRAIL is a member of the TNF superfamily, and has been implicated in the regulation of various physiological and pathological immune responses [14]. This might be because of its wide expression among cells of the immune system, including activated T cells [37], B cells [38,29],
monocytes [28], dendritic cells [39], natural killer cells [37], and neutrophils [28]. Most of these studies, however, were performed in vitro and little is known about TRAIL expression under in vivo conditions.

TRAIL induces apoptosis in dendritic cells and neutrophils, in addition to several primary cells and cancer cells, suggesting its critical role in the regulation of the adaptive and innate immune responses [40]. It has been reported that apoptosis via TRAIL is important for homeostasis of T cells [41]. TRAIL deficient mice showed increased susceptibility to experimentally induced AD [42] and autoimmune arthritis [43]. On the other hand, TRAIL application improved the clinical signs of experimental atopy. Heishi et al 2002[44] reported that apoptosis via TRAIL might be implicated in AD and that TRAIL is expected to be a useful marker for evaluating AD. Patient phobia from the steroid treatment is one of the obstacles that the physician can face [45,46]. Heishi et al, 2002 [44] reported that in vitro activated peripheral blood mononuclear cells (PBMC) from AD patients with poor response to steroid, demonstrated low TRAIL expression on their peripheral blood CD4+ T and CD8+ T cells, compared to mononuclear cells from AD patients with good response to steroid. That is why we aimed to assess the in vivo difference in the level of TRAIL expression between those receiving steroids whether good or poor responders and those who are not receiving steroids.

The aim of this work was to examine the expression of TRAIL on peripheral blood lymphocytes and monocytes of atopic dermatitis patients before starting topical steroid therapy, to find its correlation with disease severity before starting treatment with topical steroids, and to assess the difference in the level of TRAIL expression between good steroid responders and poor steroid responders.

Atopic dermatitis patients were classified into two groups according to their response to hydrocortisone cream, class 7 and betamethasone valerate 0.1% cream, class 3 steroid therapy for 2 weeks Group 1 (good responding group) improved within one week of treatment and group 2 (poorly responding group) who did not or poorly responded after 2 weeks of treatment. Our results showed a significant difference between group 1 and group 2 as regards family history of atopy. In group II, 100% of patients had positive +ve both paternal and maternal family history of atopy, while in group I, all patients had +ve maternal family history of atopy and 50% had +ve paternal family history of atopy. We also found that by the presence of +ve F.H of atopy in both parents, the age of disease onset was smaller (inverse correlation), with a statistically significant difference between them. Our results were in agreement with Tay et al, 2002[47] and Beltrani and Boguniewicz, 2003[48], who reported that the strongest risk factor is a parental history of atopy or eczema, they also noted that maternal atopy is considered the most important risk factor for the development of atopic disorders in offspring than paternal atopy.

We found also that the average absolute eosinophil count and IgE levels in group II patients of atopic dermatitis were significantly higher than that of group I. Each of these parameters showed significant correlation with severity of the disease and showed a nonhomogeneous distribution reflected by significant association with family history of atopy, when both parents were atopic. These findings were in agreement with the gene dose effect postulated by many authors [49,50], who suggested that subjects who inherit sets of atopy genes from both paternal and maternal origin have higher levels of IgE and eosinophilic % than subjects who inherit only one set of atopy genes either of paternal or maternal origin.

Comparison between studied groups (I, II, and controls) as regards different TRAILs, revealed that TRAIL expression in both CD4+ and CD8+ T cells as well as CD14+ monocytes was

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significantly higher in AD patients (group I and II) compared with normal controls, which usually demonstrated little TRAIL expression. In particular, CD8+ T cells expressed large amounts of TRAILs in AD. Comparing group I and II, it was found that group I had statistically higher levels of all TRAILs than group II, suggesting that TRAIL may exert anti-inflammatory effects in AD the fact that was reported by many studies [31 and 32]. Also TRAIL expression was inversely correlated with objective SCORAD index (done before treatment). Our results confirm those of other studies [32] who also demonstrated increased TRAIL expression by several inflammatory cells including peripheral blood T cells (CD4+T and CD8+T cells), monocytes, eosinophils, and neutrophils under in vivo conditions in AD. To our knowledge there are no other reports on the TRAIL expression under in vivo inflammatory conditions. The inverse correlation between TRAIL expression and objective SCORAD, can be explained by the data suggesting that TRAIL expressing inflammatory cells may contribute to the epidermal activation of IL-1 receptor antagonist (IL-1Ra) in AD and that TRAIL might play an important role in pathogenesis of AD [32]. To our knowledge this is the first report describing a -ve correlation between TRAIL expression in AD patients' blood T cells and monocytes and disease activity. The only study we could find regarding this point was done by Heishi et al, 2002 [44] who reported that in vitro activated PBMC from AD patients with poor response to steroid, demonstrated low TRAIL expression on their peripheral blood CD4+T and CD8+Tcells, compared to mononuclear cells from AD patients with good response to steroid. Warnnissorn et al, 2003 [31] examined expression of TRAIL in skin samples from AD patients. They found significantly higher number of TRAIL-positive mononuclear cells in the lesions of atopic dermatitis (mostly were CD68-positive macrophages) than in nonlesional skin of atopic dermatitis, normal skin and psoriasis. This suggests that TRAIL may also be involved in keratinocyte apoptosis in atopic dermatitis. However, they could not exclude the possibility that TRAIL may have other unknown functions in AD lesions.

To better understand TRAIL functions in AD, more studies should be performed on blood and skin samples and controlled following pharmacological treatment, as TRAIL may have other unknown functions in AD. Also more studies should be done to determine the role of TRAIL in the regulation of the IL-1Ra/IL-1 system. More studies about TRAIL correlation with treatment response should be done with greater number of patients and with different treatment options to better understand the role of TRAIL.

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