INVESTIGATING THE ROLE OF THE PD-1/PD-L1 PATHWAY IN CUTANEOUS LUPUS ERYTHEMATOSUS

PhD thesis outlines

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1. Introduction

The various clinical presentations of cutaneous lupus erythematosus (CLE) are well-recognized in clinical practice. The clinical presentation, development of systemic symptoms, disease course, and response to therapies differ between the two primary forms of CLE, namely, subacute cutaneous lupus erythematosus (SCLE) and a chronic form, discoid lupus erythematosus (DLE). Another important distinction between the two forms is that immune-checkpoint-inhibitor (ICI) therapy can induce SCLE, which is unusual in the case of DLE. Based on this clinical observation, the possibility of different involvement of the target molecules of these therapies in DLE and SCLE was raised. ICI therapies are commonly used pharmaceuticals in the field of cancer treatment. They target the programmed cell death receptor 1 (PD-1) and its ligand (PD-L1). PD-1 is expressed on the surface of activated T-cells, while PD-L1 is expressed on various tissue cells, such as keratinocytes (KCs). The connection between PD-1 and PD-L1 causes the inhibition of T-cell activation. Therefore, it is essential to maintain peripheral tolerance. In recent years, the soluble variants of these two molecules, sPD-1 and sPD-L1, also gained interest as possible modulators of the PD-1/PD-L1 axis. Besides the importance of this axis in cancerous diseases, its role in autoimmunity has also been suggested. Numerous papers have been published investigating the different aspects of

the involvement of the PD-1/PD-L1 axis in systemic lupus erythematosus (SLE). Yet, data about the PD-1/PD-L1 pathway in CLE is lacking.

2. Objectives

Study I: To evaluate the expression of PD-1 and PD-L1 in the skin of DLE and SCLE patients

The main objective of our first study was to investigate the expression patterns of PD-1 and PD-L1 proteins in the skin of patients with DLE and SCLE, both compared to healthy skin and each other. We aimed to discern any differences in expression levels between these two forms of CLE and healthy skin, shedding light on potential differences specific to each subtype. Additionally, we wanted to explore the PD-1 and PD-L1 expression in the skin of idiopathic SCLE and SCLE induced by ICI therapy. Understanding these variations could provide valuable insights into the underlying mechanisms of CLE pathophysiology.

Study II: To evaluate the levels of sPD-1 and sPD-L1 in the sera of DLE and SCLE patients

Our second study aimed to address three primary objectives regarding soluble sPD-1 and sPD-L1 levels in patients with DLE and SCLE compared to healthy

individuals. Firstly, we wanted to investigate any alterations in sPD-1 and sPD-L1 levels in DLE and SCLE patients compared to healthy controls, providing insights into potential dysregulation of immune responses in these conditions. Secondly, we aimed to discern differences in serum levels of sPD-1 and sPD-L1 between DLE and SCLE, elucidating potential distinctions in these two subtypes of CLE. Lastly, we aimed to explore the relationship between sPD-1/sPD-L1 levels and the activity of skin symptoms in DLE and SCLE, potentially uncovering associations with disease activity and severity.

3. Methods

Methods of study I.

1.

Ten skin biopsy specimens from nine individuals were collected from patients with active skin lesions. Among them, four had SCLE, two triggered by PD-1-inhibitors (PD1-SCLE), and two without such history (non-PD1-SCLE). Four had DLE, and one had a history of SLE developing toxic epidermal necrolysis (TEN)-like symptoms. SCLE patients showed widespread symptoms in sun-exposed areas, while DLE patients exhibited involvement in facial areas, scalp, and ears. Antinuclear antibodies (ANA) were detected in all SCLE and TEN-like lupus cases. Non-PD1-SCLE patients tested positive for

anti-SSA, one also for anti-SSB and RF, and one PD1-SCLE patient for SSA. Anti-dsDNA was positive in the TEN-like lupus case. DLE patients showed no antibody positivity. Histological analysis revealed varying stages of interface dermatitis in all cases, with DLE cases showing more robust follicular plugging and perifollicular lymphocytic infiltrate compared to SCLE.

2.

The LEICA Bond Max automated staining system and Bond Polymer Refine Detection Kit were used for immunohistochemical staining. The antibodies used targeted PD-1, PD-L1, CD3, CD4, CD8, Granzyme B (GB), CD123, and CD163 antigens. Manufacturer instructions were followed, although PD-L1 staining involved a unique antigen retrieval method. Slides were scanned at x40 magnification. Human tonsils and healthy skin were used as positive and negative controls, respectively. KC PD-L1, CD123, and CD163 stainings were assessed across the whole sample. Positive KC PD-L1 staining was defined as >1% of cells with moderate to high-intensity staining. The quantity of PD-1, PD-L1, CD3, CD4, CD8, and GB-positive lymphocytes was assessed by two pathologists in relevant areas of the sample. All staining procedures were performed at Semmelweis University's Department of Pathology and Experimental Cancer Research.

3.

A descriptive analysis of the immunohistochemical findings was performed to compare the various forms of CLE. However, cases 9 and 10 were excluded from this analysis due to the patient's involvement of multiple organs. Given the limited size of the sample, statistical tests were not conducted.

Methods of study II.

1.

Serum samples from patients with DLE, SCLE, and SLE were collected before treatment, along with samples from healthy controls. Diagnosis criteria were applied, excluding patients with concomitant autoimmune conditions. DLE was characterized by scarring plaques on the head and neck without autoantibodies, while SCLE exhibited annular lesions in sun-exposed areas with anti-SS-A/Ro antibodies. Skin symptoms were observed in all SLE patients, with varying organ involvement. ANA and anti-SS-A/Ro antibodies were prevalent in SCLE and SLE patients. Anti-dsDNA antibodies were specific to SLE.

2.

CLASI is a validated scoring system for assessing activity and damage in CLE. CLASI-A evaluates erythema, scale/hypertrophy, mucosal involvement, recent hair loss, and non-obvious scarring alopecia. CLASI-D measures

dyspigmentation, scarring/atrophy/panniculitis, and evident scarring alopecia across 13 regions, with scores ranging from 0 to 3, except for scarring alopecia, scored from 0 to 6. Higher scores signify greater disease activity or damage.

3.

Serum samples from CLE and SLE patients were analyzed for specific antibodies: ANA, dsDNA, SS-A/Ro, SS-B/La, and phospholipids (Cardiolipin and β2-GPI). ANA levels were assessed using a HEp-2 substrate immunofluorescence assay, while dsDNA, CL, and β2-GPI antibodies were quantified via chemiluminescent immunoassays. Commercial ELISA kits were used to detect SS-A/Ro and SS-B/La antibodies. All assessments followed manufacturer instructions.

4.

Serum sPD-1 and sPD-L1 levels were measured using ELISA kits designed for human PD-1 and human/cynomolgus monkey PD-L1/B7-H1 (Quantikine, R&D Systems). Monoclonal antibodies specific to human PD-1 and human B7-H1 were pre-coated on 96-well plates, with samples and standards added in seven-point serial dilutions. Controls for each experiment were included. Enzyme-linked monoclonal anti-PD1 and polyclonal anti-PD-L1 antibodies were used for detection, followed by TMB substrate. Measurements were taken at

450 nm using a microplate reader (Multiskan® EX, Thermo Fisher Scientific). Concentrations (pg/ml) were estimated using a 4-point-fit calibration curve. Detection ranges were 15.6-1,000 pg/ml for sPD-1 and 25.0-1,600 pg/ml for sPD-L1, with minimum detectable amounts of 3.27 pg/ml and 4.52 pg/ml, respectively.

5.

The Shapiro-Wilk test confirmed non-normal distribution for sPD-1 and sPD-L1 serum levels. Thus, the Mann-Whitney U-test was employed for two-group comparisons. Spearman's rank correlation coefficient assessed connections between sPD-1, sPD-L1, and CLASI-A. A p-value <0.05 signified statistical significance.

4. Results

Results of study I.

PD-L1 expression was observed in epidermal KCs across all samples, contrasting with healthy skin. KC PD-L1 expression was lower in DLE than in SCLE (65% vs. 5%). Dermal inflammatory cell analysis revealed CD3+ T cells in the superficial dermis, with follicular localization in DLE. DLE showed higher median values for CD4, GB, PD-1, and GB+/CD8+ ratio compared to SCLE. CD123+ pDCs were present in all cases, with clustering in DLE. CD163+ histiocyte numbers varied. In non-PD1-SCLE,

GB+ cell presence in the dermis was greater than in PD1- SCLE. KC PD-L1 expression, GB+ cytotoxic T-cell number, and GB+/CD8+ ratio increased over time in TEN-like lupus cases.

Results of study II.

1.

In the DLE, SCLE, SLE, and HC groups, the median serum levels of sPD-1 were 225,35 pg/mL, 200,97 pg/mL, 420,12 pg/mL, and 177,01 pg/mL, respectively. Remarkably, sPD-1 serum levels were significantly higher in SLE patients than in HCs ($p=0.002$). No statistically significant distinctions were found between the DLE and SCLE groups when comparing them to each other ($p=0.933$) or to the HC group. Nevertheless, compared to the SLE group, the DLE and SCLE groups also showed significant differences in sPD-1 ($p=0.002$ and $p=0.004$, respectively).

2.

Regarding the serum concentrations of sPD-L1, the median values in the DLE, SCLE, SLE, and HC groups were 53.52 pg/mL, 66.4 pg/mL, 76.55 pg/mL, and 64.1 pg/mL, respectively. Notably, the DLE group showed a significant decrease in sPD-L1 serum levels compared to the HC group ($p=0.009$). Nevertheless, no significant differences were observed between the SCLE and SLE

groups compared to the HC group. Additionally, a significantly lower sPD-L1 level was detected in the DLE group compared to the SCLE and SLE groups (p=0.027 and p=0.003, respectively).

3.

The median CLASI-A scores for both DLE and SCLE patients did not reveal any statistically significant differences between the two groups (p=0.18). Additionally, no significant correlation was observed between serum levels of sPD-1, sPD-L1, and CLASI-A scores in either the DLE or SCLE groups.

5. Conclusions

Study I.

1. In our study, we first investigated the potential role of the PD-1/PD-L1 axis in CLE.
2. Alterations of the PD-1/PD-L1 pathway appear to play a role in CLE pathogenesis.
3. Lower KC PD-L1 expression was observed in the chronic form of CLE (DLE) compared to the subacute form (SCLE).
4. Clinicopathological and immunohistochemical resemblances were established between PD-1-inhibitor-induced SCLE and non-PD-1-inhibitor-

induced SCLE, although some subtle distinctions were also identified.

Study II.

1. This is the first study investigating the sPD-1 and sPD-L1 in the context of CLE.
2. The serum levels of sPD-L1 were notably lower in the DLE group compared to the HCs, SCLE, and SLE groups.
3. In our study, we observed no statistically significant correlation between the activity of skin symptoms and the levels of sPD-1 and sPD-L1.

Collectively, the results of the two studies suggest that the insufficient inhibitory impact of PD-L1 and sPD-L1 on T-cell activity could possibly promote the chronic form of CLE.

6. Publications

Publications directly related to this thesis

1. **Király Z**, Szepesi Á, Sebestyén A, Kuroli E, Rencz F, Tóth B, Bokor L, Szakonyi J, Medvecz M and Hidvégi B. Immunohistochemical Study of the PD-1/PD-L1 Pathway in Cutaneous Lupus Erythematosus. Pathol. Oncol. Res. 2022 28:1610521

IF: 2,874

2. **Király Z**, Nagy E, Bokor L, Kovács A, Marschalkó M, Hidvégi B. The Possible Clinical Significance of a Decreased Serum Level of Soluble PD-L1 in Discoid Lupus Erythematosus, but Not in Subacute Cutaneous Lupus Erythematosus—A Pilot Study. J. Clin. Med. 2023 12, 5648
IF: 3,9

Publications not directly related to this thesis

1. **Király Z**, Róbert L, Joura MI, Hidvégi B. Dermatoskopie von granulomatösen und Autoimmunerkrankungen der Haut [Dermoscopy of granulomatous and autoimmune skin diseases]. Dermatologie (Heidelb). 2023 Apr;74(4):243-249. German.
IF: 0,8

2. **Király Z**, Kovács A, Medvecz M, Róbert L, Bokor L, Kuroli E, Szepesi Á, Marschalkó M, Hidvégi B. A lupus erythematosus panniculitis lefolyásának jellegzetességei 17 betegünk retrospektív vizsgálata alapján [Characteristics of the course of lupus erythematosus panniculitis in a retrospective analysis of 17 patients]. Orv Hetil. 2023 Feb 5;164(5):172-178. Hungarian.
IF: 0,6

3. Hidvégi B, **Király Z**, Marschalkó M. A cutan lupus bőr vagy szisztémás autoimmun betegség? [Cutaneous lupus erythematosus, skin or systemic autoimmune disease?]. Bőrgyógyászati és Venerológiai Szemle. 2021 97(3):120-127. Hungarian
IF: -

4. Hidvégi B, Horváth N, Kovács A, **Király Zs**, Marschalkó M, Holló P. Myositis specifikus antitest diagnosztika dermatomyositisben – mi haszna a klinikai gyakorlatban [Myositis specific antibodies – What is the clinical usefulness?]. Bőrgyógyászati és Venerológiai Szemle. 2023; 99(1):55-59. Hungarian
IF: -