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Study

Apoptotic View to What Happens at Periphery in Psoriasis

Semih Tatlıcan,*[1] MD, Özlem Gülbahar,[2] MD, Cemile Eren,[1] MD, Fatma Eskioğlu,[1] MD, Mehmet Ali Ergun,[3] MD, Akın Yılmaz,[3] MD

Address: Department of Dermatology, Ministry of Health Dışkapı Yıldırımı Beyazıt Education and Research Hospital;[1] Departments of Medical Biochemistry,[2] and Medical Genetics,[3] Gazi University Faculty of Medicine, Ankara, Turkey
E-mail: semihtatlican@gmail.com
* *Corresponding author*: Semih Tatlıcan, MD, Becerikli Sokak No: 7/19 Kocatepe, Ankara, Turkey, 06650

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Abstract

Introduction: Psoriasis vulgaris is characterized by T cell alterations both in skin and peripheral blood. There are reports indicating that apoptotic changes in keratinocytes and T lymphocytes may take role in the pathogenesis of psoriasis vulgaris.

Objective: The aim of the current study is to find out the apoptotic changes in peripheral lymphocytes of psoriasis patients.

Material and Methods: 57 psoriasis vulgaris and 27 healthy control subjects were included in the study. The levels of caspase-8 and caspase-9 in the sera of the patients and control subjects were measured by Enzyme-linked Immunosorbent Assay (ELISA) method and the number and the percentage of apoptotic lymphocytes were calculated.

Results: General demographic features of the study groups were similar. There was statistically significant difference between the mean apoptotic index of the patients (12.35 ± 3.50) and control group (5.27 ± 1.56), (p=0). The mean caspase-9 levels of the patients (2.2839 ± 0.0653 ng/mL) were also significantly higher than the levels of control subjects (1.9489 ± 0.0214 ng/mL), (p=0.017). The mean caspase-8 levels of the patients (0.1909 ± 0.0653 ng/mL) were significantly lower than the levels of control subjects (0.1919 ± 0.0214 ng/mL), (p=0.042).

Conclusions: Increased apoptosis of peripheral lymphocytes of psoriasis patients can be interpreted as a part of the complex relationship of lymphocytes between periphery and skin. The major pathway of apoptosis in peripheral lymphocytes seems to be the intrinsic pathway as mean caspase-9 levels were higher and the mean caspase-8 levels were lower than the levels of control subjects.

Introduction

Psoriasis is a chronic, inflammatory skin disease and activated T lymphocytes reported to be the pivotal cells in the pathogenesis of psoriasis [1, 2].

There is evidence that both disturbances occur in peripheral blood and psoriatic skin related with T lymphocytes in the development of disease [3, 4].

Apoptosis is a type of cellular killing which the cells go under programmed death [5].

Studies focusing on the changes related with apoptosis of T lymphocytes in psoriasis such as sensitivity to interferon (IFN)-α [6] and expression of perforin [7, 8] demonstrated differences which seem to be important in pathogenesis of the disease.

Apoptosis of circulating T cells was of inter-

est in some chronic skin disorders such as psoriasis, contact dermatitis and atopic dermatitis patients [**9**].

Two signaling pathways take role in apoptosis; one is the extrinsic pathway in which caspase-8 take role and the other is the intrinsic pathway which involves caspase-9. It is showed that peripheral lymphocytes may use both intrinsic and extrinsic pathway in certain circumstances [**10**].

In this study, by comparing psoriatic patients with healthy subjects in terms of the percentage of peripheral lymphocyte apoptosis, we discussed its possible role in the pathogenesis of psoriasis. Also by measuring the levels of caspase-8 and caspase-9 both in psoriatic patients and control group we investigated whether there was a switch in favor of one of the apoptotic pathways.

Materials and Methods

Patients and Controls

57 untreated psoriasis vulgaris patients and 27 age and sex matched healthy control subjects were included in the study. Psoriasis vulgaris diagnosis was made clinically and confirmed histopathologically. The extent of the lesions and severity of the disease was calculated according to *Psoriasis Area and Severity Index* (PASI) scores. Patients and control subjects having a history of systemic illnesses such as diabetes mellitus, renal and hepatic insufficiency, internal malignancies, using any systemic drugs and, having smoking habit were not included in the study.

Blood Collection

After the approval of the study protocol by the local ethical committee of our hospital, an informed consent was obtained from all enrollees. None of the patients used any systemic agent for the treatment of the disease previously. Topicals were ceased at least two weeks prior to the blood sampling. Blood samples (10 mL) were collected by a 25-gauge needle in sitting position through antecubital vein, avoiding haemolysis, after a rest of 30 minutes at 9:00 a.m. following an overnight fast.

Laboratory Methods

Measurement of Serum Caspase 8 and Caspase 9 Levels

Serum was obtained by the centrifugation of the collected blood and immediately stored at -80° C until use. Serum caspase-8 levels were measured Enzyme-linked Immunosorbent Assay (ELISA) method using commercial kits (*Bender MedSystems, Vienna, Austria*). Minimum detectable concentration for caspase-8 was 0.10 ng/mL. Intra-assay and inter-assay variation coefficients for caspase-8 were <6.7% and <8.5%, respectively [**11**].

Serum caspase-9 levels were measured Enzyme-linked Immunosorbent Assay (ELISA) method using commercial kits (*Bender MedSystems, Vienna, Austria*). Minimum detectable concentration for caspase-9 was 0.40 ng/mL. Intra-assay and inter-assay variation coefficients for caspase -9 were <6.6% and <9.0%, respectively [**12, 13**].

Lymphocyte Isolation and Morphological Assessment of Apoptosis

Lymphocyte Isolation

Peripheral venous blood was drawn from patients and controls into heparinized Vacutainer™ tubes. Blood samples were layered on Ficoll and centrifuged at 500 g for 10 min to separate mononuclear cells. The buffy coat was recovered and washed twice with RPMI 1640 (Biological Industries).

Morphological Assessment of Apoptosis

After isolation of the lymphocytes, the cell pellets were collected on a glass slide, stained with 1 uL of a mixture of acridine orange (Sigma A-6014, 100 µg/mL) and ethidium bromide (100 ug/mL, Sigma E-8751) in PBS and immediately examined under a fluorescence microscope at a 490 nm excitation wavelength. Acridine orange, a vital dye, enters cells through an intact cytoplasmic membrane and intercalates into DNA making it appear green, with structure variations in fluorescence intensity in normal nuclei due to the relative distribution of euchromatine and heterochromatin. In contrast, apoptotic nuclei have condensed chromatin, which is uniformly stained, and takes the form of crescent or numerous featureless bright spherical bodies. Passive diffusion of acridine orange induces, in addition, a green cytoplasmic coloration. Ethidium bromide is only taken up by cells with a damaged cytoplasmic membrane and stains the nucleus in red, with the same characteristic apoptotic features in the case of secondary necrosis or intact nuclear structure in cell death due to primary necrosis [**14, 15**].

Analysis of the Lymphocytes

The analyses were performed under a Fluorescent microscope. Viable and apoptotic cells were calculated. Apoptotic index is defined as the ratio of the number of the apoptotic cells to the total cell number.

Statistical Analysis

Statistical analyses were carried out with SPSS for Windows version 15.0 statistical software (*SPSS Inc., Chicago, IL, USA*). Continuous variables are presented as mean ± standard deviation and categorical variables as percentages.

Continuous variables were examined for normality by *Shapiro-Wilks* test. For normally distributed variables, differences between the groups were determined by t test. Mann Whitney test was used for not normally distributed variables. Associations between the continuous variables were investigated by *Pearson* correlation coefficient or *Spearman* rank correlation coefficient. *Wilcoxon* signed rank test was used to examine the difference between before and after the treatment. Significance value considered as 0.05.

Results

The general features of study groups were similar (**Table 1**). The mean age and gender distribution of psoriasis vulgaris patients (40.09 ± 14.04 years; 29 female, 28 male) and, control subjects (38.89 ± 14.12 years; 11 female, 16 male) were similar. Mean disease duration was 184.37 ± 156.73 months.

Whereas the mean serum caspase-8 levels of patients (0.1909 ± 0.0653 ng/mL) were significantly lower than the levels of control subjects (0.1919 ± 0.0214 ng/mL), (P=0.042); the mean serum caspase-9 levels of the patients (2.2839 ± 0.0653 ng/mL) were significantly higher than the levels of control subjects (1.9489 ± 0.0214 ng/mL), (P=0.017).

Mean apoptotic index of the patient group (12.35 ± 3.50) was significantly higher than the mean apoptotic index of the control group (5.27 ± 1.56), (p=0).

There was no correlation between the caspase-8, caspase-9 and apoptotic index both in patient and control groups, as well as PASI scores.

Discussion

Psoriasis is a chronic skin disorder in which the T cell infiltration of the skin is one of the characteristic finding [**16**]. Studies strengthened the major role of T cells and drew attention to the changes in peripheral T lymphocytes such as type 1 cytokine production [**4, 17**], increased sensitivity to IFN-α [**6**] and expression of perforin [**8**].

The possible role of biological molecules related with apoptosis such as IFN-α, perforin and peroxisome proliferator-activated receptor (PPAR) δ were discussed in relevant studies [**6, 8, 18**].

Peripheral T cell apoptosis was investigated in atopic dermatitis previously. The relevant study also dealt with the apoptosis of peripheral T cell apoptosis in four psoriasis patients but the results of these patients were not primarily compared with controls [**9**].

Although the exact target cell was not explained, it is demonstrated that the expression of perforin which is involved in apoptosis is higher among peripheral blood T lymphocytes in severe psoriasis when compared to mild disease [**8**] also in exacerbation of disease [**7, 19**].

Eriksen et al in their study with three psoriasis patient and three healthy subjects showed that IFN-α signaling was increased

Table 1. Clinical Features of Patients, Demographic Features and Laboratory Results of Study Groups and P Values

Variables	Patients	Controls	P values
Age*	40.09 ± 14.04	38.89 ± 14.12	P>0.05
Gender (F/M), (n, (%))	29/28 (50.9/49.1)	11/16 (40.7/59.3)	P>0.05
Disease duration*	184.37 ± 156.73	-	-
Nail involvement (n / %)	18 / 31.57	-	-
Arthritis (n / %)	7 / 12.28	-	-
PASI scores	21.9 ± 12.8	-	-
Caspase 8*	0.1909 ± 0.0653	0.1919 ± 0.0214	P=0.042
Caspase 9*	2.2839 ± 0.6078	1.9489 ± 0.3891	P=0.017
Apoptotic index*	12.35 ± 3.50	5.27 ± 1.56	P=0

*: Data is given as mean ± standard deviation, F: Female, n: number, %: percentage, M: Male, PASI: Psoriasis Area and Severity Index

in peripheral blood mononuclear cells (PBMC) of psoriatic patients. IFN-α signaling was associated with growth arrest of peripheral T cells but only apoptosis of T lymphocytes in psoriatic skin was studied and data about apoptosis of peripheral T lymphocytes of psoriatic patients were not given [**6**].

Yacoub et al found PPARδ to be expressed in peripheral blood T cell of healthy subjects and in T cells from skin lesions of psoriatic patients. They ascribed a role for PPARδ in the pathogenesis of psoriasis and showed that IFN-α signaling induces PPARδ expression. PPARδ results in proliferation of T lymphocytes and protects from apoptosis induced by IFN-α [**18**]. However the peripheral blood T cells were from healthy subjects and it is questionable whether the results are valid in case of psoriatic patients. Also it should be kept in mind that the primary immunologic changes in the beginning of disease may result in increase or decrease of T cell apoptosis and the results in relevant studies may in fact show the compensatory responses.

We found that the apoptosis of peripheral lymphocytes in psoriasis patients were significantly higher when compared to control subjects. During the development of psoriatic plaque T lymphocytes from the peripheral blood infiltrate skin [**20**]. Our finding that increased apoptosis of peripheral lymphocytes is not discordant with the evidence that even in the absence of subsequent PBMC, the previously infiltrated T lymphocytes which are triggered by inciting event of psoriasis, sustain the psoriasis clinic [**21, 22**]. The correlation of the improvement of psoriatic plaque with the decrease in the T lymphocytes in the skin but not in circulation [**23**], suggest that PASI correlates with the density of T lymphocytes infiltrating the plaque. It is consistent with our finding that the percentage of apoptosis did not correlate with PASI scores.

We found serum caspase-8 levels to be significantly lower in patients when compared to controls whereas caspase-9 levels were higher in psoriatic patients. It is demonstrated that in certain conditions both intrinsic and extrinsic way of apoptosis take role for different subsets of T lymphocytes according to the pathogenesis of disease [**10**].

Also Yacoub et al showed that PPARδ does not protect from Fas induced, namely extrinsic apoptosis but protects from apoptosis by intrinsic pathway [**18**]. This finding may be related with our results that caspase-8 levels were lower but caspase-9 levels were higher in patients.

There is a nonlinear relation between skin and periphery in pathogenesis of psoriasis and it is not known where the trigger resides [**24**]. The results of the relevant studies reflect the dynamics between skin and immune system components.

Considering the remarkable number of our patients and their untreated state, we suggest that increased apoptosis of peripheral lymphocytes provides insight into the pathologic disturbances related with apoptosis and reflects the complex regulation of apoptotic process in peripheral blood mononuclear cells of patients with psoriasis.

Also significantly higher levels of caspase-9 whereas lower levels of caspase-8 psoriatic patients compared to controls, made us to think that there is a switch in apoptotic pathway in favor of intrinsic pathway.

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