Dermatology Practical & Conceptual

Investigation of the PD-1/PD-L1 Expression in the Lesional Skins of Patients With Psoriasis

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ABSTRACT

Introduction: Psoriasis is an immune-mediated, chronic and inflammatory disease whose pathogenesis is affected by the interactions of several immune cells and cytokines. PD-1 is an inhibitor receptor that is expressed to a large extent in T lymphocytes and responsible for regulating autoimmunity and self-tolerance.

Objectives: In this study, we aimed to investigate the expression of PD-1/PD-L molecules in the lesioned skins of psoriasis patients.

Methods: The study included 30 psoriasis patients, and 15 healthy volunteers as the control group. Anti PD-1 and PD-L1 antibodies were applied to the skin biopsy samples that were collected from the patient and control groups. Cytoplasmic and membranous staining of PD-1 and PD-L1 were considered positive. The number of stained immune cells that was examined for each case.

Results: The percentage of the tissues with high PD-1 (+) and PDL-1 (+) immune cell counts were significantly higher in the psoriasis patients compared to healthy controls (P values = 0.004 and 0.002, respectively). A negative and statistically significant correlation was detected between PDL-1(+) immune cell numbers and PASI scores (P = 0.033, r=-0.57).

Conclusions: In the lesioned skin samples of psoriasis patients, the PD-1 and PD-L1 expressions were significantly higher in immune cells than that in the skin samples of the healthy controls. This study was the first investigation of the expression of PD-1/PD-L molecules in the immune cells in found the lesioned skins of psoriasis patients.

Introduction

Psoriasis is an immune-mediated, chronic and inflammatory disease whose pathogenesis is affected by the interactions of several immune cells and cytokines. Over the last two decades, highly significant advancements have been achieved regarding the pathogenetic mechanisms of psoriasis where T helper 1 (Th1) and Th 17 lymphocytes play a major role [1].[1] Recently, new molecules and pathways responsible for regulating autoimmunity and tolerance have been defined. One of these molecules, programmed death-1 (PD-1) (CD279) is an immune check point that sends inhibitor signals to the immune system to maintain a balance between T cell activation and self-tolerance in peripheral tissues. PD-1 is an inhibitor receptor in the form of a transmembrane glycoprotein that belongs to the CD28/CTLA-4 family with the weight of 50-55 kDa. PD-1 is expressed in active T cells, B cells, thymocytes, natural killers (NK) and double negative thymocytes ($CD4^-CD8^-$). PD-1 has two ligands: Inhibitor signals are achieved by the bonding of PD-1 to its ligands – PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). While PD-L1 is widely expressed in T cells, B cells, macrophages and dendritic cells, PD-L2 is expressed in active macrophages and dendritic cells. The PD-1/PD-L pathway participates in the pathogenesis of autoimmune diseases. Signals that are generated by PD-1 and its ligands, PD-L1 and PD-L2, regulate central and peripheral tolerance by means of several mechanisms [2-4]. In experimental autoimmunity and pathogenic polymorphism models, it was seen that a decrease in the expression of PD-1 and its ligands is associated with some autoimmune disorders such as Type 1 diabetes mellitus, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, and encephalomyelitis [2,3].

Regulatory T-cells (Treg) is a T-cell subtype responsible for suppressing the immune response and maintaining immune tolerance. PD-1 surface molecules on the surface of Treg cells play a key role in immune tolerance. PD-1 binds to PD-L1, inhibiting the function of the effector T-cells and supporting the Treg cells activity. Likely to be dysfunctional in psoriatic patients, T-cell surface molecules are unable to inhibit the activity of the inflammatory cells [4,5]. In addition, the PD-1 and PDL-1 pathways are among the most important mechanisms in tumor cells escape from the immune system. PD-L1 expression of tumors is a key determinant of the response to immune check point inhibitors. It was reported that, in patients treated with anti-PD-1 molecules, eczema, lichenoid dermatoses, vitiligo and psoriasis lesions emerged, or previously existing psoriasis became more severe [7-9]. The fact that anti-PD-1/PD-L1 antibodies trigger psoriasis suggests that PD-1 and PD-L1 pathways are somehow involved in the pathogenesis of psoriasis. Few studies have investigated the expression of PD-1 and PD-L in the T lymphocytes of the peripheral blood of psoriasis patients. Even fewer studies investigate the expression of PD-1 and PD-L1 in the lesional skins of psoriatic patients.

Objectives

In this study, we aimed to investigate the expression of PD-1/PD-L molecules in the lesional skins of psoriasis patients.

Methods

Thirty psoriasis patients aged between 18 and 70 years who were admitted to the dermatology outpatient clinic were included in the study. Skin biopsy specimens of the patients who were histopathologically diagnosed with psoriasis, and had the appropriate characteristics for the study, were included in the immunohistochemical study. Those who had another autoimmune disease such as Type 1 diabetes mellitus, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, vitiligo, alopecia areata or encephalomyelitis were excluded. None of the patients included in the study, had any malignity or was being treated with anti-PD-1 molecules for malignity. Intact skin biopsy samples of healthy volunteers aged 19 to 60 years who did not have any disease were included in the study as the control group. All participants were informed about the study and their written consent was obtained prior to their enrollment in the study. Before starting the study, approval was received from the local ethics board of our hospital with the decision date 05.03.2018 and number 52.

The skin biopsy samples collected from the patients were put into formalin solutions. After formaldehyde fixation, the tissues were embedded in paraffin. Four cross-sections were taken from the paraffin blocks. One of the cross-sections was stained with H&E. The diagnosis of psoriasis was histopathologically confirmed by the H&E cross-sections.

Immunohistochemical Study Procedure

All cases were applied, a 1/250 dilution rate of anti PD-1 antibody (ab137132, abcam), 1/200 dilution rate of anti PD-L1 antibody (ab205921, abcam) and 1/100 dilution rate of anti PD-L2 antibody (ab200377, abcam). From the formalin-fixed paraffin blocks, 3-4-µ-thick sections were prepared on slides coated with poly-L-lysine.

The cross-sections were left overnight in a stove at 37-40 °C, and in the morning, they were kept in a stove at 65 °C for 45 minutes so that the paraffin could melt. The cross sections were then kept in xylol for 20 minutes to deparaffinize. They were rehydrated in alcohol and hydrated in distilled water.

In the immunohistochemical staining process that was carried out using a Leica Bond-Max automated immunohistochemical staining device, the slides were boiled in water containing a citrate buffer (pH 6.0) solution at 95-99 °C for 10 minutes to recover the antigen before applying the antibody. They were then left to cool at room temperature for 15-20 minutes. The slides were washed with distilled water, treated with 3% hydrogen peroxide for 15 minutes at room temperature to block endogenous peroxidase activity, and put in distilled water once more. Superblock was dripped on the cross-sections which were washed with PBS (0.01 M Phosphate Buffer Saline) and left for 3-5 minutes. Then, primary antibodies were dripped on the cross sections, which were left for 30-45 minutes. Following the PBS washing process, the cross-sections were kept at room temperature for 20 minutes by dripping biotinylated secondary antibodies, and then, washed with PBS for 5 minutes. Conjugated streptavidin enzyme was dripped on the cross-sections, which were kept at room temperature for 10 minutes, and washed again with PBS. Later, DAM chromogen was dripped, and the cross-sections were washed with distilled water. Following a preliminary staining with Harris Hematoxylin for 10 seconds, and washing with distilled water, the slides were dried by dipping into alcohol and coated with balm.

Analysis of the Immunohistochemical Study Results

Anti PD-1 and PD-L1 antibodies were applied on the cross-sections obtained from the prepared paraffin blocks. Cytoplasmic and membranous staining of PD-1 and PD-L1 was considered positive. The number of the stained immune cells was examined, counted and recorded for each case. Additionally, tissues were divided into two groups according to their PD-1 and PD-L1 positive immune cell counts: Tissues with low PD-1 and PD-L1 (+) immune cell counts (number of PD-1 and PD-L1 positive immune cell <10) and tissues with high PD-1 and PD-L1 (+) immune cell counts (number of PD-1 and PD-L1 positive immune cells ≥10)

Statistical Analyses

All statistical analyses were conducted using IBM Statistical Package for the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM SPSS Corp.). Categorical variables were presented as frequency and percentage. Visual methods, Kolmogorov–Smirnov test and Shapiro-Wilk test were used to evaluate if the numerical variables were normally distributed. Normally distributed variables were presented as mean (standard deviation, SD) and continuous variables which were not normally distributed were presented as median (interquartile range, IQR). Categorical variables were analyzed using chi-squared test or Fischer exact and numerical variables were analyzed using Student t-test or Mann–Whitney U test. Spearman and Pearson tests were used to evaluate the correlations of continuous variables. The level of statistical significance was accepted as $P < 0.05$.

Results

Among the patients in the study, 22 were male, 8 were female. Among the individuals in the control group, 13 were female, 2 were male. The mean age of the patients was 40.07 ± 16.99, the mean age of the control group was 44.13 ± 12.35, and there was no significant difference between the groups in terms of age ($P = 0.415$) (Table 1).

For all the samples studied, only the immune cells in the dermis showed positive staining with PD-1 and PDL-1. Epidermis did not show any staining. 86.7% of the psoriasis patients had high PD-1 (+) immune cell counts (Figure 1). In the control group, 40% of the tissues had high PD-1 (+) immune cell counts, which was significantly lower than the

Table 1. Basic characteristics of patient and control groups, comparison of PD-1 and PDL-1 results.

	Patients (N = 30)	Controls (N = 15)	P
Age (years, mean ± SD)	40.07±16.99	41.13±12.35	0.415
Gender (n (%)) Female Male	 8 (26.7) 22 (73.3)	 13 (86.7) 2 (13.3)	 <0.001
PASI (median (IQR))	5.4 (4.6-6.9)	-	-
Disease duration (years, median (IQR))	2 (1.5-9)	-	-
PD-1 positive cell number (n (%)) Low (<10) High (≥10)	 4 (13.3) 26 (86.7)	 9 (60) 6 (40)	 0.004
PDL-1 positive cell number (n (%)) Low (<10) High (≥10)	 17 (56.7) 13 (43.3)	 15 (100) 0 (0)	 0.002

IQR = interquartile range; PASI: Psoriasis Area and Severity Index; SD = standard deviation.

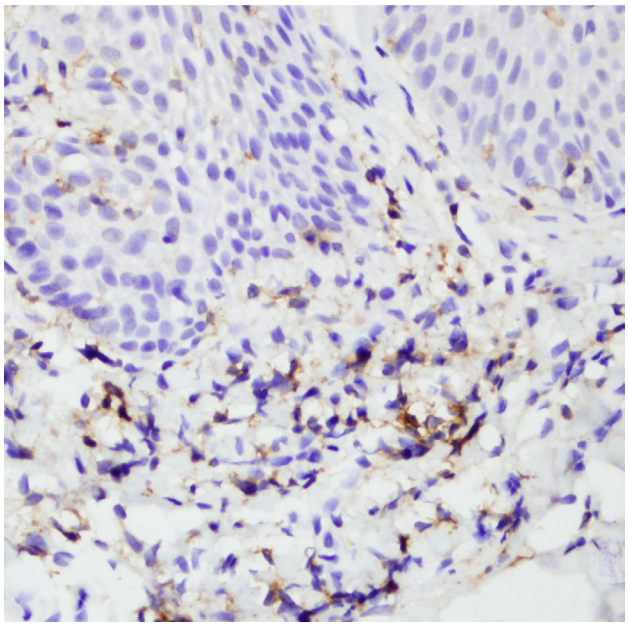

Figure 1. Positive staining of more than 10 cells is present in the immune cells in the upper dermis under the epidermis (PD-1, x400).

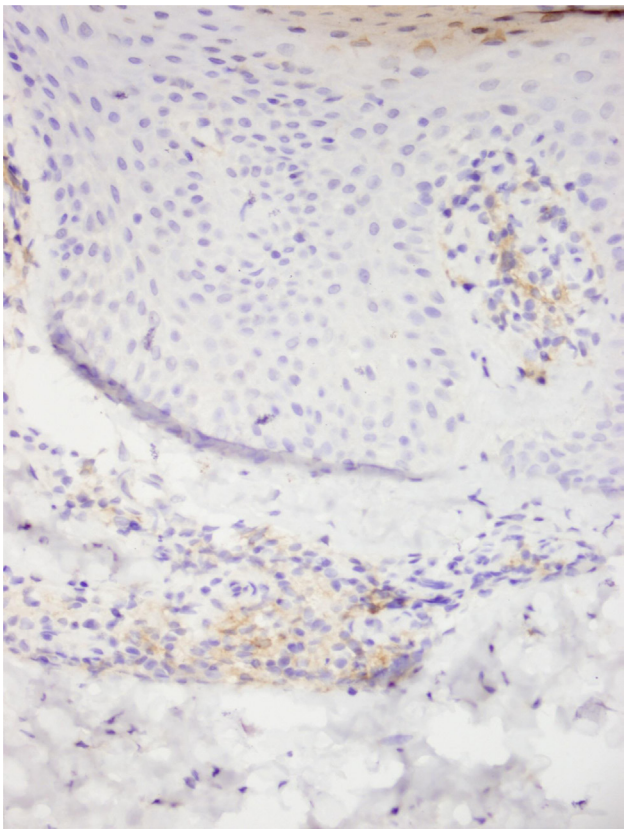

Figure 2. Positive staining is present in more than 10 cells in the immune cells of the upper dermis under the epidermis (PD-L1, x400).

psoriasis patients (P = 0.004). Similarly, the percentage of the tissues with high PDL-1 (+) immune cell counts was significantly higher in the psoriasis patients compared to healthy controls (43.3% versus 0%, P = 0.002) (Figure 2).

The median PASI score was calculated as 5.4 (IQR: 4.6-6.9) and the median disease duration was 2 years (IQR: 1.5-9). A negative and statistically significant correlation was detected between PASI scores and PDL-1 (+) immune cell counts (P = 0.033, r = -0.57). There was no significant correlation between PASI scores and PD-1 (+) immune cell counts (P = 0.51, r = -0.19). Disease duration did not correlate with the PD-1 (+) immune cell counts or PDL-1 (+) immune cell counts (P = 0.43, r = 0.29; P = 0.61, r = 0.19, respectively).

Among psoriasis patients, one patient had pustular psoriasis, one had guttate and one had palmoplantar psoriasis. All three patients had high PD-1 (+) immune cell counts however PDL-1 (+) immune cell counts differed with subtype. Patient with pustular psoriasis had high PDL-1 (+) immune cell counts whereas patients with guttate and palmoplantar psoriasis had low PDL-1 (+) immune cell counts.

Conclusions

PD-1 is an inhibitor receptor that is highly expressed in T lymphocytes. When the PD-1 pathway is activated, its inhibiting effect on the immune system emerges [10]. It was reported in previous studies that the PD-1 signal pathway regulates the production of cytokines such as INF, IL-2, IL-17, and TNF-α, thus regulating the axes of PD-1, Th1, and Th17. In murine models, PD-1 deficiency induced psoriasiform dermatitis while stimulation of the PD-1 pathway inhibited the Th-1/Th17-mediated cutaneous reactions [6,11].

High-affinity anti-PD-1 or anti-PD-L1 monoclonal antibodies that block the PD-1 and PD-L1 interaction may eliminate the control mechanism over T cells by reversing the immune control point. In recent years, anti-PD-1 antibodies have been used in cancer treatment, controlling tumor progression and mobilizing the immune system. Using anti-PD-1 antibodies for treatment has brought about some side effects. Skin toxicity is the most frequently observed side effect encountered in anti-PD-1 treatments. Some skin diseases such as vitiligo, photosensitivity, lichenoid eruption and psoriasis were triggered in patients, on whom the anti-PD-1 antibody was used. In the literature, lichenoid reactions, eczema, pruritus and vitiligo are reported as the most frequently encountered cutaneous side effects [7,9,12,13]. Psoriasis is a well-established side effect secondary to PD-1 and PD-L1 blockade, and the number of anti-PD-1-induced psoriasis cases in the literature continues to increase [8,14-17]. How the PD-1/PD-L1 pathway affects psoriasis or other skin diseases is unknown. In patients receiving anti-PD-1 therapy, PD-1 blockade may be inducing a pro-inflammatory Th-1/Th-17 response by increasing interferon-gamma, tumor necrosis factor-alpha (TNF-alpha), and IL-2, IL-6, and IL-17 [17].

A few studies have been conducted to investigate PD-1 and PD-L1 expression in the T lymphocytes in the peripheral blood of psoriasis patients. Bartosinska et al examined the PD-1 expressions in the CD4+ and CD8+ T-cells in the peripheral blood samples of the patients with psoriasis and psoriatic arthritis [18]. They concluded that the PD-1 expression in the CD4+ and CD8+ T lymphocytes of the patient group was significantly lower than the controls. The authors argued that reduced PD-1 expression may be responsible for immune dysregulation in the pathogenesis of psoriasis. In a later study of theirs, Bartosinska et al reported that there was no difference between psoriatic patients with and without PsoA in terms of CD4+ and CD8+ T lymphocytes PD-1 in peripheral blood, and both groups had lower CD4+ and CD8+ PD-1 levels than the healthy controls [19]. The authors stated that, regardless of the clinical type, mutual inflammatory pathways and mediators play a role in psoriasis. In contrast, Peled et al reported that the PD-1 expression in the T lymphocytes in the peripheral blood of psoriatic arthritis patients was significantly higher than the healthy controls [10]. They showed that this was correlated with the activity of arthritis. The authors argued that PD-1 could be a marker that shows psoriatic arthritis activity. Bommarito et al showed that Rheumatoid arthritis (RA) and psoriatic arthritis (PsoA) patients had an increased expression of PD1 on their CD4+ and CD8+ T cells in their peripheral blood and sinovial fluids [20]. The authors showed that IL-1β, IL-6, and TNF-α raised the soluble PD-1 levels, and that increased expression of soluble PD-1 interfered with the PD-1/PD-L1 pathway.

Few studies investigate the PD-1 and PD-L1 expression in the lesional skins of psoriatic patients and reported variable results. Kim et al showed that the PD-L1 expression in keratinocytes in psoriatic epidermis was significantly reduced compared to normal skin, pityriasis rosea, liken planus and allergic contact dermatitis epidermis samples [21]. The authors suggested that T cell activation was not suppressed due to the reduced PD-L1 and 2 in psoriasis. On the other hand, Jung et al. [20] reported that among the chronic plaque-type psoriatic patients, the group with a higher PD-1 in epidermis had a higher PASI score and more typical histopathological changes than the lower PD-1 expression group. Authors suggested that epidermal PD-1 upregulation in chronic plaque psoriasis is correlated with a more chronic and severe disease. They showed that guttate psoriatic patients with a longer disease duration had a lower dermal PD-1 expression than those with a shorter disease duration, and downregulated dermal PD-1 expression is correlated with a poorer prognosis in guttate psoriasis. Çetinözman, et al also found increased levels of PD-1 in the CD8+ T-cells in the epidermis and dermis of the skin biopsies taken from the lesions of 6 patients with psoriatic erythroderma [22].

In our study, we examined PD-1 and PD-L1 expression in the immune cells in the epidermis and dermis. There was no PD-L1 and PD-1 expression in the keratinocytes in psoriatic skin and healthy controls. In patient group, we found the PD-1 and PD-L1 expression in the immune cells in the dermis of psoriatic skin to be significantly higher in comparison to the healthy controls. Since PD-1 is an inhibitor co-receptor in the immune system, it may seem unreasonable that its expression increases in autoimmune diseases, whereas studies in autoimmune diseases including SLE and rheumatoid arthritis show that the PD-1/PD-L1 pathway is modulated under the permanent chronic inflammation conditions [5]. It is reported that T-cells express higher amounts of PD-1 in autoimmune diseases, neoplasms and chronic infections, limiting the protective immunity. In addition, since PD-1 is also a marker of the T-cell activation, it is not surprising to see that it is increased in psoriatic lesions [23].

In this study, we also evaluated the association of clinical features such as disease duration and PASI scores of psoriasis patients with PD-1 and PD-L1 expressions. Our results showed a significant negative correlation between PDL-1 (+) immune cell counts and PASI. PDL-1 (+) immune cell counts did not correlate with disease duration. PD-1 (+) immune cell counts did not correlate with PASI or disease duration. Few studies investigated the association of clinical features with PD-1 and PD-L1 expressions. In the study by Peled et al, no statistically significant correlation was shown among the percentage of PD-1 expressing T cells and PASI scores of patients but the percentage of PD-1 expressing T cells negatively correlated with psoriatic arthritis articular disease activity [10]. Jung et al showed that psoriasis patients with high epidermal PD-1 expression had higher PASI scores and longer disease durations [20]. However, dermal PD-1 expression and disease duration had an opposite relationship. Psoriasis patients with lower PD-1 expressions had longer disease durations. Dermal PD-1 expression did not correlate with PASI. These differences between the results of the studies may be due to the small number of patients included in the studies, the fact that the presence of psoriatic arthritis was not considered in every study, different sides of the PD-1 and PDL-1 positivity (epidermal or dermal area), and the low PASI scores of the patients included in the studies.

This study showed that, the PD-1 and PD-L1 expressions in immune cells in the lesioned skin samples of psoriasis patients are significantly higher than that in the skin samples of healthy controls. Previous studies and our study are preliminary studies with small numbers of patients with psoriasis. To the best of our knowledge, the present study is one the first investigations of the expression of PD-1/PD-L molecules in the immune cells in the lesioned skins of psoriasis patients. The role that the degradation of the PD-1/PD-L1 axis plays in psoriatic patients is backed by evidence. However, there is

not ample evidence and the results of the present study are not sufficient for a thorough understanding of the subject matter. For a better understanding of this subject, it is necessary to investigate the PD-1 and PD-L1 expressions in psoriasis patients with reference to the studies conducted with more patients and employing more detailed research designs. We believe that this may unearth new pathways in the pathogenesis of psoriasis. In the future, medications modulating the function of the PD-1/PD-L1 pathway may emerge as a promising option in the treatment of psoriasis.

PD-1 and PD-L1 expressions in the immune cells in the psoriatic skin are significantly higher in comparison to the healthy controls. Increased immune system activity in the psoriatic lesions may trigger PD-1 and PD-L1 expression in the immune cells of the lesioned skin.

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