

Expression of TNF α Receptors on Immunocompetent Cells Is Increased in Atopic Dermatitis

Julia A. Lopatnikova^a Alina A. Alshevskaya^a Olga L. Krugleeva^{a, b}
Vera M. Nepomnyschih^a Victor S. Gladkikh^{c, d} Vitaliy L. Lukinov^{c, d}
Alexander V. Karaulov^e Sergey Vitalievich Sennikov^a

^aFederal State Budgetary Scientific Institution “Research Institute of Fundamental and Clinical Immunology,”

^bNovosibirsk State Medical University, ^cEOL Labs Ltd., and ^dInstitute of Computational Mathematics and Mathematical Geophysics SB RAS, Novosibirsk, and ^eI.M. Sechenov First Moscow State Medical University of the Ministry of Health, Moscow, Russia

Keywords

Membrane-bound receptors · Tumor necrosis factor · Receptor expression density · Atopic dermatitis

Abstract

Background: Expression levels of cytokine and growth factor receptors have been found to be important in the regulation of their action. Tumor necrosis factor- α (TNF α) is actively involved in inflammation processes in atopic dermatitis (AD), but the role of TNF α membrane receptors (TNFR) and their regulatory function in AD remains unclear. **Aim:** We aimed to determine the associations of parameters of TNFR α expression on immunocompetent cells with disease severity before and after therapy in AD patients. **Methods:** TNFR α expression on T cells, B cells, and monocytes was evaluated by flow cytometry. To determine receptor numbers on the cells, Quantibrite PE beads were used. The content of soluble mediators was evaluated by ELISA. To reveal linear relationships between the index scoring AD (SCORAD) and the studied parameters, multiple linear regression

model building was used. **Results:** TNFR1 and TNFR2 expression in lymphocyte and monocyte populations of AD patients was higher than in healthy individuals (HI). At the same time an increased percentage of positive cells was not associated with high receptor density, and vice versa. Serum content of TNF α , both soluble receptors, the number of TNFR2/T cells, and the percentage of TNFR2+ monocytes were found to be strongly associated with the SCORAD index. **Conclusion:** AD patients had increased TNFR expression on immune cells. Changes in the parameters of TNFR α expression compared to HI were associated with the disease severity index SCORAD.

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Introduction

Tumor necrosis factor- α (TNF α) is a pleiotropic cytokine that exerts a regulatory effect on all major subsets of human immunocompetent cells through its 2 receptors, TNF receptor 1 (TNFR1) and TNFR2. Depending on

Table 1. Baseline characteristic of the patients with atopic dermatitis (AD) and healthy individuals (HI)

Characteristics	Patients with acute AD (n = 20)	HI (n = 103)	p value
Females, n (%)	10 (50)	47 (45.6)	0.808
Median age on admission (IQR), years	23.5 (22–26)	25 (22–32)	0.357
Median SCORAD (IQR)	70.75 (58.6–76.7)	–	
Comorbidity			
Allergic rhinitis, n (%)	6 (30)	–	
Bronchial asthma, n (%)	2 (10)	–	
Both, n (%)	5 (25)	–	
Concomitant treatment			
Antihistamine (cetirizine), n (%)	20 (100)	–	
Emollients, n (%)	20 (100)	–	

several factors, TNF α signaling can lead to either prolonged inflammatory proliferative processes or the activation of proapoptotic pathways. The balance between these two main mechanisms of TNF action varies in a number of pathological processes, including atopic dermatitis (AD), and it can be critical both to the prognosis of disease development and progression and choice of the treatment strategy. AD is a chronic inflammatory skin disease that is associated with allergic processes and, in general, is accompanied by significant abnormalities in the cytokine profile [1], in particular in the production of TNF α . A number of studies have shown that AD patients have changes in proinflammatory mediator production by different cell types in inflamed areas of the skin [2]. Certain allelic variants of the TNF α gene were shown to be associated with AD [3]. These abnormalities lead to a reduced activity of TNF α in the acute phase of the disease and increased skin sensitivity to microorganisms in AD. In contrast, keratinocytes, mast cells, monocytes, and dendritic cells can have upregulated TNF α production in AD. However, the use of anti-TNF α agents in AD demonstrates conflicting results [4]. This may be due to anti-TNF α agents inhibiting the entire cytokine pool, both circulating and membrane-bound forms [5], while TNF α -mediated abnormalities are largely related to changes at the cellular level (in particular, in TNFR α expression) and require a targeted approach for their correction. Furthermore, TNF α activity varies depending on the cytokine form, either soluble or membrane bound [6, 7]. In a number of pathologies, the role of certain combinations of particular forms of receptors and cytokines has been demonstrated (e.g., the role of reverse signaling of membrane-bound TNF α in inflammatory colon diseases) [8, 9]. However, the role of TNF α and interactions between this important proinflammatory cy-

tokine and its receptors in AD remains unclear. Because one of the key factors capable of regulating both the efficacy and variability in cytokine action on targeted cells is receptor expression density (as previously demonstrated for a number of cytokines and growth factors) [10–13], investigation of not only the percentage of cells expressing TNFR1 and TNFR2 in cell subsets but also the amount of receptors expressed is very important. A number of studies have demonstrated that major leukocyte and monocyte subsets of immunocompetent peripheral blood cells are either actively involved in the development and progression of AD or are subjected to changes [14, 15], but the specific mechanisms require more detailed investigation. Also, we found recently that the density of receptors to IL-1, another proinflammatory cytokine, was associated with disease activity in AD patients [16]. However, these 2 cytokines may act together or at different AD stages (acute or chronic). Therefore, the purpose of this study was to carry out a screening of the TNF α membrane-bound receptor system to identify potential differences and abnormalities associated with AD activity.

Materials and Methods

Patients and Peripheral Blood Mononuclear Cells

Human peripheral blood mononuclear cells (PBMC) were obtained from AD patients hospitalized at the Clinic of Immunopathology under the Federal State Budgetary Scientific Institution “Research Institute of Fundamental and Clinical Immunology” in Novosibirsk, Russia, as described in detail previously [16]. The severity of AD was determined using the SCORAD index in the acute stage of the disease. Twenty persons (10 males and 10 females) aged 19–46 years (median 23.5, interquartile range, IQR, 22–26), formed the “AD, acute stage” group; their median SCORAD was 70.75 (IQR 58.6–76.7, min–max 42.10–91.80; Table 1). Patients received a course of steroid-based therapy (dexamethasone, 8 mg i.v.

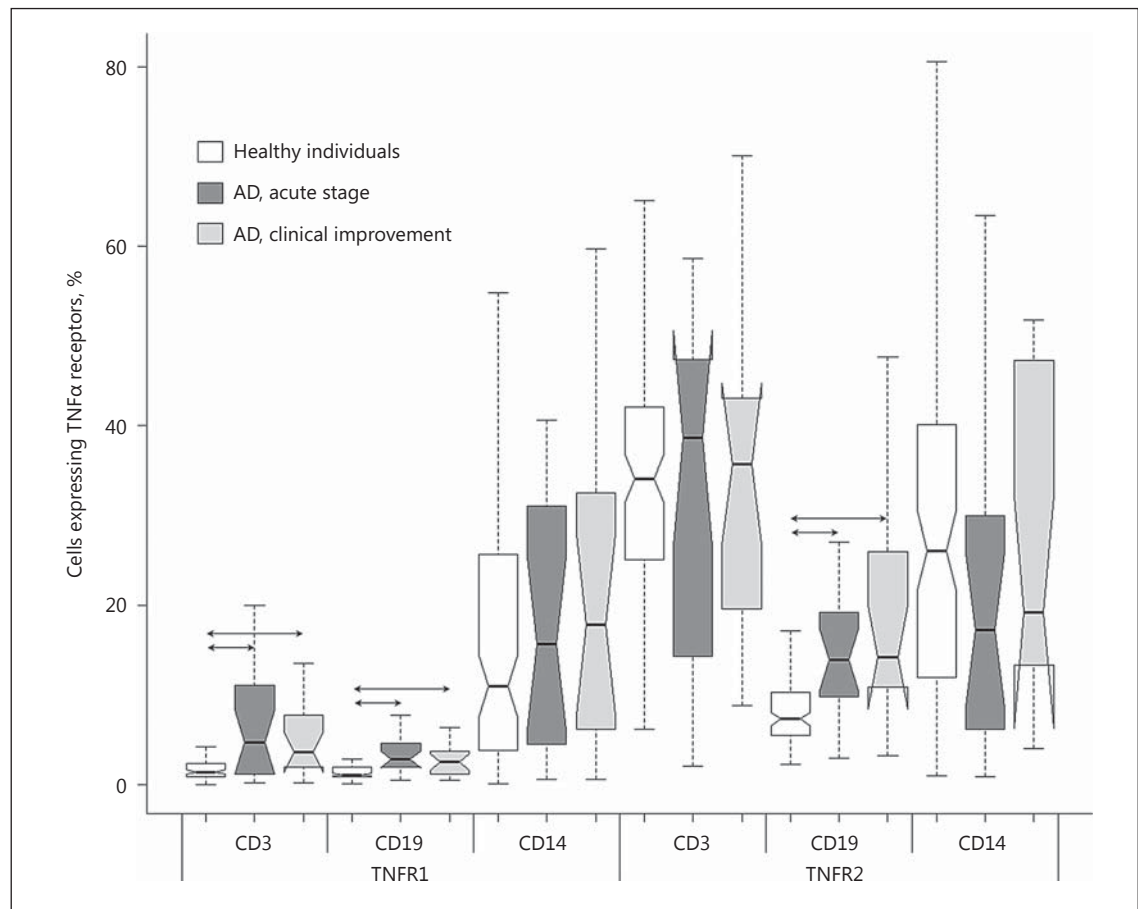


Fig. 1. Expression of tumor necrosis factor receptors TNFR1 and TNFR2 determined by flow cytometry in peripheral blood mononuclear cell subsets of healthy individuals ($n = 103$) and atopic dermatitis (AD) patients during the acute stage ($n = 20$) and clinical improvement of AD ($n = 17$). The arrows denote significant intergroup differences by Kruskal-Wallis analysis: $p < 0.05$.

once a day for 3–4 days and topical betamethasone), H_1 antagonists (cetirizine, 2.68 mg i.m. [1 ampoule] twice a day for 5–6 days), and concomitant therapy (emollients). A SCORAD decrease by 10 points was considered a clinically significant indicator of therapy response. SCORAD response data were available for 17 patients; all of them showed significant responses ($p < 0.001$) and formed the group of responders (“AD, clinical improvement,” median SCORAD 15.45, IQR 7.8–25.6, min–max 3.90–37.70, median SCORAD decrease 45.5, IQR 40.8–57.9, min–max 31.8–61.7). The group of healthy individuals (HI) comprised 103 HI from our previous study [17] matched for age and gender (age 18–46 years, median 25, IQR 22–32; 56 males and 47 females) with the AD group from the Novosibirsk Blood Center.

Informed consent was obtained from HI and AD patients before collecting peripheral blood samples. PBMC were isolated from whole blood by Ficoll (Pharmacia Fine Chemicals, Sweden) density centrifugation ($\rho = 1.077$ g/L) using a standard procedure [18].

All work was conducted in accordance with the Declaration of Helsinki (1964). The study was approved by the local ethics committee of the Federal State Budgetary Scientific Institution “Re-

search Institute of Fundamental and Clinical Immunology” (protocol No. 52, 23.06.2010).

Flow Cytometry

To identify subsets of T cells, B cells, and monocytes expressing different types of TNFR α , we employed flow-cytometric analysis. The full gating strategy, protocol, and reagents were described previously [17]. Mouse anti-human monoclonal antibodies anti-CD3-APC, anti-CD14-FITC, anti-CD19-PE-Cy7 (eBioscience, San Diego, CA, USA), and anti-human TNFR1 and TNFR2 PE (R&D Systems, Minneapolis, MN, USA) were used. We used BD Quantibrite™ PE beads (BD Biosciences, San Jose, CA, USA) to count the number of receptors per cell by converting fluorescence data.

Content of Soluble Cytokine TNF α and Its Receptors in Serum

Mediator levels in peripheral blood serum were evaluated using enzyme-linked immunosorbent assay (ELISA) kits for soluble TNFR (sTNFR) 1, sTNFR2 (RayBiotech, Norcross, GA, USA), and TNF α (JSC Vector-Best, Novosibirsk, Russia), as per the manufacturer’s instructions.

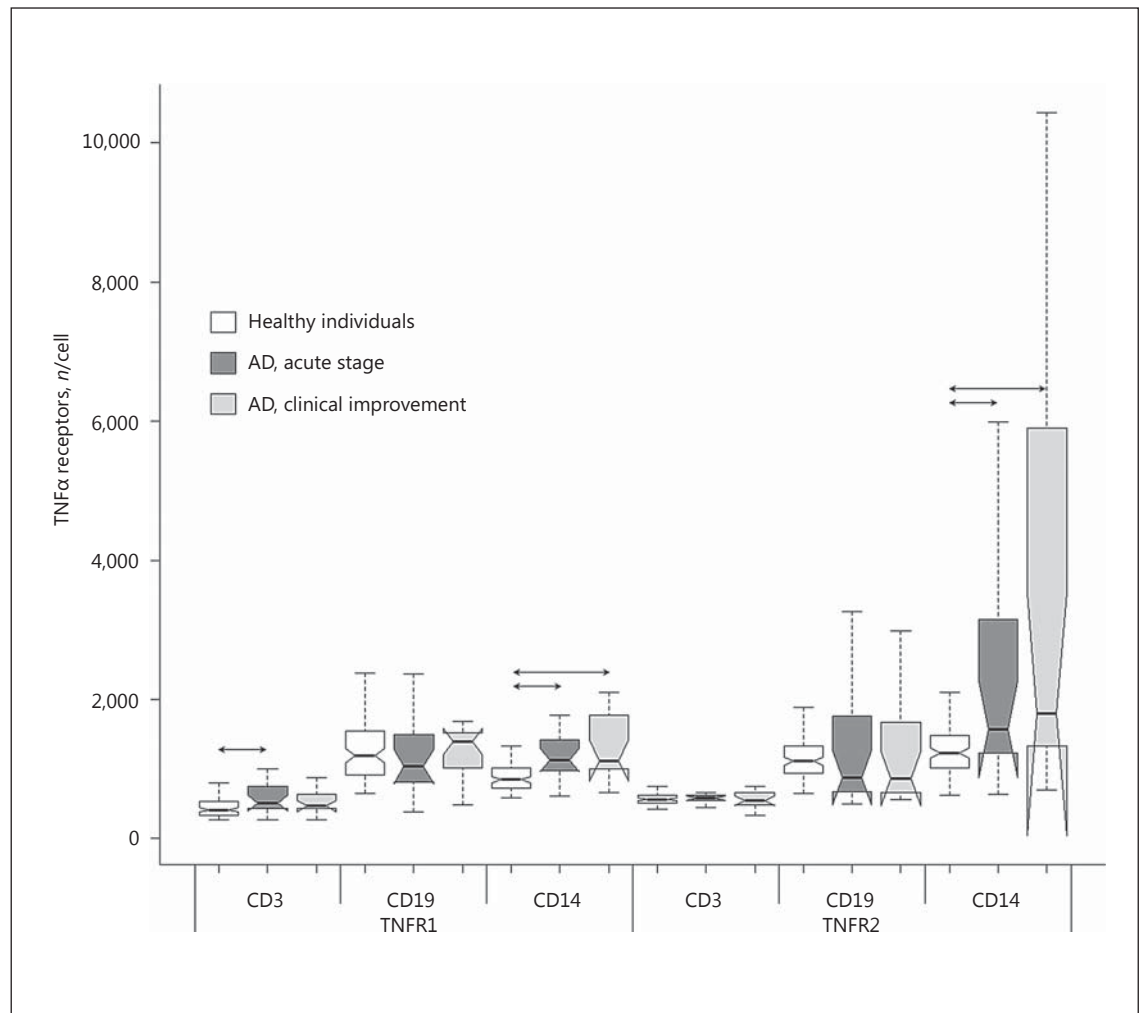


Fig. 2. Number of tumor necrosis factor receptors TNFR1 and TNFR2 on cells calculated with fluorescence intensity from flow-cytometric data in peripheral blood mononuclear cell subsets of healthy individuals ($n = 103$) and atopic dermatitis (AD) patients during the acute stage ($n = 20$) and clinical improvement of AD ($n = 18$). The arrows denote significant intergroup differences by Kruskal-Wallis analysis: $p < 0.05$.

Statistics

We used STATISTICA 7.0 software (StatSoft, Tulsa, OK, USA) and RStudio software version 0.99.484 (Free Software Foundation, Inc., Boston, MA, USA) with R packages (version 3.2.2; The R Foundation for Statistical Computing, Vienna, Austria) to perform statistical analysis. Statistical significance was determined using the Mann-Whitney test and Kruskal-Wallis analysis (for multiple comparisons) for all independent variables.

Data on the figures are presented with notched box plots with the IQR shown as a box with a line showing the median of the data. The whiskers add 1.5 times the IQR to the 75th percentile and subtract 1.5 times the IQR from the 25th percentile and show 95% confidence intervals of the median.

The correlations between the parameters studied were evaluated using the Pearson test and the Spearman test with p values

<0.05 considered statistically significant. The contribution of each studied parameter (serum levels of cytokines and receptors, percentage of cells with receptors in subsets, and number of receptors/cell) to changes in the SCORAD level in each group before and after therapy was studied. To study relationships between the SCORAD index and the study parameters of intact cells and of the soluble content of mediators, we used a multiple linear regression model (MLRM) similar to the one described previously for IL-1 receptors in AD [17]. To identify the most accurate version of MLRM, the reduction in parameters with low significance or low Pearson correlation coefficient (<0.3) was applied. To enable comparisons of coefficients, values of the independent variables were normalized. The quality control of the model was carried out after each remote factor. The target level of the determination coefficient R^2 was 0.8 and higher.

Table 2. Balance between expression of membrane-bound tumor necrosis factor receptors TNFR1 and TNFR2 on intact cells in patients with atopic dermatitis (AD) during the acute stage ($n = 20$) and clinical improvement ($n = 17$) and in healthy individuals (HI; $n = 103$)

	By cell percentage			By receptor number		
	HI	AD, acute stage	AD, clinical improvement	HI	AD, acute stage	AD, clinical improvement
T cells <i>p</i> value	TNFR2 <0.001	TNFR2 <0.001	TNFR2 <0.001	TNFR2 <0.001	0.52	0.554
B cells <i>p</i> value	TNFR2 <0.001	TNFR2 <0.001	TNFR2 <0.001	0.077	0.064	0.266
Monocytes <i>p</i> value	TNFR2 <0.001	0.153	0.332	TNFR2 <0.001	TNFR2 0.02	TNFR2 0.001

Appropriate cells show a subset in which the percentage of TNFR2+ cells or the number of TNFR2/cell is significantly higher (Wilcoxon matched-pair test) than those of TNFR1.

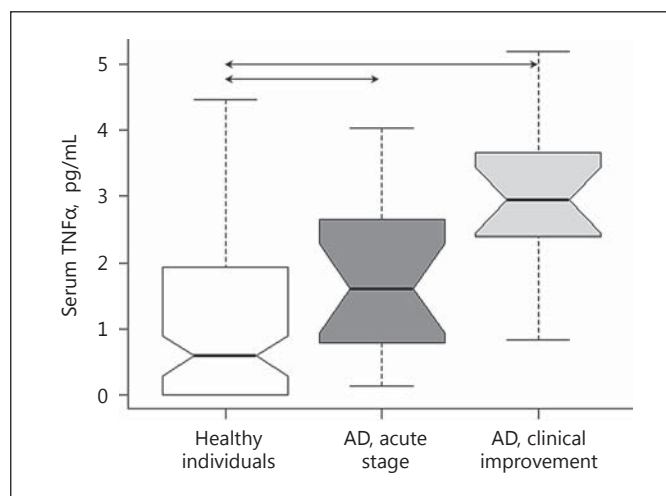


Fig. 3. The level of soluble tumor necrosis factor- α (TNF α) determined by ELISA in the serum from healthy individuals ($n = 103$) and atopic dermatitis (AD) patients during the acute stage ($n = 19$) and clinical improvement of AD ($n = 17$). The arrows denote significant intergroup differences by Kruskal-Wallis analysis: $p < 0.05$.

Results

Expression of TNFR1 and TNFR2 in PBMC Subsets

To understand the changes in the TNF α system in AD, main subsets of peripheral blood immunocompetent cells (CD14 monocytes, CD19 B cells, and CD3 T cells) were analyzed in patients during exacerbation and after response to therapy. The percentage of cells expressing

TNFR1 and TNFR2 (Fig. 1) and the number of TNFR/cell were evaluated (Fig. 2).

An analysis of the data demonstrated that all 3 subsets had individual characteristics of TNFR α expression. T cells of patients during disease exacerbation demonstrated an increase both in the percentage of TNFR1+ cells and in the number of receptors per cell, with the TNFR expression parameters being unchanged. Regardless of the disease phase, B cells of AD patients were characterized by higher percentages of TNFR1+ and TNFR2+ cells compared to HI, with the number of receptors on cells being constant. In contrast, monocytes of AD patients were characterized by an increased expression density of both receptor types, with the percentage of positive cells being unchanged. Both in healthy donors and in patients with AD, there was no subpopulation in which the expression of TNFR1 would be higher than that of TNFR2 expression (Table 2).

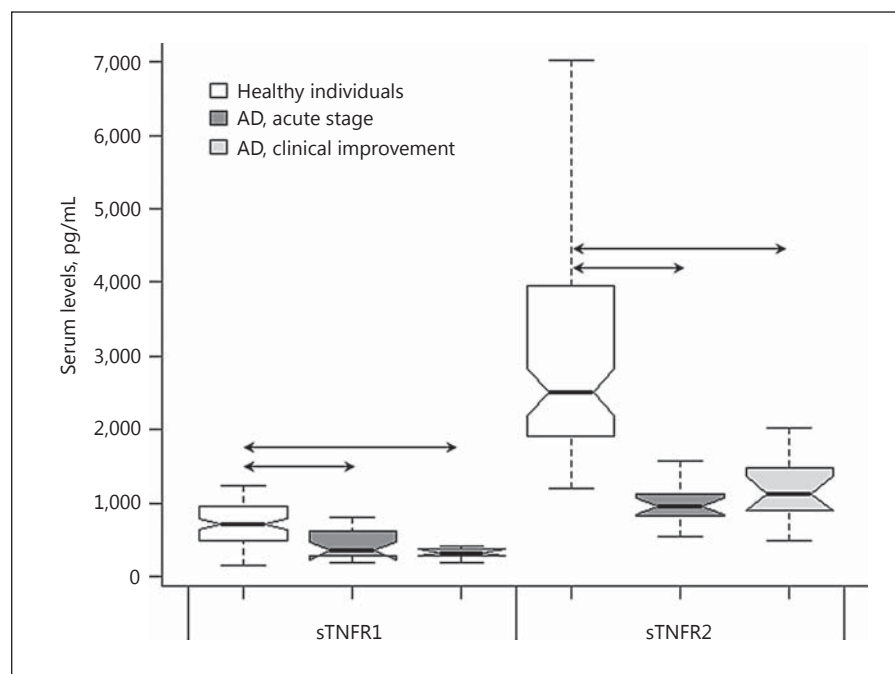
No statistically significant differences between AD patients before and after treatment were found.

Blood Serum Levels of TNF α and sTNFR1 and sTNFR2

As the serum levels of sTNFR α receptors and TNF α itself greatly influence the effect of TNF α on cells, the serum levels of TNF α (Fig. 3) and its soluble receptors (sTNFR1 and sTNFR2; Fig. 4) were studied in AD patients during exacerbation and after response to therapy using appropriate ELISA kits.

A higher serum cytokine level and lower levels of both types of soluble receptors were detected in AD patients

Fig. 4. Levels of the soluble tumor necrosis factor receptors sTNFR1 and sTNFR2 determined by ELISA in the serum of healthy individuals ($n = 103$) and atopic dermatitis (AD) patients during the acute stage ($n = 19$) and clinical improvement of AD ($n = 17$). The arrows denote significant inter-group differences by Kruskal-Wallis analysis: $p < 0.05$.



compared with HI. Notably, TNF α and sTNFR2 levels in patients were significantly increased after treatment.

Associations between TNFR Expression and Disease Activity

MLRMs were built to assess the impact of and identify linear relationships between the SCORAD index and studied cell parameters, as well as parameters of soluble mediators.

First, factors of each group, e.g., serum cytokine and receptor levels (“soluble factors”), percentage of cells with receptors in subsets (“percentage factors”), and number of receptors per cell (“number factors”), were used separately and in combination to identify the best-quality model that explains the change in the SCORAD index after therapy (SCORADdiff). A comparison of the models showed that the full model, including all the factors studied, has the best quality indicators. In particular, multiple R^2 levels were as follows: 0.505 for the full model, 0.037 for the model containing only soluble factors, 0.233 for the model containing only percentage factors, and 0.076 for the model containing only number factors. Low R^2 levels meant that the full set of studied parameters was not sufficient to predict the change in the SCORAD index and confirmed that there were other factors that could affect response to therapy.

As all of the quality indicators were low, in the second stage of the study individual factors were included in the complete model. For the group of patients with acute AD, the final MLRM explained the correlation of the SCORAD index with indicators from different groups – soluble factors, percentage factor, and number factors: $\text{SCORAD} \sim 7.446 \times \text{TNF}\alpha + 0.038 \times \text{sTNFR1} - 0.035 \times \text{sTNFR2} + 0.842 \times \text{CD3}(\%) \text{TNFR2} + 0.064 \times \text{CD3}(N) \text{TNFR2} - 4.412 \times \text{CD19}(\%) \text{TNFR1} + 0.511 \times \text{CD14}(\%) \text{TNFR1} - 0.012 \times \text{CD14}(N) \text{TNFR1} - 0.492 \times \text{CD14}(\%) \text{TNFR2} - 0.004 \times \text{CD14}(N) \text{TNFR2}$. The model explained the variation in the SCORAD index with selected predictors by 87%, thus only 13% of the variation in the SCORAD index remained unexplained (Table 3). For the group of patients with clinical AD improvement, the final MLRM explained the association with the SCORAD index: $\text{MLRM: SCORAD} \sim 15.48 \times \text{TNF}\alpha + 0.175 \times \text{sTNFR1} + 0.036 \times \text{sTNFR2} - 8.367 \times \text{CD3}(\%) \text{TNFR1} - 0.063 \times \text{CD3}(N) \text{TNFR1} + 0.099 \times \text{CD3}(N) \text{TNFR2} - 1.902 \times \text{CD19}(\%) \text{TNFR1} - 0.012 \times \text{CD19}(N) \text{TNFR1} + 0.651 \times \text{CD19}(\%) \text{TNFR2} + 1.061 \times \text{CD14}(\%) \text{TNFR2} + 0.002 \times \text{CD14}(N) \text{TNFR2}$. The model also had good statistical quality indicators (Table 4) and explained the variation in the SCORAD index with selected predictors by 95%, thus only 5% of the variation in the SCORAD index remained unexplained.

Table 3. Reduced multiple linear regression model for the SCORAD index of atopic dermatitis patients in the acute stage

Predictor	Coefficient	Coefficient SE	<i>p</i> value
Intercept	48.656	13.127	<i>0.006</i>
TNF	7.446	2.789	<i>0.028</i>
sTNFR1	0.038	0.014	<i>0.029</i>
sTNFR2	−0.035	0.013	<i>0.024</i>
CD3(%)TNFR2	0.842	0.215	<i>0.004</i>
CD3(N)TNFR2	0.064	0.017	<i>0.006</i>
CD19(%)TNFR1	−4.412	1.176	<i>0.006</i>
CD14(%)TNFR1	0.511	0.222	<i>0.050</i>
CD14(N)TNFR1	−0.012	0.005	<i>0.043</i>
CD14(%)TNFR2	−0.492	0.125	<i>0.004</i>
CD14(N)TNFR2	−0.004	0.002	0.082

Significant predictors are italicized ($p < 0.05$). Residual mean: 0 ($<10^{-15}$), SE: 6.584; F statistics: 5.29 (10 and 8 degrees of freedom), $p = 0.004$; Akaike information criterion statistics: 133.10; Shapiro-Wilk normality test for residuals: $W = 0.98$, $p = 0.90$; multiple R^2 : 0.87.

Table 4. A reduced multiple linear regression model for the SCORAD index in atopic dermatitis (AD) patients with clinical improvement

Predictor	Coefficient	Coefficient SE	<i>p</i> value
TNF	15.48	4.242	<i>0.022</i>
sTNFR1	0.175	0.040	<i>0.012</i>
sTNFR2	0.036	0.012	<i>0.043</i>
CD3(%)TNFR1	−8.367	2.223	<i>0.020</i>
CD3(N)TNFR1	−0.063	0.018	<i>0.026</i>
CD3(N)TNFR2	0.099	0.035	<i>0.046</i>
CD19(%)TNFR1	−1.902	0.912	0.105
CD19(N)TNFR1	−0.012	0.003	<i>0.030</i>
CD19(%)TNFR2	0.651	0.191	<i>0.027</i>
CD14(%)TNFR2	1.061	0.219	<i>0.008</i>
CD14(N)TNFR2	0.002	0.001	0.095

Significant predictors are italicized ($p < 0.05$). Residual mean: 0 ($<10^{-17}$), SE: 4.935; F statistics: 7.14 (11 and 4 degrees of freedom), $p = 0.036$; Akaike information criterion statistics: 100.31; Shapiro-Wilk normality test for residuals: $W = 0.97$, $p = 0.82$; multiple R^2 : 0.95.

Discussion

The proinflammatory cytokine TNF has a wide range of both local and systemic effects during the acute and chronic phases of AD. However, at the molecular level, these effects are based not only on a change in TNF production but also on changes in systems of signal inhibition and transduction into immunocompetent cells and, accordingly, in the implementation of their biological functions. Soluble and membrane-bound forms of TNF were previously shown to be involved at the local level in AD [19, 20]. However, changes in the system of membrane-bound receptors have been studied to a much lesser extent. Membrane-bound TNFR α (TNFR1 and TNFR2) enable complex interaction with the cytokine. TNFR1 is expressed by almost all cell types and, in addition to its proinflammatory effects, can signal TNF α -induced apoptosis. In contrast, TNFR2 is predominantly expressed by blood, lymphoid, and epithelial cells and is involved in proliferative processes [21, 22].

In this study, we conducted a comprehensive evaluation of membrane-bound TNFR expression by major subsets of immunocompetent PBMC: T cells, B cells, and monocytes. We demonstrated that intact cells of PBMC subsets differ both in the relative percentage of cells expressing TNFR1 and TNFR2 and in the absolute number of these receptors (Table 5).

T and B cells are actively involved in various processes associated with the development and progression of AD [23, 24], but the role of monocytes still remains ambiguous. Most researchers believe that the changes in monocyte functions are secondary. In particular, there is evidence that some of the abnormalities in monocyte functions in AD are the consequence, but not the cause, of recurrent skin infections [15], and that monocytes are activated by the production of cytokines/chemokines by other cells [25]. In contrast, other data confirm the independent involvement of monocytes in pathogenic processes and the association of disease severity with certain allelic TNF variants [26]. However, the expression level of major proinflammatory cytokine receptors in main immunocompetent cell subsets of AD patients has not been evaluated previously. Our data demonstrate that all 3 studied peripheral blood subsets (T cells, B cells, and monocytes) significantly differ in their TNFR1 and TNFR2 expression. Notably, there is no single trend for the subsets analyzed in the expression change, and each subset is characterized by a particular combination of changes compared with healthy controls.

Study of built MLRM indicated that to evaluate the prognostic significance of the studied indicators, it was necessary to take a combination of factors from different groups into account. Only serum levels of the mediators or only the percentage of positive cells cannot explain the

Table 5. Summary of changes in the expression of membrane-bound tumor necrosis factor receptors TNFR1 and TNFR2 on intact cells and serum levels of cytokine TNFα and soluble TNFR1 and TNFR2 in atopic dermatitis (AD) patients during the acute stage (*n* = 20) and clinical improvement (*n* = 17) compared to the parameters of healthy individuals (*n* = 103)

	AD, acute stage		AD, clinical improvement	
	TNFR1	TNFR2	TNFR1	TNFR2
TNFR expression				
T cells	↑% + ↑ <i>N</i>		↑%	
B cells	↑%	↑%	↑%	↑%
Monocytes	↑ <i>N</i>	↑ <i>N</i>	↑ <i>N</i>	↑ <i>N</i>
Serum level				
TNFα	↑		↑	
Soluble receptor	↓	↓	↓	↓

Arrows (↑, increase; ↓, decrease) indicate statistically significant changes (*p* < 0.05; Mann-Whitney U test) of either TNFR1+/TNFR2+ cells in cell subsets (%) or numbers of receptors/cell (*N*) compared to the parameters of apparently healthy individuals.

variability in the SCORAD index. A model comparison allowed to reveal that there are 5 factors affecting the SCORAD level in both the acute stage of AD and after effective therapy. At the same time, higher levels of serum TNFα, sTNFR1, and the number of TNFR2 on T lymphocytes correlated with a more severe SCORAD level, regardless of active disease exacerbation. However, the effect of sTNFR2 and percentage of monocytes expressing TNFR2 varied for the acute stage and after the response to therapy: high levels of parameters were associated with a lower SCORAD index during exacerbation and a higher one after therapy. This may be due to the fact that the steroid-based therapy was more affected by TNFR2 expression on monocytes and needs a more detailed study. The associations found differed significantly from analogously analyzed data on receptors for IL-1 in AD [16].

Given the different functions of TNFR1 and TNFR2 and their competition for binding to the cytokine, the balance of the different receptor types reflects the state of the regulatory system of the biological effect of TNFα. The differences in the percentage of receptor-expressing cells in a subset and the differences in the mean density of TNFR1 and TNFR2 expression (as well as the changes in these ratios in AD patients compared with HI) can modulate the effect of cytokines by increasing the likeli-

hood of TNFα binding to a particular type of receptor on cells of a certain subset or to trigger specific signaling pathways. We demonstrated that the percentage of TNFR2 -expressing cells in each of the HI subsets was significantly higher than the percentage of TNFR1-expressing cells, while this ratio was retained only for lymphocytes, but not for monocytes, in AD patients (Table 2).

Regarding the number of receptors on cells, the TNFR1/TNFR2 ratio of HI is retained in AD patients for B cells and monocytes, but changes for T cells. This may be one of the consequences of active involvement of this subset in the pathological process, which was previously demonstrated in various experimental studies [27, 28].

Most studies have established the fact of changed TNF production at the local level in inflamed tissue in both the acute and the chronic AD phase, but the direction of these changes has been subject to controversy [29, 30]. Soluble TNFRα, which are commonly identified in pathologies, are essentially natural anti-TNFα agents. However, they not only bind TNFα and act as neutralizing agents but also play a role as a reserve pool for subsequent gradual mediator release [31]. In the present study, the total serum TNF level (total, free, and bound TNF in blood) was higher in AD patients than HI, but it increased after effective treatment (2.93 ± 0.54 times, on average). During disease exacerbation, proinflammatory mediators are produced locally [29], and some portion of TNF is neutralized due to successful treatment (partially by soluble receptors, which may account for the significant increase in sTNFR2 observed in this study, a receptor with high affinity for TNF). However, some portion of TNF enters the systemic circulation owing to a low level of soluble receptors. Another possible explanation is a disturbance in the mechanisms of soluble receptor formation in AD. Structurally, soluble receptors are an enzymatically cleaved extracellular domain of a membrane receptor [32, 33]. In addition, the type 2 receptor may be produced via alternative splicing [34, 35]. An increased serum TNF level in AD patients may also indicate changes in the sensitivity of immune cells to the cytokine, which leads both to a change in the receptor expression density on the cell surface and to the formation of soluble forms. A decrease in the level of soluble receptors, in turn, prevents effective neutralization of the serum TNF, inducing a chain reaction during disease exacerbation.

Conclusion

Our findings indicated that AD patients had increased TNFR expression on immune cells which presented as an increase in either the percentage of positive cells or the number of receptors per cell, or both. We demonstrated the importance of determining not only the relative percentage of cells expressing TNFR1 and TNFR2, but also the number of membrane-bound receptors, because receptor expression density was characterized by changes not evident upon a standard evaluation of the percentage of positive cells.

Acknowledgments

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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