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

PhD thesis

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List of Abbreviations

ACLE	acute cutaneous lupus erythematosus
ANA	anti-nuclear antibody
APS	antiphospholipid syndrome
BAFF	B-cell activating factor
β2-GPI	β2-glycoprotein I
CCLE	chronic cutaneous lupus erythematosus
CHLE	chilblain lupus erythematosus
CL	cardiolipin
CLASI	Cutaneous Lupus Disease Area and Severity Index
CLE	cutaneous lupus erythematosus
CTLA4	Cytotoxic T-lymphocyte associated protein 4
CXCL	chemokine (C-X-C motif) ligand
PD1-SCLE	PD-1-inhibitor-induced subacute cutaneous lupus erythematosus
DLE	discoid lupus erythematosus
DNA	deoxyribonucleic acid
dsDNA	double-stranded deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EULAR/ACR	European League Against Rheumatism/American College of Rheumatology
GB	granzyme B
ICLE	intermittent cutaneous lupus erythematosus
ICI	immune-checkpoint-inhibitor
IFN	interferon
IL	interleukin
HC	healthy control
Non-PD1-SCLE	non-PD-1-inhibitor-induced subacute cutaneous lupus erythematosus
JAK	Janus kinase
KC	keratinocyte
LE	lupus erythematosus
LEP	lupus erythematosus panniculitis

LET	lupus erythematosus tumidus
mAb	monoclonal antibody
MxA	myxovirus resistance protein A
NET	neutrophil extracellular trap
NK-cell	natural killer cell
NSCLC	non-small cell lung cancer
PBMC	peripheral blood mononuclear cell
PD-1	programmed death receptor 1
PD1-SCLE	PD-1-inhibitor-induced subacute cutaneous lupus erythematosus
pDC	plasmacytoid dendritic cell
PD-L1	programmed death ligand 1
PD-L2	programmed death ligand 2
RF	rheumatoid factor
PRR	pattern recognition receptor
RNA	ribonucleic acid
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCLE	subacute cutaneous lupus erythematosus
SLE	systemic lupus erythematosus
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
Sm	Smith
sPD-1	soluble PD-L
sPD-L1	soluble PD-L1
SS-A	Sjögren's syndrome-related antigen A
SS-B	Sjögren's syndrome-related antigen B
STAT	signal transducer and activator of transcription
TEN	toxic epidermal necrolysis
TLR	toll like receptor
TNF-α	tumor necrosis factor - α
UV	ultraviolet

1. Introduction

1.1. Lupus erythematosus in general

Lupus erythematosus (LE) encompasses a spectrum of various autoimmune conditions that may affect different kinds of internal organs (systemic lupus erythematosus (SLE)) or only the skin (cutaneous lupus erythematosus (CLE)). Moreover, the diverse clinical subtypes of CLE are accompanied by varying risks for developing systemic disease. The diagnosis of SLE is based on the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria. [1]

1.1.1. Epidemiology

The diverse clinical forms of CLE contribute to a lack of comprehensive data on its epidemiology. In a population-based Swedish cohort study, the incidence of CLE was found to be 4/100000 cases per year, while in the Danish population, a yearly incidence rate of 2.74/100000 was observed.[2,3] The female: male ratio varies between 3:1 and 4:1 based on different population-wide studies.[2–4] The initial onset of CLE typically occurs in adulthood, with a mean age ranging between 43 and 54 years.[2,5] According to the study of Petersen et al., 24% of CLE patients have a prior diagnosis of SLE, and 8% of CLE patients develop SLE in a 15-year follow-up period.[3] Other authors have reported varying rates of developing systemic symptoms, ranging from 5% to 25%; however, the duration of the follow-up period differs among these studies.[5–7] The skin is one of the most frequently affected organs in SLE, with approximately 70% to 85% of the patients experiencing some form of skin involvement during the disease.[8]

1.1.2. Clinical forms of cutaneous lupus erythematosus

The distinct clinical forms of CLE are categorized by the Düsseldorf classification shown in Table 1. Based on these criteria, the subtypes of CLE are acute CLE (ACLE), subacute CLE (SCLE), chronic CLE (CCLE), and intermittent CLE (ICLE). CCLE is further

divided into discoid lupus erythematosus (DLE), lupus erythematosus panniculitis/lupus profundus (LEP), and Chilblain lupus erythematosus (CHLE).[9]
The two primary clinical subtypes, SCLE and DLE, may take work to differentiate clinically in some cases. However, it's important to note that the prognosis of these two subtypes is markedly different. Moreover, the therapeutic response also differs between the two, with DLE often showing resistance to therapy.

Table 1. The Düsseldorf classification of CLE, according to Kuhn et al.[9]

Acute cutaneous lupus erythematosus (ACLE)
Subacute cutaneous lupus erythematosus (SCLE)
Chronic cutaneous lupus erythematosus (CCLE) - Discoid lupus erythematosus (DLE) - Lupus erythematosus profundus/panniculitis (LEP) - Chilblain lupus erythematosus (CHLE)
Intermittent cutaneous lupus erythematosus (ICLE) - Lupus erythematosus tumidus (LET)

1.1.2.1. Acute cutaneous lupus erythematosus

The two clinical forms of ACLE are localized and generalized ACLE. Localized ACLE manifests as a butterfly rash (or malar rash) on the malar area and may only affect the skin temporarily. This symptom can precede the subsequent development of systemic symptoms in weeks or months. Approximately half of the SLE patients present with a malar rash at the initial diagnosis.[10] At first, the malar rash appears as small, symmetric, erythematous macules and papules on the central facial region, sometimes accompanied by scaling. Over time, these lesions may merge, resulting in the characteristic clinical presentation of the butterfly rash. The butterfly rash typically involves the central area of the face while sparing the nasolabial folds and the mental area.[10] The generalized form of ACLE manifests as widespread, symmetric, erythematous macules and papules, sometimes accompanied by itching. (Figure 1a) Typically, the rash appears on sun-exposed areas of the body, such as the shoulders, arms, V-area, trunk, hands, and feet.

Importantly, these lesions do not tend to leave scars or cause dyspigmentation after they heal.[11] The likelihood of developing SLE in the presence of ACLE skin symptoms is approximately 80%. In the case of systemic involvement, antibodies such as anti-nuclear antibody (ANA), anti-dsDNA, and anti-Sm are often present.[10,12]

Beyond these two forms, another extremely rare, life-threatening form of ACLE is toxic epidermal necrolysis (TEN)-like lupus. (Figure 7 a,g) TEN-like lupus is a hyperacute, vesicobullous form of ACLE, but unlike in TEN, no provoking drug can be identified, and some lupus-specific antibodies are present. The prognosis of TEN-like lupus is better compared with TEN. Differentiating TEN and TEN-like lupus might be challenging in clinical practice.[13]

1.1.2.2. Subacute Cutaneous Lupus Erythematosus

SCLE comprises approximately 15% of the various subtypes of CLE.[5] Lesions can be present in two distinct morphological variations; however, they might combine in some cases. The annular form appears as polycyclic, scaling papules and plaques with a clear center, while the papulosquamous form presents as psoriasiform lesions. Symptoms typically develop on sun-exposed areas, such as the arms, shoulders, V-neck, upper back, and dorsal side of the palms; however, the facial area and the scalp are rarely affected. (Figure 1d) [10] Dyspigmentation or vitiligo-like residuals are expected after the resolution of the SCLE lesions, but they do not leave scars. To date, there is no specific classification system for diagnosing SCLE; however, some progress has been made in this direction.[14]

Among all SCLE cases, approximately 10-15% of patients develop SLE, while mild systemic manifestations are common in the remaining cases.[10,15] SS-A antibodies are relatively prevalent in SCLE, present in approximately 60% of cases, while SS-B is detected only in 10% of all SCLE cases.[16]

In addition to UV light, the provoking effect of many pharmaceuticals has been a subject of interest in recent years. Among these, antifungals (terbinafine), calcium-channel blockers (diltiazem), anticonvulsants (phenytoin), and tumor necrosis alpha inhibitors might induce SCLE in susceptible individuals.[17] Lately, immune checkpoint inhibitors (ICIs) have been associated with the induction of SCLE.[14]

1.1.2.3. Chronic Cutaneous Lupus Erythematosus

1.1.2.3.1. Discoid Lupus Erythematosus

DLE is the most common variant of CLE and constitutes the majority of CCLE cases. The skin symptoms appear over the shoulders (particularly on the scalp, neck, face, and ears) in 80% of the cases (localized DLE), whereas in 20% of the patients, lesions extend to the trunk and extremities (generalized DLE). The active stage of DLE is characterized by well-demarcated, erythematous, or livid plaques with infiltration, central atrophy, and scarring. (Figure 1b) [10] Hyperkeratosis and telangiectasias are frequently present. Following the resolution of DLE lesions, scarring and hyperpigmentation often persist, and cicatricial alopecia of the scalp may develop. In recent years, diagnostic criteria for DLE have been developed, as shown in Table 2. These criteria encompass atrophic scarring, localization in the conchal bowl, a predilection for the head and neck, dyspigmentation, follicular hyperkeratosis/plugging, and a color spectrum ranging from erythematous to violaceous.[18,19]

The chance of developing SLE is around 5% in the case of localized DLE, while in generalized DLE, this probability ranges from 20-40%. Many individuals with DLE lesions experience a slowly progressing disease that can lead to scarring and changes in skin pigmentation.[20] SS-A antibodies are present in approximately 25% of DLE patients, while SS-B is found in a maximum of 10% of DLE cases.[16] According to one study, the number of therapy-refractory cases is high among generalized DLE patients.[20]

1.1.2.3.2. Lupus Erythematosus Panniculitis

Among all CLE cases, approximately 1-3% of these patients suffer LEP, making LEP a relatively rare form of CLE. The disease is characterized by the appearance of deep, painful, erythematous subcutaneous nodules on the thighs, gluteal region, upper arms, or face. (Figure 1e) In some cases, DLE lesions might cover the LEP. Subsequently, lipoatrophy and deep scars develop. The concomitant presence of DLE or SLE in LEP patients is common. In a cohort study in 2020, the presence of DLE or SLE was observed

in most of the LEP patients (62%).[21] In a recent study conducted by our group, concomitant SLE was observed among LEP patients in 41% of the cases.[22] During the progression of the disease, the chances of developing SLE is 10-15%.[21]

Table 2. Classification criteria of discoid lupus erythematosus by Elman et al.[18]

Clinical feature	Points assigned
Atrophic scarring	3
Location in the conchal bowl	2
Preference for head and neck	2
Dyspigmentation	1
Follicular hyperkeratosis/plugging	1
Erythematous to violaceous in color	1

1.1.2.3.3. Chilblain Lupus Erythematosus

CHLE is a rare form of CCLE characterized by itchy, painful, livid, edematous plaques typically presenting in acral localization (fingers, toes, ears, nose). (Figure 1f) Ulceration and fissures might develop in some of the cases. According to the diagnostic criterion for CHLE - evaluated in 1994 – both major and two out of three minor criteria must be present for the diagnosis. One of the major criteria is that the lesions induced by cold develop on acral areas. The other major criterion is the presence of the typical picture seen by histopathology or direct immunofluorescence. Minor criteria are concomitant SLE or DLE symptoms, response to anti-lupus therapy, and negative cryoprotein and cold agglutinin studies results.[23]

Just like LEP, CHLE often presents together with DLE or SLE. According to Hedrich et al., the progression into SLE during CHLE is 18%.[24]

1.1.2.4. Lupus Erythematosus Tumidus

Intermittent cutaneous lupus erythematosus (ICLE), or lupus erythematosus tumidus (LET), is a distinct, rare subtype of CLE. Characteristics of LET are oedematose, erythematous, annular, or semi-annular plaques with induration but without the

involvement of the epidermis. (Figure 1c) Extreme sun exposure sensitivity is a typical feature of this disease; lesions generally exacerbate in the summer. Once the lesions are resolved, this subtype does not tend to scarring or dyspigmentation. The prognosis of LET is usually good, and association with SLE is infrequent; there are only a few reported cases.

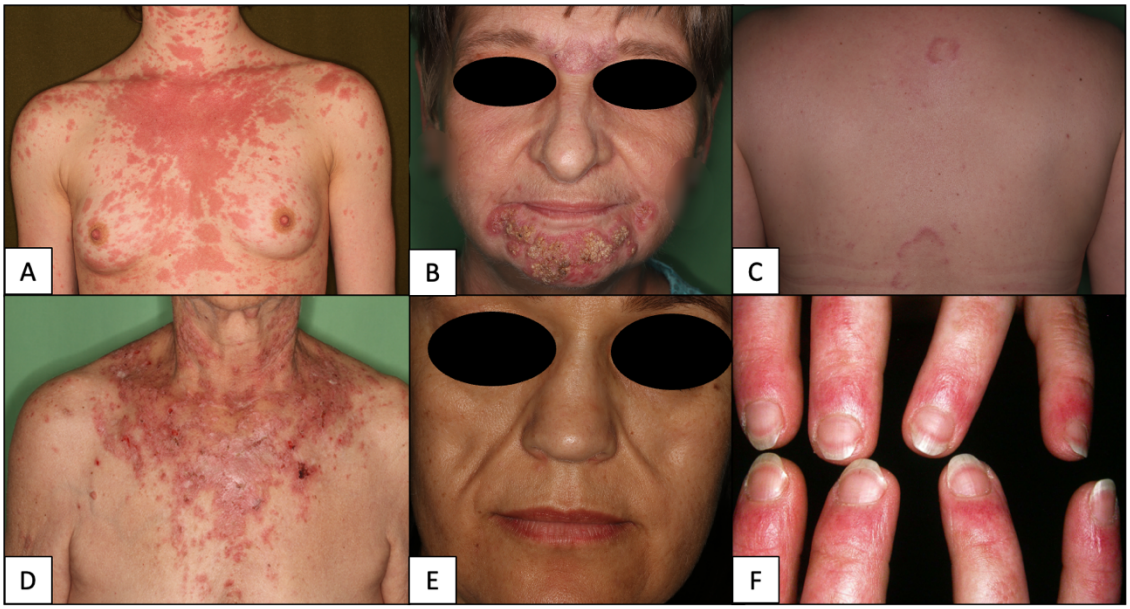


Figure 1. The broad clinical spectrum of CLE. Clinical pictures of ACLE (A), DLE (B), lupus tumidus (C), SCLE (D), LEP (E) and CHLE (F)

1.1.3. Pathomechanism

The complex development of CLE skin lesions is influenced by a combination of genetic susceptibility and the interplay of environmental factors. These factors trigger molecular mechanisms and activate both the innate and adaptive immune system, leading to the manifestation of CLE skin lesions in affected individuals. (Figure 2)

1.1.3.1. Genetic factors

Recently, numerous genetic networks that might contribute to the development of CLE were identified. Key genes involved in inflammation, apoptosis, and immunity pathways have been revealed as potential contributors to CLE pathogenesis.[25] Detailed

knowledge about genetic factors of CLE subtypes might help clarify key differences between subtypes of CLE, such as prognosis and response to treatments.

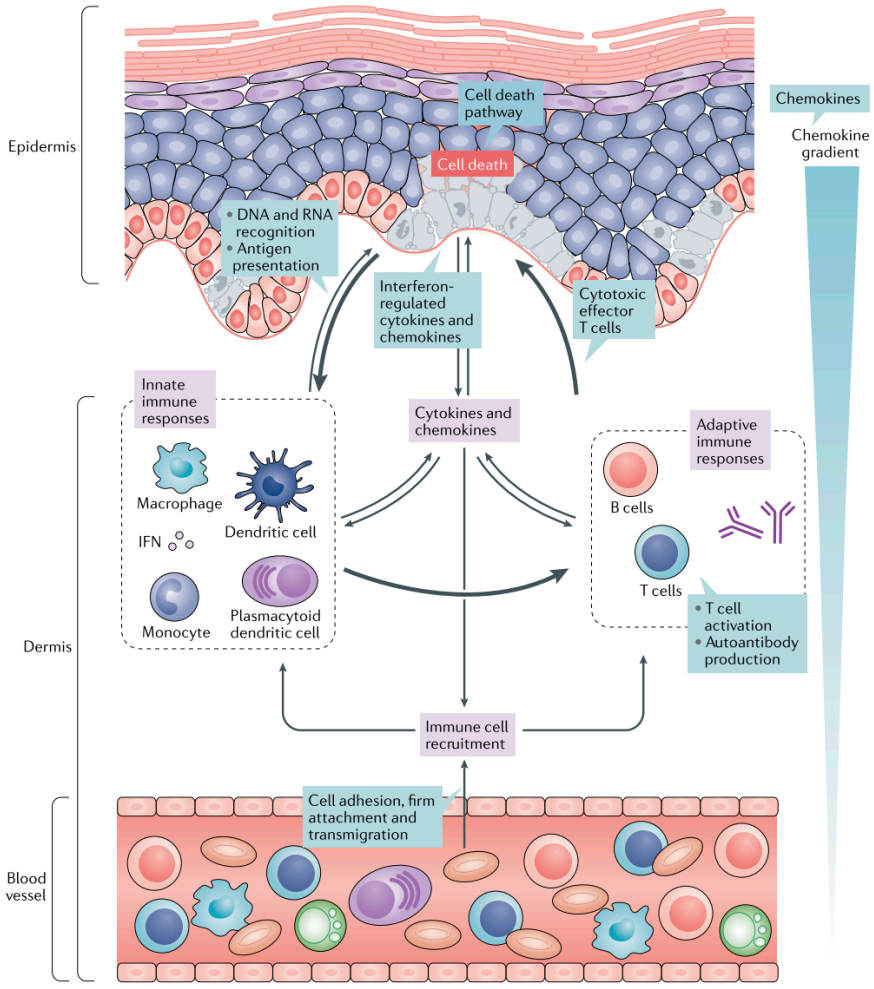


Figure 2. The pathomechanism of CLE.[26] Typically, a triggering event, such as UV-light exposure or drug administration, initiates keratinocyte apoptosis, coupled with impaired clearance of apoptotic cells. This results in the entry of nuclear components into the tissue, activating both the innate and adaptive immune systems and subsequently developing a self-amplifying cascade.

1.1.3.2. Environmental factors

The role of ultraviolet (UV) light as a triggering factor of CLE is widely known. Photosensitivity is a common feature of both CLE and SLE patients. In a study from 2013, they found that 72% of CLE patients suffer photosensitivity, and in the case of 93,4%,

the lesions develop on sun-exposed areas.[5] UV-irradiation might induce the apoptosis of keratinocytes (KCs) in many ways, such as the damage of the mitochondria through reactive oxygen species, the activation of apoptotic membrane receptors (without their ligands), or direct DNA damage.

Besides UV radiation, smoking has long been known to worsen the symptoms of lupus erythematosus. Smokers among DLE patients tend to have skin symptoms with higher activity than those of never or past smoker patients. Due to smoking, the level of inflammatory cytokines is increased; the higher activity of neutrophil granulocytes induces the production of neutrophil extracellular traps (NETs), and phototoxic agents are induced, all together triggering the symptoms of lupus erythematosus. Notably, smoking might reduce the efficacy of antimalarials in CLE; however, more recent studies found no influence of smoking regarding the plasma level of antimalarials.[27,28]

Among CLE subtypes, SCLE might be triggered by many other drugs, such as Ca-channel inhibitors, angiotensin convertase enzyme inhibitors, proton pump inhibitors, thiazide-type diuretics, statins, or terbinafine. With the rise of biological agents in recent years, many monoclonal antibodies (mAbs) were identified as a triggering factor of SCLE, such as tumor necrosis factor - α (TNF-α)-inhibitors and immune-checkpoint inhibitor (ICI) molecules. According to the systematic review by Bolton et al., approximately 50% of mAb-induced SCLE cases are due to TNF-α-inhibitors, such as adalimumab, infliximab, and etanercept, while 25% of cases are due to ICI-s, like nivolumab or pembrolizumab.[14] Nivolumab and pembrolizumab both inhibit the programmed death receptor 1/ programmed death ligand 1 (PD-1/PD-L1) pathway. Therefore, they enhance the activity of T-cells, which might cause various autoimmune side effects, including SCLE.

1.1.3.3. Innate immune system

The various triggering factors mentioned above subsequently lead to the damage of KCs within the epidermis, thereby inducing apoptosis in these cells. Additionally, the accumulated apoptotic cells subsequently undergo necroptosis, releasing proinflammatory agents and potential autoantigens.[29]

Uncleared nuclear debris originating from dying cells might induce the heightened production of pro-inflammatory cytokines within KCs primarily via pattern recognition receptors (PRRs). Upon PRR activation, KCs produce extensive levels of type I and III interferons (IFNs) (especially IFN-κ and IFN-λ). Additionally, several other proinflammatory mediators such as various cytokines and chemokines (e.g., CXCL9, CXCL10, CXCL11), TNFα, and B-cell activating factor (BAFF) are generated. The release of interferons sets in motion an autocrine feedback loop that amplifies the pro-inflammatory cytokine production capacity of the affected KCs, primarily through the JAK-STAT signaling pathway. It is highly plausible that the dysfunction of the innate immune system – particularly concerning the type I IFN pathway – plays a central role in the pathogenesis of LE.

The pivotal role of plasmacytoid dendritic cells (pDCs) in the pathogenesis of CLE was first revealed in 2001 by Farkas et al.[30] Characterized by their resemblance to plasma cells, pDC-s represent a distinct population of dendritic cells known for their excessive production of type I IFN-s (particularly IFN-α/β). Notably, previous research has observed the accumulation of pDC-s within the skin of LE patients in contrast to their absence in the skin of healthy individuals.[30] It is believed that the initial high number of pDC cells is attributed to the inflammatory microenvironment characterized by various factors such as cytokines, complement fragments, and adhesion molecules, which are absent in healthy skin. Necroptotic cell-derived DNA/RNA immunocomplexes, when bound to CD32 on pDCs, undergo endocytosis and subsequently engage toll-like receptor 7 (TLR7) or TLR9 receptors in endosomes, triggering signaling pathways for type I and III IFN production.[31] Beyond the generation of IFN-s, pDC-s also potentially release other proinflammatory cytokines, such as interleukin-6, BAFF, and TNF-α. Collectively, these cytokines play an essential role in orchestrating T-, B- and natural killer cell activities.[32] Importantly, type I IFN-s, mainly INF α, can further induce the production of proinflammatory agents such as CXCL9 and CXCL10 through the JAK-STAT signaling pathway, which is highly active in CLE skin. CXCL9 and CXCL10 molecules subsequently facilitate the recruitment of more cytotoxic T-lymphocytes and pDC-s into the skin. This cascade of events generates the constant activation of the innate and adaptive immune system, thus establishing an ongoing and self-perpetuating interplay.

1.1.3.4. Adaptive immune system

T lymphocytes, comprising the main component of the infiltrate, are attracted to the lesion site via their CXCR3 receptor, which binds to CXCL10 expressed by KCs and other skin cells induced by type I IFNs. Wenzel et al. previously found that the immune response in CLE is a Th1-biased immune response, although the role of cytotoxic T-cells is non-negotiable.[33] Lesional T-cell-generated cytotoxic agents induce the death of KCs, especially basal KCs, amplifying cell death and releasing immunostimulatory debris, reinforcing the self-perpetuating cycle.

Among T-cells, specific B-cell subsets are also found in CLE skin. The inflammatory microenvironment perpetuates the activation of B-cells, which adds to the continuous inflammation of the skin.[34] Antigen-presenting cells, keratinocytes, and T-cells can all activate B-cells. Subsequently, clonal expansion of B-cells follows, and they develop into antibody-producing plasma cells. These antibodies bind to the nuclear agents coming from the dead KCs and facilitate the immune response.[35]

Recently, the role of B-cells in different types of CLE skin has been extensively investigated. In their study, Abernathy-Close et al. found that the presence of CD20+ B-cells in DLE skin is more robust than in other forms of CLE.[36] A similar approach was conducted by de Vos et al., who observed more prominent B-cell infiltration in DLE skin than in SCLE.[37]

1.1.4. Diagnosis

The diagnosis of CLE is primarily based on the evaluation of the clinical presentation (as described previously) and the histopathological features, often complemented by direct immunofluorescent measurements and immunohistochemistry. In recent years, dermoscopy has also been recognized as a helpful tool to support the diagnosis of LE.[38] The assessment of complete blood count, immunoserological parameters, complement measurements, and internal organ involvement is necessary for any patient suspected of having LE. This evaluation is essential to confirm or exclude the diagnosis of SLE.

1.1.4.1. Histopathology

The histopathological picture of CLE typically reveals interface dermatitis, characterized by a mononuclear cell infiltrate at the dermo-epidermal junction, the degeneration of the basal cell layer, perivascular and periadnexal lymphocytic infiltration, hyperkeratosis, and mucin depositions.[10] The dermatopathology seen in LE skin lesions is quite uniform among the different clinical forms. It is rarely used to distinguish between subtypes but rather to support the diagnosis of CLE. However, some characteristic features might be observed. In SCLE, mild epidermal atrophy might be present, while the hyperkeratosis and the periadnexal infiltrate are less pronounced than in DLE. DLE skin lesions tend to demonstrate a more prominent hyperkeratosis, follicular plugging, and deeper extent of the infiltration to the dermal area (Figure 3.) than SCLE skin lesions. In LEP, the inflammation of the subcutaneous tissue is also present, sometimes accompanied by hyaline necrosis and calcium deposits. [21,22] In the rare form of lupus tumidus, the high amount of mucin deposits and the involvement of mainly the superficial periadnexal and perivascular areas might be characteristic.[10] The histopathological picture of ACLE is usually not as striking as seen in other forms of CLE.[16]

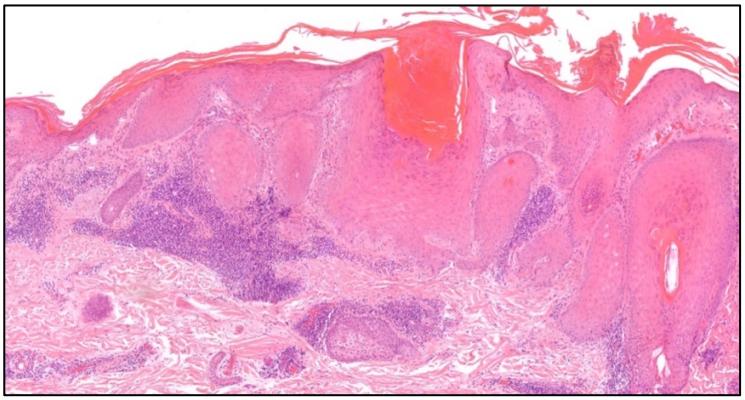


Figure 3. The histopathological picture of one of our DLE patients. (Magnification: ×5)

1.1.4.2. Direct immunofluorescence

The lupus band test is a tool to assess the localized immunoglobulins in the dermo-epidermal junction in lesional and non-lesional sites of the skin in both SLE and CLE patients. According to this technique, IgG, IgM, IgA, and C3 deposits present as a

homogenous, thready, or stippled band in the dermo-epidermal junction. A recent study evaluating the sensitivity and specificity of the lupus band test found that this tool is less sensitive (17,6%) but highly specific (98,8%) for LE. The lupus band test was found to have a positive predictive value of 92,8% and a negative predictive value of 58,2%.[39] false negative cases are unlikely due to the high specificity and positive predictive value. This testing method is optional in diagnosing LE; however, it can support the diagnosis, especially in challenging cases.

1.1.4.3. Immunohistochemistry

In recent years, immunohistochemical parameters of CLE skin lesions have gained attention as valuable indicators to address differential diagnostic challenges, distinguish between clinical subtypes, and aid in selecting appropriate therapeutic approaches.
In the study of Wenzel et al., the domination of T-cells in the inflammatory infiltrate was found in all CLE skin samples, regardless of the clinical subtype. This group also observed that CLE subtypes with scarring or fibrosing skin (DLE and LEP) present with more CD8+ T-cells in the infiltrate than CD4+ T-cells. Conversely, the dominance of CD4+ T-cells over CD8+ T-cells was observed in SCLE and lupus tumidus.[40]
The presence of CD123+ pDCs is a prevalent characteristic across the various CLE subtypes, and it is associated with the subsequent presence of myxovirus resistance protein A (MxA) induced by IFN-alpha. The expression of MxA is characteristic in LE skin; however, the staining's extent and unique localization can differentiate between subtypes. In DLE and SCLE, the same pattern of MxA distribution is seen in the lesional epidermis, the follicular epithelium, and the inflamed perivascular and periadnexal areas. However, the staining is noticeably milder in SCLE than in DLE. In LEP, characterized by lobular panniculitis, a prominent MxA staining can be seen in the subcutaneous tissue. This feature, together with CD123 staining, is important in differentiating LEP from subcutaneous panniculitis-like T-cell lymphoma.[37,41]
Recently, immunohistochemical analysis has gained theoretical and practical significance regarding therapeutic choices of the future. According to the study by de Vos et al., multiplex immunohistochemical analysis of the various pathogenic pathways in the skin

samples of CLE patients could support selecting appropriate targeted treatment options.[37]

1.1.4.4. Immunoserology

The immunoserological assessment of suspected LE patients is a cornerstone of the diagnostical algorithm since it aims to evaluate the degree of systemic involvement.

ANA positivity in a titer of at least 1:80 is an entry criterion for SLE according to the 2019 EULAR/ACR criteria.[1] In systemic involvement, positivity often occurs in even higher (1:160, 1:320) titers, while high titer positivity is less likely to present in CLE without systemic involvement.[42]

The dsDNA antibodies tend to be highly specific for SLE, and they are also indicators of the disease activity since higher levels of dsDNA are observed in case of SLE flares and lower levels or absence during remission.[10,16] Anti-Sm antibodies are also specific for SLE, although present in only a quarter of SLE cases.[10,16] Anti-histone antibodies tend to be associated with drug-induced SLE.[16]

SS-A and SS-B antibodies might also be observed in SLE; however, they are not specific to the disease. SS-A antibodies are relatively common among SCLE patients, with 60% showing positivity, while only 25% of DLE patients have SS-A positivity. Regarding SS-B and anti-U1 ribonuclear protein, they are detected in only 10% of both SCLE and DLE patients.[16]

Antiphospholipid antibodies, including lupus anticoagulant antibodies, anticardiolipin antibodies (aCL), and β2-glycoprotein I (β2-GPI) antibodies, can also present in the context of antiphospholipid syndrome (APS) since the most common underlying factor of secondary APS is SLE.[16]

1.1.4.5. Other blood parameters

Other laboratory findings, such as leukopenia, thrombocytopenia, hemolytic anemia, and elevated sedimentation rate, are often seen in SLE but are less likely to present in CLE. The low levels of C3 and C4 complements are also common findings in SLE, while complement levels are usually in between normal ranges in CLE.[16]

1.1.5. Treatment

The therapeutic algorithm of CLE has been summarized by many groups in the past years. The first line of these algorithms is usually the use of topical corticosteroids (or calcineurin inhibitors, depending on the availability in each region) combined with oral antimalarials, such as chloroquine or hydroxychloroquine (or quinacrine, if available). In case of high disease activity, oral corticosteroid therapy might be considered. In case of ineffectiveness, a second line of treatment might be considered, namely methotrexate, retinoids, or dapsone. In selected refractory patients, thalidomide therapy might be considered (depending on local availability). It is generally not suggested to use azathioprine, cyclosporin, cyclophosphamide, lenalidomide, intravenous immunoglobulin, belimumab, rituximab, or anti-TNF-α antibodies in the treatment of CLE without systemic involvement.[43–46]

Nowadays, therapies targeting the specific elements of the pathogenic pathways in CLE, such as pDCs, the JAK/STAT pathway, or type I-IFN receptor, are tested and developed. Furthermore, a novel tape RNA method might be employed in the future to assess the specific gene signature of the specific pathogenic pathways affected in CLE. This could support both diagnostics and aid in treatment choices in a non-invasive manner.[47]

1.2. The PD-1/PD-L1 axis in general

1.2.1. Structure and function of PD-1/PD-L1 axis

Programmed cell death protein 1 (PD-1, also known as CD279) is a co-inhibitory receptor that belongs to the CD28/CTLA4 family. The PD-1 receptor is expressed on the surface of T-cells, B-cells, natural killer (NK) cells, and thymocytes.[48] The inhibitory signal is transmitted after one of the two classical ligands of PD-1 (PD-L1 and PD-L2) is connected to the receptor. It is believed that the binding affinity of PD-1 to PD-L1 is generally more potent compared to binding to PD-L2; therefore, PD-L1 is known as the dominant ligand of PD-1.[49] The expression of PD-L1 is observed on various tissue

cells, including T and B cells, dendritic cells, mast cells, vascular endothelial cells, astrocytes and keratinocytes (KCs).[49] Among many cytokines, PD-L1 expression, can be induced by all three types of interferons (alpha, beta, and gamma); however, IFN-gamma appears to be the strongest inducer.[50]

PD-1 and PD-L1 are transmembrane proteins consisting of one extracellular domain, one transmembrane domain, and one cytoplasmic domain.[48,51] The cytoplasmic domain of PD-1 is connected to two tyrosinase-based signaling motifs (immunoreceptor tyrosinase-based inhibitory motif and immunoreceptor tyrosinase-based switch motif). The extracellular interaction between PD-1 and PD-L1 leads to the phosphorylation of these two motifs, which subsequently attenuates the T-cell response. The activation of PD-1 by PD-L1 modifies T-cell functions in many ways, such as their cytokine production, proliferation, and survival, all together inhibiting the T-cell response.[52,53]

Taken together, the PD-1/PD-L1 axis is crucial in maintaining peripheral tolerance and immune homeostasis. Previous research has shown the importance of this pathway in various levels of self-tolerance. In the experiments of Nishimura et al., the development of lupus-like symptoms or fatal autoimmune cardiomyopathy developed in PD-1 deficient mice.[54,55] Moreover, their research group reported that PD-1 deficiency may cause significant alterations in the maturation of thymocytes.[56] Additionally, the PD-1/PD-L1 pathway plays a vital role in pregnancy since suppressing maternal immunity toward paternal antigens is crucial. According to the study conducted by Guleria et al., the impairment of fetomaternal tolerance could be observed in a murine model after PD-L1 inhibition.[57]

In their pioneering study, Agata et al. demonstrated that the stimulation of T-cells with antigens induces their PD-1 expression, possibly to prevent autoimmune tissue damage.[58,59] In chronic viral infections and tumorous diseases, antigen elimination is insufficient, and T-cells are continuously stimulated with the antigen-expressing target cells. Subsequently, this persistent T-cell stimulation leads to the exhaustion of these antigen-specific lymphocytes.[60] In addition, exhausted T-cells are characterized by high expression of PD-1 receptors and loss of effector functions such as the production of cytokines, proliferation, or cytotoxic capacity.[60] The blockage of PD-1 or PD-L1 in chronic viral infections or cancerous diseases can recover the effector functions of T-cells with exhausted phenotype and, therefore, serves as a therapeutic option in these

diseases.[61] Importantly, it is well known that tumorous cells can express PD-L1 to silence the immune system and exert immune escape by reducing T-cell activity.[62] To resolve this, immune checkpoint inhibitors such as PD-1- or PD-L1-inhibitors are widely used in cancer treatment. According to clinical data, some tumors show better response for these therapies (eg. melanoma, non-small-cell lung cancer, gastric cancer), while others offer less or no response.[63]

In the past few years, the soluble variants of PD-1 and PD-L1 have been detected in the plasma and serum, further complicating the mechanism of this already complex pathway.

1.2.2. Soluble variants

A growing number of articles regarding the roles of soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) in mediating immune responses are published.

sPD-1 is known to be generated through alternative splicing mechanisms. Many splice variants of PD-1 have been discovered; however, only one can code sPD-1.[64]. This variant lacks the exon encoding the transmembrane domain of PD-1 but retains the exon corresponding to the extracellular domain, allowing it to bind PD-L1.[64] Due to this function, sPD-1 can disrupt the interaction between membrane-bound PD-1 and PD-L1, therefore increasing T-cell activity.

sPD-L1 can also originate from alternative splicing, although it might be the product of the metalloprotease-related shedding of membrane-bound PD-L1.[65,66] Functionally, sPD-L1 is akin to its membrane-bound counterpart; it can bind to PD-1, ultimately exerting an inhibitory effect on T-cell activity.

1.3. The role of PD-1/PD-L1 in autoimmunity

Immune checkpoints play a crucial role in T-cell and overall immune system function, maintaining a healthy baseline when these pathways are balanced. An overactive PD-1/PD-L1 pathway can result in insufficient immune activity, thereby promoting tumor development, while its impairment can lead to enhanced immune activity and, consequently, autoimmunity. (Figure 4)

The heterogeneous group of autoimmune disorders is characterized by the immune system's abnormal response against the body's tissues. Lately, the loss of peripheral tolerance has gained theoretical and practical significance in developing autoimmunity. As previously discussed, the PD-1/PD-L1 pathway plays a pivotal role in regulating the immune system's response to both self and foreign antigens. Therefore, it is essential for maintaining peripheral tolerance. The dysregulation or dysfunction of the PD-1/PD-L1 axis is believed to alter immune homeostasis and might lead to the loss of peripheral tolerance. Based on this idea, an increasing number of studies address the possibility that the disruption of the PD-1/PD-L1 axis – either by membrane-bound or soluble protein variations – influences the pathogenesis and prognosis of several autoimmune conditions such as rheumatoid arthritis, inflammatory bowel diseases, systemic sclerosis or SLE.

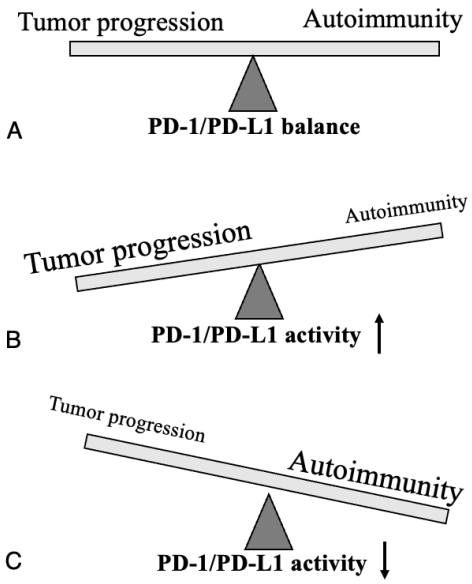


Figure 4. The simplified representation of the balance and imbalance of the PD-1/PD-L1 axis. In a balanced condition, neither tumor progression nor autoimmunity develops. **(A)** In case of enhanced activity of the PD-1/PD-L1 pathway, the chance of tumor progression increases. **(B)** The inhibition of the PD-1/PD-L1 pathway increases the risk of developing autoimmunity. **(C)** Modified figure after Szekanecz et al.[67]

1.4. The role of PD-1/PD-L1 axis in inflammatory skin diseases

According to experimental models, the PD-1/PD-L1 pathway seems to be involved in the interactions between KCs and lymphocytes. [68,69] Therefore, it could be involved in inflammatory skin diseases too. This axis had been investigated only in a few inflammatory skin conditions, such as psoriasis and oral lichen planus. In psoriasis, the expression level of KC PD-L1 was investigated by two separate groups. Kim et al. found lower KC PD-L1 expression in the lesional skin of psoriatic patients compared to the skin of healthy individuals.[70] In another study, no PD-L1 staining keratinocytes were observed neither in psoriatic skin, nor in the skin of healthy controls.[71] These contradictory findings might be attributed to the differences between antibodies, immunohistochemical protocols and laboratory devices. Concerning sPD-1 levels in psoriasis, one study observed no significant alteration compared to healthy controls, while sPD-L1 levels were not estimated in this study.[72] In case of oral lichen planus, one group found no PD-L1 staining KCs in the majority of their patients.[73]

1.5. The role of PD-1/PD-L1 axis in lupus erythematosus

The PD-1/PD-L1 axis seems to be involved in the complex, multifactorial pathogenesis of lupus erythematosus. In recent years, an emerging number of publications have investigated how these proteins are embedded into the system of the many signaling pathways affected in the development of SLE.

The two key pathogenetic pathways of interest in SLE are the toll-like receptor (TLR) signaling and the type I IFN signaling; both are highly active in SLE.[74,75] Nevertheless, these two pathways are also involved in regulating the PD-1/PD-L1 axis. TLR signaling induces the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the production of type I IFNs. Following this, type I IFNs trigger the activation of the signal transducer and activator of transcription 1 (STAT1). Both NF-κB and STAT1 function as transcription factors, governing the expression of PD-1 and PD-L1 on cell membranes.[50,76] Consequently, their roles are pivotal in the pathological mechanisms of the PD-1/PD-L1 axis in SLE.

Several research groups in SLE have explored the expression of PD-1 and PD-L1 on cell membranes. According to earlier publications, it seems that the expansion of some subsets of PD-1+ CD4 T follicular helper cells positively correlates with disease activity (indicated by the SLEDAI-2K score) in SLE.[77,78] In their pioneering study, Lee et al.

evaluated the immune profiling of peripheral blood mononuclear cells (PBMCs) in SLE patients and healthy individuals. They observed a higher concentration of PD-1+ and a lower concentration of PD-L1+ PBMCs in the blood of SLE patients compared to the HC group. Furthermore, after treatment with immunosuppressants, the changes in disease activity (indicated by the SLEDAI-2K score) were found to correlate negatively with PD-L1+ monocytes and positively with CD4 T cells and PD-1+ CD8 T-cells. [79]

The possible roles of sPD-1 and sPD-L1 in the imbalance of immune regulation in SLE have been recognized in recent years. One research group found elevated levels of both soluble proteins in SLE patients compared to HCs, while another observed this difference only in the case of sPD-1.[80,81] According to the study by Hirahara et al., there is a moderate positive correlation between the levels of sPD-1 and the activity of the disease (indicated by the SLEDAI-2K score).[80]

The clinical observation that ICI therapies might induce SLE development as a side effect also points toward the involvement of the PD-1/PD-L1 axis in the pathogenesis of SLE.[82,83] These pharmaceuticals enhance the immune system by blocking the connection between PD-1 and PD-L1. Importantly, this immune boost might cause the development of new-onset autoimmune diseases or trigger subclinical factors of autoimmune conditions in cases of susceptibility.

Besides SLE, SCLE is another disease on the spectrum of LE that might be induced by ICI therapy, according to case reports.[84–87] In the systematic review of Bolton et al., they found that ICI-s are the second most common cause of monoclonal antibody-induced SCLE. Notably, the ratio of SCLE cases among annual ICI inhibitor users was far higher than that of other monoclonal antibodies.[14] Based on these clinical observations, it is plausible that the disruption of the PD-1/PD-L1 axis influences the development of SCLE. Notably, the induction of DLE, which is a chronic form of CLE, by ICI-s is extremely rare — the reason why these pharmaceuticals tend to trigger SCLE, but not DLE is yet uncovered.

2. Objectives

While the clinical presentation and prognosis of DLE and SCLE are somewhat distinct, knowledge about differences in their pathomechanism is still being explored. Given the clinical observation that ICI therapy may induce SCLE in contrast with the sporadic development of DLE besides ICI therapy, it is plausible that these two diseases differ regarding the PD-1/PD-L1 axis. The pathologic activation of some T-cell-associated pathways by ICIs might cause the development of SCLE in susceptible individuals.
There are no studies investigating the PD-1/PD-L1 axis in CLE yet, although there is extended research on this pathway in SLE.

The present research aimed to investigate the PD-1/PD-L1 axis in CLE with special regard to the following questions:

1. Study I: To evaluate the expression of PD-1 and PD-L1 in the skin of DLE and SCLE patients
    a. Are PD-1 and PD-L1 proteins expressed differently in the skin of DLE (chronic form of CLE) and SCLE (subacute form of CLE) patients compared to healthy skin and each other?
    b. Are there any differences in the expression of PD-1 and PD-L1 in the skin between idiopathic and ICI-induced SCLE?

2. Study II: To evaluate the levels of sPD-1 and sPD-L1 in the sera of DLE and SCLE patients
    a. Are there any alterations in the sPD-1 and sPD-L1 levels in DLE and SCLE compared to healthy individuals?
    b. Are there any differences between the DLE and SCLE in the serum levels of sPD-1 and sPD-L1?
    c. Is there a relationship between sPD-1/sPD-L1 levels and the activity of skin symptoms in DLE and SCLE?

3. Methods

This study involving human participants was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was waived by the Regional Institutional Scientific and Research Committee of Semmelweis University, Budapest, Hungary (license number: 5/2021, date of approval: 24 February 2021).

All patient samples utilized in our research were obtained from individuals diagnosed with CLE or SLE at the Department of Dermatology, Venereology, and Dermatooncology of Semmelweis University. All the cases were confirmed by histopathology and Immunoserology. All samples were gathered from untreated patients during diagnostic procedures.

3.1. Methods of study I.

3.1.1. Clinical and histological characteristics of the study population

Ten skin biopsy specimens from 9 individuals were gathered for analysis. All selected samples were obtained from patients with active skin lesions at the time of the skin biopsy, and biopsies were consistently taken from these active lesions. Immunoserological parameters were in accordance with the expected profile for the respective clinical type of CLE in all instances. Among these selected patients, four were diagnosed with SCLE, out of which two were triggered by treatment with PD-1-inhibitors due to malignant melanoma (labeled as PD-1-inhibitor-induced SCLE or PD1-SCLE) – specifically nivolumab (6^{th} dose) and pembrolizumab (1^{st} dose). The remaining 2 SCLE patients did not have any SCLE-provoking drug in their history (labeled as non-PD-1-inhibitor-induced-SCLE or non-PD1-SCLE). Additionally, four patients were diagnosed with DLE, while one patient had SLE in her medical history and developed TEN-like lupus symptoms (without provoking drugs in her history). In the case of the latter patient, two separate skin biopsies were conducted at different time points due to the rapid

progression of skin symptoms: the first was taken at the onset of the symptoms, and the second was collected one week later when the skin detachment occurred.

The SCLE patients in this study typically presented with widespread symptoms in sun-exposed areas, whereas the involvement of the facial area, the scalp, and ears were characteristic for DLE patients. ANA was detected in all SCLE and the TEN-like lupus cases. Both idiopathic SCLE patients tested positive for anti-SSA, with one also testing positive for anti-SSB and rheumatoid factor (RF), while one PD1-SCLE patient tested positive for SSA. Anti-dsDNA was found to be positive in the TEN-like lupus patient. None of the DLE patients showed positivity for any antibody. Examination of the tissue samples through histological analysis showed varying stages of interface dermatitis in each case. A more robust follicular plugging and perifollicular lymphocytic infiltrate were found in the DLE cases compared to SCLE, where this localization was uncommon. The clinicopathological characteristics of the patients are summarized in Table 3.

Table 3. Clinicopathological characteristics of our patients.

Case No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9-10**
				Patient demographics					
Sex/age	M/78	F/71	F/72	F/77	M/43	M/37	F/31	F/45	F72
Diagnosis	PD1-SCLE (nivolumab 6th dose)	PD1-SCLE (pembrolizumab 1st dose)	Non-PD1-SCLE	Non-PD1-SCLE	DLE	DLE	DLE	DLE	TEN-like lupus
				Autoantibody positivity					
ANA	+	+	+	+	-	-	-	-	+
anti-SS-A/Ro	+	-	+	+	-	-	-	-	-
anti-SS-B/La	-	-	-	+	-	-	-	-	-
Anti-dsDNA	-	-	-	-	-	-	-	-	+
RF	-	-	-	+	-	-	-	-	-

3.1.2. Immunohistochemistry

The immunohistochemical staining process utilized an entirely automatized staining system, the LEICA Bond Max. The Bond Polymer Refine Detection Kit (LEICA) was used for antigen localization. The antibodies used targeted the following antigens: PD-L1 (DAKO, clone 22C3), PD-1 (Cell Marque, clone NAT105), CD3 (DAKO, clone A0452), CD4 (LEICA, clone NCL-L-CD4-368), CD8 (BioSB, clone EP334), Granzyme B (GB) (DAKO, clone Ret40f), CD123 (Cell Marque, clone 6H6), CD163 (LEICA, clone 10D6). All procedures adhered to the instructions provided by the manufacturer. Unique antigen retrieval was used for PD-L1 staining, involving a 25-minute treatment in a pressure cooker with DAKO retrieval solution (Target Retrieval Solution Low pH). The slides were incubated for 2 x 60 min with the primary antigen. Positive and negative controls were established with tonsil and healthy skin samples, respectively.

Stained slides were scanned at a magnification of x40 using a Pannoramic scan instrument (3D Histech, Budapest, Hungary) equipped with a Carl Zeiss objective (NA = 0.83; Carl Zeiss MicroImaging Inc., Jena, Germany). The relative proportion of the positive KCs and the number of inflammatory cells were determined on the scanned slides.

Assessment was conducted across the whole sample for KC PD-L1, CD123, and CD163 stainings. KC PD-L1 staining was deemed positive when moderate to high-intensity staining was present in >1% of the cells. CD123 positive pDCs were quantified in the dermis and graded as 1–5% (1+), 6–15% (2+), and 16–30% (3+). CD163-positive histiocytes in the dermis were evaluated as 1–20% (1+), 21–50% (2+), and >50% (3+). The quantity of the PD-1, PD-L1, CD3, CD4, CD8, and GB positive lymphocytes was evaluated by two independent pathologists in the most diagnostically relevant area of the sample (7 x 3–4mm wide) at x20 magnification in the dermis. All immunohistochemical staining was conducted in the Department of Pathology and Experimental Cancer Research, Semmelweis University.

3.1.3. Data analysis

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.

3.2. Methods of study II.

3.2.1. Clinical characteristics of the study population

Serum samples were obtained from 21 patients with DLE, 18 with SCLE, and 13 with SLE. All samples were collected before administering topical or systemic medication for lupus erythematosus. Additionally, sera from 20 healthy age- and sex-matched subjects were used as healthy controls (HCs). All samples were stored at -80 °C until use.

None of the DLE and SCLE patients were diagnosed with concomitant SLE or other systemic autoimmune conditions. However, all SLE patients fulfilled the 2019 EULAR/ACR criteria for the diagnosis of SLE. The diagnosis of CLE was established based on clinical characteristics and histological findings.

Further characterization into the subgroup of DLE was determined by the presence of scarring plaques distributed on the head and neck region, coupled with the absence of autoantibodies. On the other hand, SCLE was identified by the characteristic annular or papulosquamous lesions in sun-exposed areas, along with the presence of autoantibodies, primarily anti-SS-A/Ro.

Among the DLE patients, symptoms presented as localized DLE, and generalized symptoms were not observed in any of these cases. In the SCLE group, 13 patients displayed annular lesions, while five presented with psoriasiform symptoms. Remarkably, skin symptoms were exhibited in all SLE patients in this study, including 3 ACLE, 5 SCLE, 3 DLE, and two other CLEs. The activity of skin symptoms was assessed with the Cutaneous Lupus Disease Area and Severity Index (CLASI) score system at the time of the blood sample collection. The median values of CLASI-A scores were estimated in the DLE, SCLE, and SLE groups and were found to be 6 for all three patient groups. Mild organ manifestations were present in some of our CLE patients: 2 SCLE patients had arthritis, 1 SCLE patient had leukopenia, and one other proteinuria. Moreover, our SLE patients displayed varying organ manifestations of SLE besides their skin symptoms: 8 cases of arthritis, three proteinuria, five leukopenia, and one

thrombocytopenia. Hypocomplementemia was found in 8 among all the SLE cases. The median value of Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) scores in our SLE group was found to be 8. Concerning their antibody profiles, ANA tested positive in 4 out of 21 patients with DLE and 12 out of 18 with SCLE, with a dilution of 1:160. Anti-SS-A/Ro antibodies were identified in 3 patients with DLE, 11 with SCLE, and 7 with SLE. Additionally, anti-SS-B/La antibodies were found in 1 DLE patient, 10 SCLE patients, and 5 SLE patients. Anti-dsDNA antibodies were detected in 9 out of 13 SLE patients, while none of the DLE or SCLE patients tested positive for these antibodies. Moreover, two patients with DLE, 1 with SCLE, and 2 with SLE tested positive for aCL antibodies. Additionally, 1 DLE patient, 1 SCLE patient, and 4 SLE patients had anti-β2-GPI antibodies. Characteristics of the patient groups are shown in Table 4.

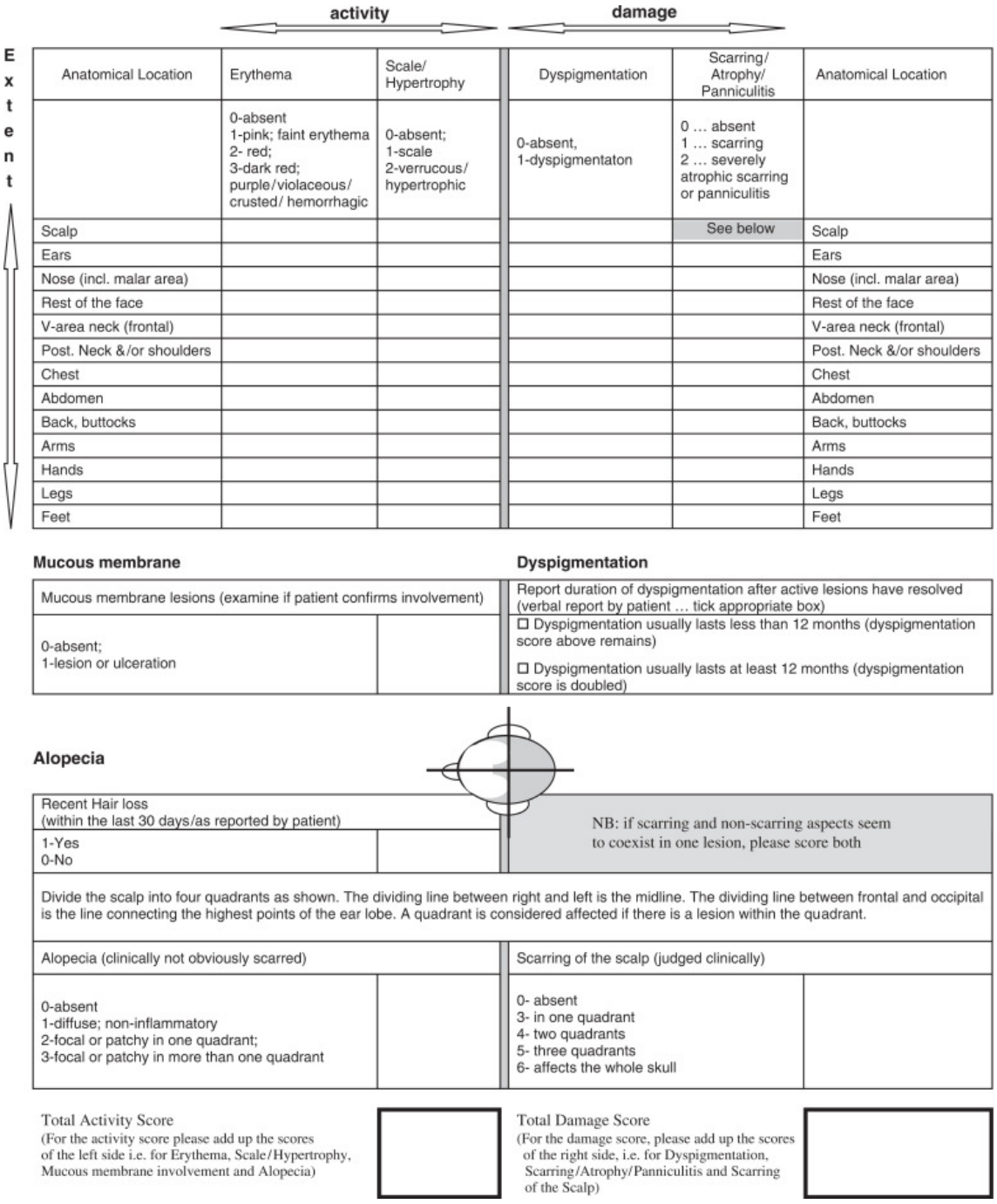
3.2.2. Cutaneous Lupus Disease Area and Severity Index (CLASI)

CLASI is a validated and reliable scoring system for measuring the activity and the resultant damage in CLE. Comprising two distinct domains, the CLASI encompasses measuring clinical signs indicative of activity and assessing damage.

The activity domain (CLASI-A) assesses the following clinical signs: erythema, scale/hypertrophy, mucosal involvement, hair loss within the preceding 30 days, and the extent of clinically non-obviously scarring alopecia. Parameters tested within the damage domain (CLASI-D) include dyspigmentation, scarring/atrophy/panniculitis and extent of clinically evident scarring alopecia. Covering 13 anatomical regions, both scales assign a value ranging from 0 to a maximum of 3 points for each region, except for the severity assessment of scarring alopecia lesions, which is scored on a scale of 0 to 6. Higher values indicate higher disease activity or damage. (Figure 5.)

Table 4. Characteristics of the patient groups**.**

	DLE	SCLE	SLE
Patient demographics			
Female/male ratio	19/2	13/5	9/4
Age (mean ± SD)	47.6 ± 14.5	62.9 ± 15	49 ± 17.9
Autoantibody positivity			
ANA	4/21	12/18	13/13
anti-SS-A/Ro	3/21	11/18	7/13
anti-SS-B/La	1/21	10/18	5/13
Anti-dsDNA	0/21	0/18	9/13
Anti-CL	2/21	1/18	2/13
anti-β2-GPI	1/21	1/18	4/13
Organ manifestations			
Rash	21/21	18/18	13/13
Oral ulcer	0/21	0/18	2/13
Alopecia	13/21	4/18	2/13
Arthritis	0/21	2/18	8/13
Proteinuria	0/21	1/18	3/13
Leukopenia	0/21	1/18	5/13
Thrombocytopenia	0/21	0/18	1/13
Hypocomplementemia	0/21	0/18	8/13
CLASI-A score (median, min-max)	6(1-14)	6(1-18)	6(3-21)
SLEDAI-2K score (median, min-max)	NA	NA	8(2-20)



activity

damage

Extent

Anatomical Location	Erythema	Scale/ Hypertrophy	Dyspigmentation	Scarring/ Atrophy/ Panniculitis	Anatomical Location
	0-absent 1-pink; faint erythema 2- red; 3-dark red; purple/violaceous/ crusted/ hemorrhagic	0-absent; 1-scale 2-verrucous/ hypertrophic	0-absent, 1-dyspigmentaton	0 … absent 1 … scarring 2 … severely atrophic scarring or panniculitis	
Scalp				See below	Scalp
Ears					Ears
Nose (incl. malar area)					Nose (incl. malar area)
Rest of the face					Rest of the face
V-area neck (frontal)					V-area neck (frontal)
Post. Neck &/or shoulders					Post. Neck &/or shoulders
Chest					Chest
Abdomen					Abdomen
Back, buttocks					Back, buttocks
Arms					Arms
Hands					Hands
Legs					Legs
Feet					Feet

Mucous membrane

Mucous membrane lesions (examine if patient confirms involvement)	
0-absent; 1-lesion or ulceration	

Dyspigmentation

Report duration of dyspigmentation after active lesions have resolved (verbal report by patient … tick appropriate box)

☐ Dyspigmentation usually lasts less than 12 months (dyspigmentation score above remains)

☐ Dyspigmentation usually lasts at least 12 months (dyspigmentation score is doubled)

Alopecia

Recent Hair loss (within the last 30 days/as reported by patient)	
1-Yes 0-No	

NB: if scarring and non-scarring aspects seem to coexist in one lesion, please score both

Divide the scalp into four quadrants as shown. The dividing line between right and left is the midline. The dividing line between frontal and occipital is the line connecting the highest points of the ear lobe. A quadrant is considered affected if there is a lesion within the quadrant.

Alopecia (clinically not obviously scarred)		Scarring of the scalp (judged clinically)	
0-absent 1-diffuse; non-inflammatory 2-focal or patchy in one quadrant; 3-focal or patchy in more than one quadrant		0- absent 3- in one quadrant 4- two quadrants 5- three quadrants 6- affects the whole skull	

Total Activity Score
(For the activity score please add up the scores of the left side i.e. for Erythema, Scale/Hypertrophy, Mucous membrane involvement and Alopecia)

Total Damage Score
(For the damage score, please add up the scores of the right side, i.e. for Dyspigmentation, Scarring/Atrophy/Panniculitis and Scarring of the Scalp)

Figure 5. The CLASI score system.[88]

3.2.3. Measurement of autoantibodies

Analysis was performed on serum samples from both CLE and SLE patients to detect the existence of particular antibodies, such as ANA, dsDNA antibodies, SS-A/Ro antibodies, Sjögren's syndrome-related antigen B (SS-B/La) antibodies, and phospholipids

(specifically Cardiolipin (CL) and β2-glycoprotein I (β2-GPI) antibodies). Levels of ANA were assessed using an indirect immunofluorescence method on a HEp-2 substrate, utilizing a commercially available kit (NOVA Lite HEp-2 IgG, Inova Diagnostics, San Diego, CA). A chemiluminescent immunoassay was employed to quantify antibodies against dsDNA, CL (both IgG and IgM), and β2-GPI (both IgG and IgM). All of these measurements were conducted by commercially available kits (QUANTA Flash dsDNA, QUANTA Flash aCL IgG, QUANTA Flash aCL IgM, QUANTA Flash β2GP1 IgG, QUANTA Flash β2GP1 IgM, Inova Diagnostics, San Diego, CA). Moreover, antibodies against SS-A/Ro and SSB/La were detected using commercially available enzyme-linked immunosorbent assay (ELISA) kits (QUANTA Lite SS-A, QUANTA Lite SS-B, Inova, San Diego). The assessment of all autoantibodies was conducted following the manufacturer's instructions.

3.2.4. Measurement of sPD-1 and sPD-L1

The serum levels of sPD-1 and sPD-L1 were measured utilizing commercially available ELISA kits specifically designed for human PD-1 and human/cynomolgus monkey PD-L1/B7-H1 (Quantikine, R&D Systems, Minneapolis, MN, USA. Both assays were performed according to the instructions provided by the manufacturer.

This approach used monoclonal antibodies specific to human PD-1, and human B7-H1 was used to precoat 96-well plates. Samples and standards were added to these plates using a seven-point serial dilution of the standards. A three-level control set for human PD-1 and human PD-L1 (Quantikine, R&D Systems, Minneapolis, MN, USA) was also used for each experiment. An enzyme-linked monoclonal anti-PD1 antibody for sPD-1 and a polyclonal anti-PD-L1 antibody for sPD-L1 were used to identify the particular binding protein, which was subsequently detected using a TMB substrate. The reaction was halted by adding 2 N H2SO4, and the plates were measured with a microplate reader (Multiskan® EX, Thermo Fisher Scientific, USA) at 450 nm. The PD-1 and PD-L1 concentrations (measured in pg/ml) were estimated using the 4-point-fit calibration curve derived from the standard dilutions. The detection range for sPD-1 was 15.6-1,000 pg/ml, with a minimum detectable amount of 3.27 pg/ml, while for sPD-L1, it was 25.0-1,600 pg/ml, with a minimum detectable concentration of 4.52 pg/ml.

3.2.5. Data analysis

The Shapiro-Wilk test was utilized to determine the normality of sPD-1 and sPD-L1 serum levels. The findings showed that the data did not have a normal distribution. Therefore, the non-parametric Mann-Whitney U-test was used to analyze the two-group comparisons. Using Spearman's rank correlation coefficient, the connections between sPD-1, sPD-L1, and CLASI-A were investigated. P-values less than 0.05 were deemed statistically significant, indicating a substantial difference.

4. Results

4.1. Results of study I.

CD3, GB, PD-1, and PD-L1 immunostainings of SCLE and DLE cases are shown in Figure 6. In all ten samples, the expression of PD-L1 in the epidermal KCs was observed, in contrast to healthy skin where no positive staining was detected. The results of PD-L1 immunostainings are summarized in Table 5. To compare the KC PD-L1 expression between the groups with DLE and the SCLE, the percentage of positive KCs was evaluated, and subsequently, the median values were calculated and compared through descriptive analysis. Compared to the SCLE group, the DLE group had lower levels of KC PD-L1 expression (65% vs. 5%, respectively) (Figure 7a). The medians of KC PD-L1 expression in the two non-PD1-SCLE samples were greater than those in the two PD1-SCLE samples (80% vs. 45%, respectively).[89]

The localization and composition of the dermal inflammatory cells were analyzed; the data is summarized in Table 5. In all instances, CD3+ T cells were found in the superficial dermis, whereas DLE cases also had follicular localization. Only two DLE cases and one PD1-SCLE case were reported to have more CD4+ cells than CD8+, and most samples had a CD4+/CD8+ ratio of <1. The median values for the following markers were greater in the DLE group than the SCLE group when dermal cells were counted: CD4 (115 vs. 61), GB (58 vs. 33), PD-1 (30.5 vs. 12), and GB+/CD8+ ratio (0.46 vs. 0.22) (Figures 7b–e). The two groups had no noticeable differences regarding the medians of other stained markers (CD3, CD8, CD123, CD163) or the PD-1+/CD3+ ratio. CD123-positive pDCs in the dermis were identified in all DLE and SCLE cases. Notably, in DLE cases, these cells tended to aggregate into clusters, a pattern absent in the SCLE samples. Dermal CD163+ histiocyte numbers varied greatly from 1+ to 3+. Data regarding the dermal immunostainings of the non-PD1-SCLE and PD1-SCLE samples are shown in Table 5. In all cases of non-PD1-SCLE, there was a greater presence of GB+ cells in the dermis compared to the cases of PD1-SCLE. Other markers showed a wide range of cell counts. In the consecutive samples from the patient with TEN-like lupus, there was a noticeable increase in KC PD-L1 expression, as well as in the number of GB+ cytotoxic T-cells and

the GB+/CD8+ ratio. Specifically, KC PD-L1 expression increased from 22 to 43, and the GB+/CD8+ ratio rose from 0.24 to 0.49 over time, as illustrated in Figure 8.[89]

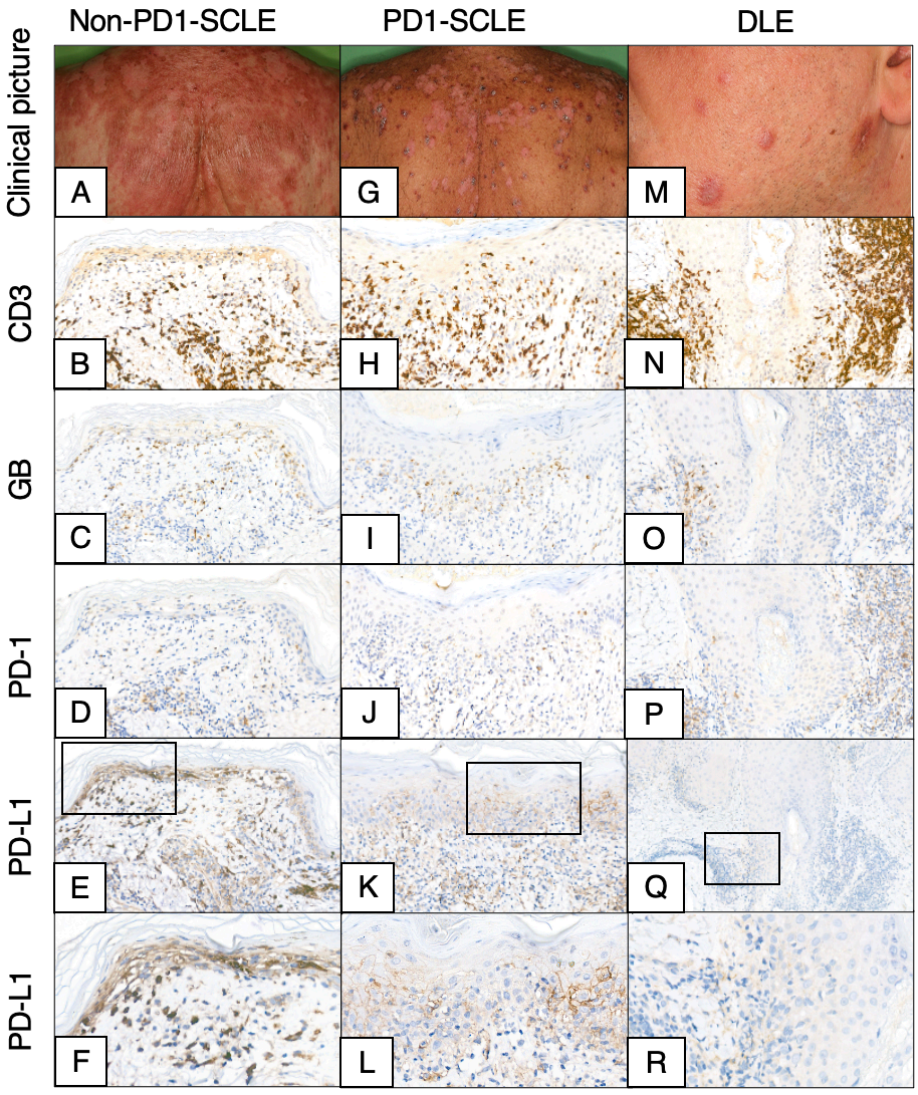


Figure 6. Clinical and immunohistochemical images of PD1-SCLE, non-PD1-SCLE, and DLE. The immunohistochemical images illustrate CD3, GB, PD-1, and PD-L1 stainings. (A–F) non-PD1-SCLE (G–L) PD1-SCLE (M–R) DLE [Magnifications: ×20, except: (Q), ×10; (F, L, R): ×40]. The immunohistochemical patterns in PD1-SCLE and non-PD-1-SCLE show many resemblances; however, more dermal GB+ cells can be observed in non-PD1-SCLE. DLE exhibits a more extensive and perifollicular dermal infiltrate compared to SCLEs. KC PD-L1 expression is present in all cases, but more remarkable staining is shown in SCLEs (irrespective of origin), while only mild staining is noted in DLE.[89]

Table 5. Results of PD-L1 immunohistochemistry. KC PD-L1 stainings were assessed across the entire sample, and they were deemed positive if there was moderate to high-intensity staining in more than 1% of the cells.[89]

Case no	Diagnosis	epidermis	dermis									
		KC PD-L1 (%)	CD3 (count/ field)	CD4 (count/ field)	CD8 (count/ field)	GB (count/ field)	GB/CD8 ratio	PD-1 (count/ field)	PD-1/ CD3 ratio	PD-L1 (count/ field)	CD123	CD163
1	PD1-SCLE	40	360	160	120	25	0.21	12	0.03	65	2+	2+
2	PD1-SCLE	50	80	18	66	13	0.20	8	0.10	68	1+	3+
3	Non-PD1-SCLE	80	280	102	230	54	0.23	48	0.17	105	2+	1+
4	Non-PD1-SCLE	80	145	20	78	41	0.53	12	0.08	28	3+	2+
SCLE group median		65	212.5	61	99	33	0.22	12	0.09	66.5		
5	DLE	5	260	190	78	48	0.62	55	0.21	38	1+	2+
6	DLE	5	400	235	140	68	0.49	21	0.05	90	3+	2+
7	DLE	5	110	10	90	35	0.39	20	0.18	22	2+	1+
8	DLE	30	230	40	180	79	0.44	40	0.17	90	3+	2+
DLE group median		5	245	115	115	58	0.46	30.5	0.18	64		
9	TEN-like lupus (early)	30	138	18	87	22	0.24	45	0.12	68	-	3+
10	TEN-like lupus (late)	70	108	26	90	43	0.49	27	0.20	13	-	3+

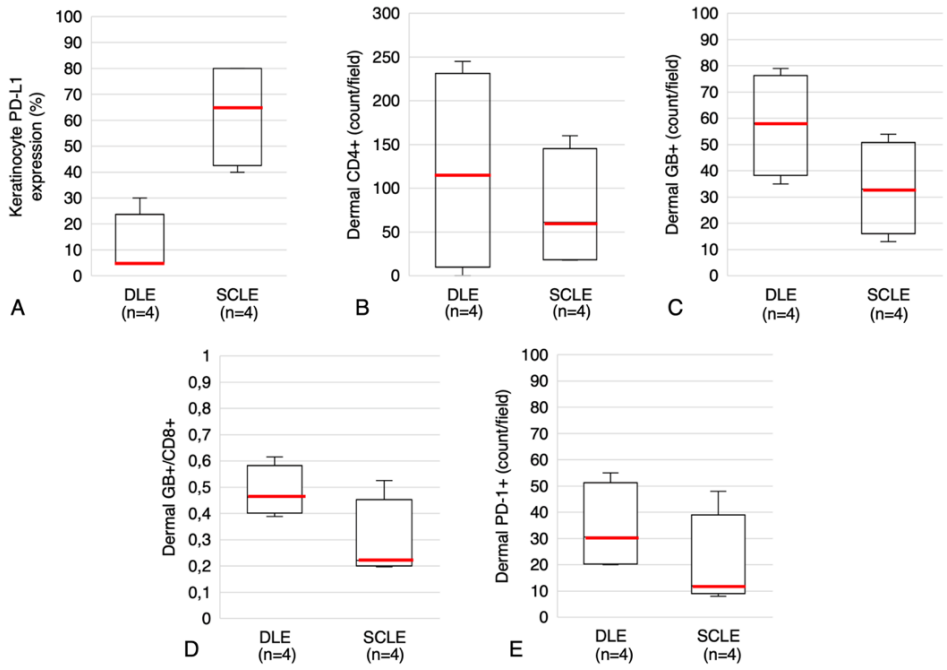


Figure 7. Boxplots illustrate the expression of KC PD-L1, counts of dermal CD4+, GB+, GB+/CD8+, and PD-1+ cells in both DLE and SCLE groups. (A) KC PD-L1 expression, (B) Dermal CD4+ cell count, (C) Dermal GB+ cell count, (D) Dermal GB+/CD8+ ratio, and (E) Dermal PD-1 cell count. Red lines indicate median values for each. The median values of these parameters exhibit variations between the DLE and SCLE groups.[89]

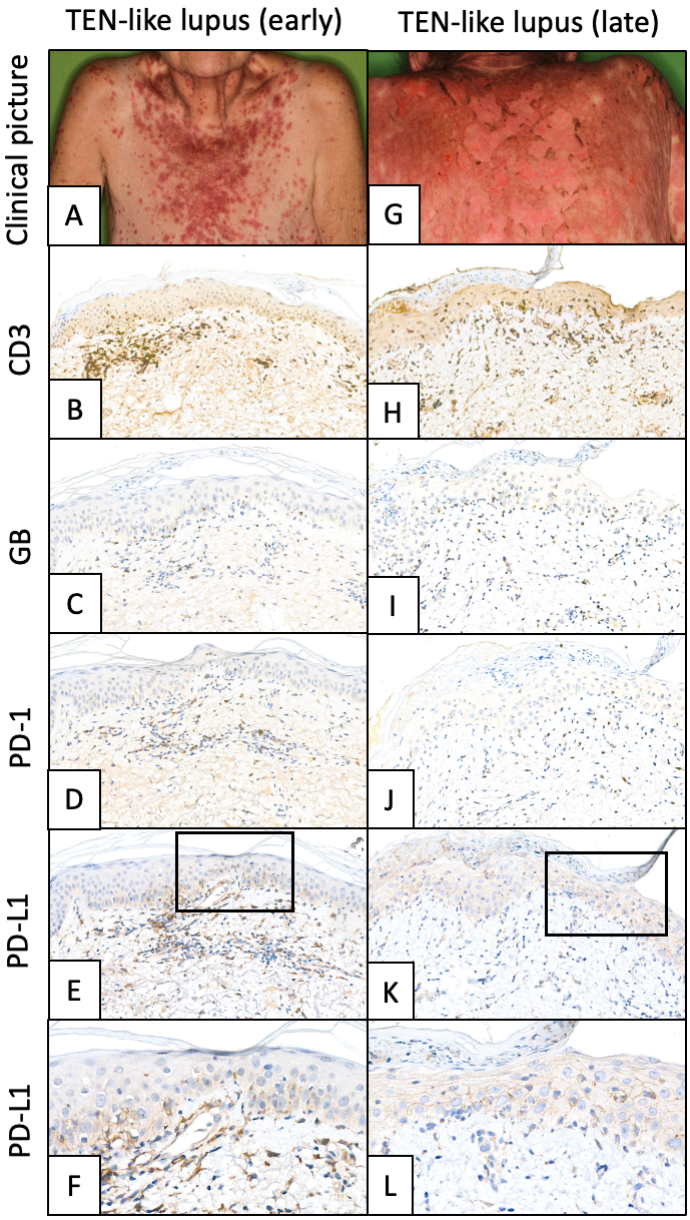


Figure 8. Representative clinical pictures and CD3, GB, PD-1, PD-L1 immunohistochemistry of earlier and later TEN-like lupus samples **(A-F)** TEN-like lupus, early sample **(G-L)** TEN-like lupus, late sample (Magnifications: 20x, except F, L: 40x) The activated T-cell numbers (GB+ T-cell numbers) and KC PD-L1 expression increased parallel to the progression of the clinical picture.[89]

4.2 Results of study II.

4.2.1. Comparison of serum sPD-1 levels

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) (Figure 9).[90]

4.2.2. Comparison of serum sPD-L1 levels

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 both the SCLE and SLE groups (p=0.027 and p=0.003, respectively), as depicted in Figure 10.[90]

4.2.3. Correlations between serum sPD-1, sPD-L1 and the CLASI

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.[90]

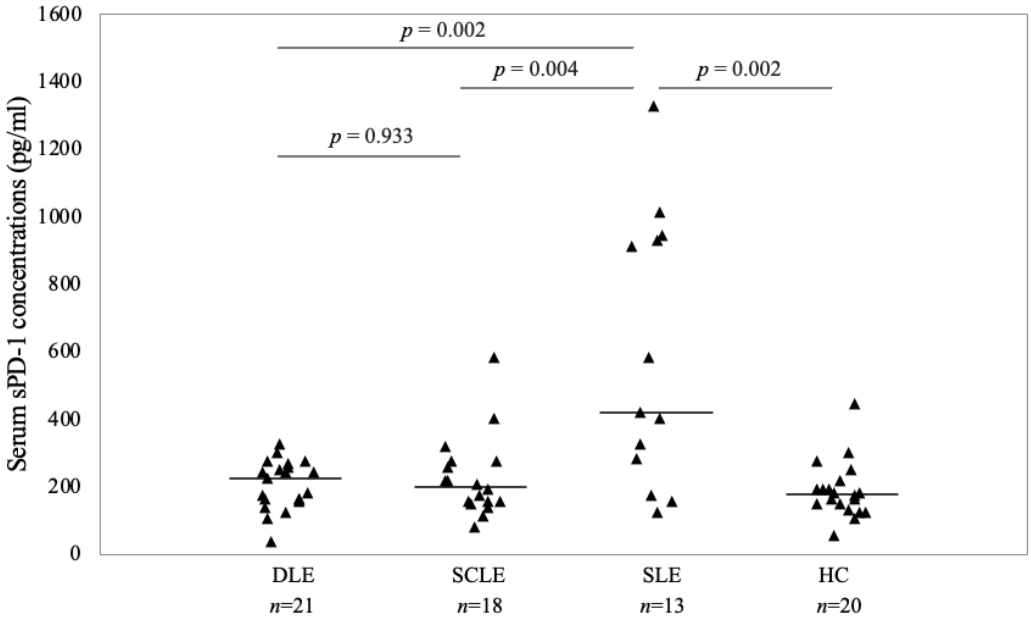


Figure 9. Serum sPD-1 levels in DLE, SCLE, SLE, and HCs. *„Serum levels of sPD-1 were significantly higher among SLE patients than in HCs (p=0.002), however no significant difference between DLE, SCLE and HCs groups was observed."*[90]

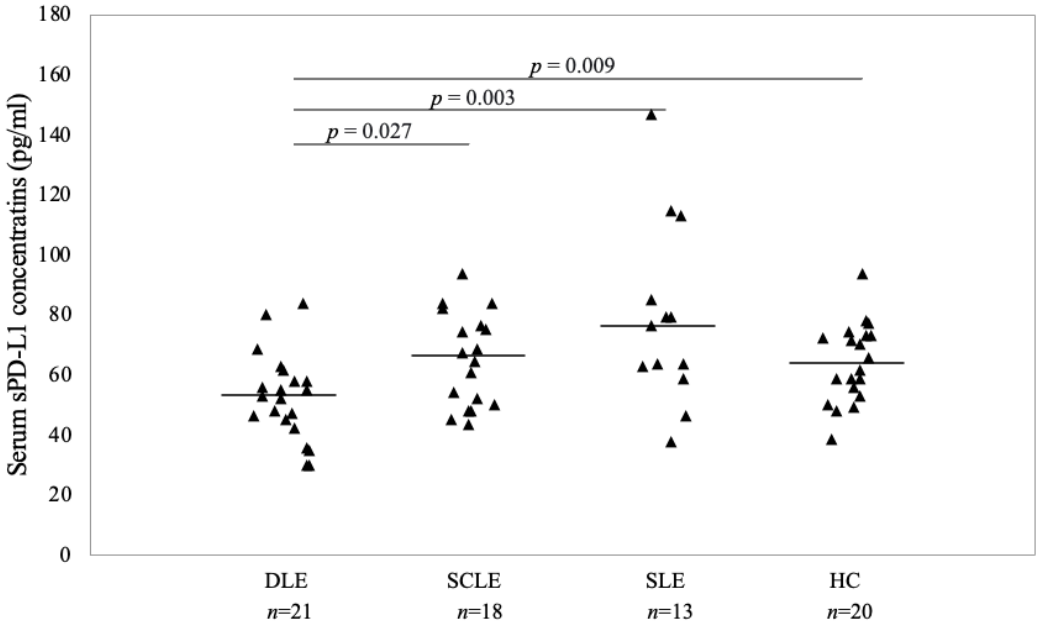


Figure 10. Serum sPD-1 and PD-L1 levels in DLE, SCLE, SLE and HCs. *„Serum levels of sPD-L1 (b) were significantly decreased in the DLE group compared to the HCs (p=0.009), while no significant differences between SCLE patients and HCs were observed."*[90]

5. Discussion

The origins and development of clinically distinct diseases within the spectrum of LE have been a longstanding challenge for researchers over the years. The complexity of the pathogenesis of CLE continues to be a subject of investigation, and there is still much to uncover regarding the distinct pathways leading to the development of various clinical subtypes within CLE. As our understanding of this complicated area deepens, it opens up new possibilities for treating this often therapy-resistant condition.

Although both DLE and SCLE are cutaneous manifestations of LE with shared histological features and pathogenic mechanisms, their clinical presentations, risk of systemic involvement, long-term outcomes, and response to therapies can differ. These clinical observations suggest the potential contribution of distinct molecular mechanisms in the development of these diseases. In recent decades, as the use of ICI therapies has expanded within the field of oncology, a connection has been noted between the development of SCLE and these therapies. However, a similar association does not appear between the presence of DLE and ICI-therapy. The reason for this distinction remains unknown and suggests the possibility of differential involvement of the PD-1/PD-L1 axis in the pathogenesis of CLE subtypes.

The PD-1 receptor and its primary ligand, PD-L1, were discovered decades ago as membrane-bound proteins, and their relevance in oncology has widely expanded in recent years. As part of the complex PD-1/PD-L1 pathway, their soluble forms, sPD-1 and sPD-L1, have been noticed as relevant factors in regulating immune responses, and their role as possible biomarkers has been suggested.

Within the field of oncology, the functions of sPD-1 and sPD-L1 as prognostic indicators have been extensively investigated. sPD-1 levels have been reported to be elevated in a variety of tumorous disorders, including non-small cell lung cancer (NSCLC), hepatocellular carcinoma, diffuse large B-cell lymphoma and metastatic melanoma.[91,92] The pretherapeutic higher plasma level of sPD-1 has been linked to disease severity, prognosis, and clinicopathological characteristics, although these findings are sometimes contradictory.[91] A recent meta-analysis has established a significant correlation between elevated levels of sPD-L1 and poorer overall survival in patients undergoing treatment with ICIs. However, the strength of this association varies

depending on the type of tumor. It is particularly robust in NSCLC but less pronounced in melanoma, renal cell carcinoma, and esophageal cancer.[93]

The clinical roles of the PD-1/PD-L1 proteins and their soluble variants as biomarkers or therapeutic targets in the field of autoimmunity and immune-mediated disorders have gained interest in recent years, and a growing number of research is being published regarding this topic. The role of this pathway has been investigated within the context of systemic autoimmune diseases. This signaling pathway is likely involved in the complex pathogenesis of these immunological conditions. While the PD-1/PD-L1 pathway has been extensively studied in the systemic form of LE, there needs to be more data concerning forms of the disease where only the skin is affected. Hence, there exists a need to address this gap in understanding autoimmune skin conditions.

In our first study, the expression of PD-1 and PD-L1, besides other cell markers, were evaluated in the skin samples of DLE and SCLE patients. First, a clear distinction in the presence of PD-L1 staining in the epidermis was noted, with a lower KC PD-L1 expression observed among DLE patients compared to the SCLE group. This finding might be attributed to the different INF profiles of the two subtypes of CLE. According to Gamblicher et al., the mRNA level of IFN-γ is higher in SCLE skin than in DLE, while no considerable differences were found in IFN-α and IFN-β mRNA levels.[94] As discussed earlier, IFN-γ is recognized for inducing PD-L1 expression more robustly than IFN-α or IFN-β, which could potentially explain the alterations between DLE and SCLE.[50] The mild expression of PD-L1 in KCs observed in DLE skin aligns with previous findings in other chronic inflammatory skin conditions from earlier investigations. In their study, Costa et al. found a low expression of PD-L1 in KCs from oral lichen planus patients.[73]

Concerning the dermal infiltrate, some differences were found between the two groups: DLE typically presented with a deeper and more robust T-cell infiltrate, which often showed perifollicular localization and featured a higher quantity of GB+ cytotoxic T-cells than SCLE. Moreover, a more prominent PD-1 staining of the dermal infiltrate was observed in the DLE samples than in the SCLE group. This is consistent with a recent study revealing increased expression of a newly discovered PD-1 homologous transmembrane protein (PD-1H) on T-cells in skin samples from individuals with DLE.[95] The frequent presence of PD-1+ on dermal T-cells in DLE skin might suggest

that these cells have an exhausted phenotype in the chronic form of CLE. Recently, a comprehensive study examining the components of the inflammatory infiltrate in chronic inflammatory skin diseases through single-cell immune profiling identified an increase in exhausted CD8+ T-cells compared to healthy skin.[96] The higher count of PD-1+ lymphocytes observed in our DLE patients might indicate a potentially higher number of T-cells with an exhausted phenotype in the chronic form of CLE. In theory, this approach would be akin to the exhausted T-cells observed in chronic viral infections and cancerous diseases, where a constant triggering factor of immune activation is present.

As part of our first study, the group of SCLE patients was further categorized into non-PD1-SCLE and PD1-SCLE. This division allowed us to investigate the similarities and differences between these patients. Many similarities in the patients' clinical and histological presentation were noted. Currently, no extensive and comprehensive study is available that directly compares the clinical presentations of non-PD1-SCLE with PD1-SCLE. Regarding the epidermal PD-L1 staining, the non-PD1-SCLE patients exhibited a slightly higher amount of PD-L1 staining KCs than the PD1-SCLE patients. Moreover, when examining other markers related to the dermal infiltrate, we noted many resemblances between the patients, although higher numbers of GB+ cytotoxic T-cells were observed in the non-PD1-SCLE group. Due to the limited number of patients examined by our team, further studies are needed to obtain more robust observations on this subject. The study by Schaberg et al. took a distinctive approach, comparing five cases of lichenoid dermatoses associated with PD-1/PD-L1 inhibitors with three cases of non-PD-1-inhibitor-related lichen planus. They observed a slightly elevated level of KC PD-L1, irrespective of the origin of the disease.[97]

Within the scope of the first study, the timeline of the immune mechanisms was explored in one case of a rare, hyperacute form of CLE by analyzing two consecutive skin samples from a TEN-like lupus patient. This approach lets us observe two distinct time points of one scenario. In the case of our patient, an elevation in the KC PD-L1 expression was observed when the initial erythematous plaques developed, which further increased as the skin symptoms progressed. According to one case report, another research group observed the same phenomenon in two consecutive samples of a (non-PD-1-inhibitor) drug-induced TEN patient: the KC PD-L1 expression increased alongside the progression of the clinical symptoms.[98]

Previous investigations have explored the potential regulatory role of the PD-1/PD-L1 axis in the interaction between KCs and lymphocytes.[68,69,99] Earlier studies utilizing cultured epithelial KCs isolated from the oral cavity of healthy individuals revealed an increase in PD-L1 expression upon stimulation with pro-inflammatory cytokines, particularly IFN-γ. Conversely, the inhibition of PD-L1 expression with specific antibodies enhanced proliferative responses of co-cultured allogeneic T-cells and increased production of IFNγ.[68] Further investigations have elucidated the regulatory roles of PD-L1 on murine KCs concerning activated T-cells. These findings indicate that PD-L1 expression by KCs might directly suppress CD8+ cytotoxic T-cells, reducing cytotoxicity and serving as a defense mechanism.[69,99] These observations imply that changes in the regulation of the PD-1/PD-L1 axis could impact the onset of skin autoimmunity. The upregulation of PD-L1 expression on KCs, concomitant with the advancement of the clinical presentation, may act as a natural defense mechanism, potentially mitigating immune attacks in a hyperacute reaction, such as TEN-like lupus. These together suggest, the changes in the PD-1/PD-L1 axis might not be specific to CLE but may extend to other inflammatory skin conditions. Furthermore, it seems that the variations of the alterations of this pathway might differ between chronic and subacute forms of CLE.

In our second study, the soluble forms of PD-1 and PD-L1 were examined in the sera of DLE, SCLE, and SLE patients. As previously stated, sPD-1 boosts T-cell activity, whereas the impact of sPD-L1 on T-cell function is currently a subject of debate. Nonetheless, sPD-L1 likely exerts an inhibitory effect on T-cells akin to its membrane-bound counterpart. In the study of Du et al., an elevated level of sPD-1 was observed in the blood of SLE patients compared to healthy individuals.[81] In a similar approach, Hirahara et al. found elevated levels of sPD-1 only in the sera of high-disease-activity SLE patients but not in low-disease-activity SLE.[80] In our study, the noted increase in serum sPD-1 levels in our SLE group is consistent with these findings, and the median values we observed closely match the measurements reported by Hirahara et al. in individuals with SLE exhibiting high disease activity (420.12 and 482.7 pg/mL, respectively). Du et al. discovered a significant rise in sPD-L1 levels in SLE patients as opposed to Hirahara et al.'s findings, which showed no significant alteration.[80,81] Between our SLE and HC groups, no statistically significant difference was observed in

sPD-L1 levels in our study. Notably, we observed a statistically significant lower level of sPD-L1 level in DLE compared to the other groups. Variations in the detection ranges and sensitivities of the ELISA kits used for measurement, as well as differences in the disease type and activity, may be partially responsible for the variances in findings regarding sPD-1 and sPD-L1 levels in immune-mediated disorders.[100]

Data on sPD-1 and sPD-L1 in autoimmune and autoinflammatory skin-affecting diseases is limited. In their study concerning psoriasis, Bartosinska et al. explored the serum sPD-1 levels in patients with psoriasis and observed no significant difference between patients and HC group.[72] In another study focusing on immune thrombocytopenia (ITP), increased levels of sPD-1 were observed in both newly diagnosed and chronic ITP patients. Notably, sPD-L1 was lower only in the newly diagnosed group compared to HCs but not in the chronic ITP group.[101] In a different approach by Yanaba et al., patients with diffuse cutaneous systemic sclerosis exhibited increased levels of sPD-1, whereas those with limited cutaneous systemic sclerosis did not show such elevation. Conversely, sPD-L1 was elevated in both groups compared to HCs. Notably, in their study, serum sPD-1, but not sPD-L1, showed a positive correlation with the severity of skin sclerosis.[102] Drawing from our results mentioned above, it can be inferred that the elevated level of sPD-1 might play a role in the development of systemic autoimmune diseases. At the same time, its impact seems less pronounced in the pathogenesis of diseases primarily affecting the skin. In the course of our study, we assessed the severity of lupus skin lesions using the CLASI-A score. However, no correlation was observed between the serum levels of sPD-1 or sPD-L1 and the activity of skin symptoms, unlike it was presented in systemic sclerosis patients.[102] Based on this result, it appears less likely that sPD-1 and sPD-L1 can be applied as markers of disease activity.

When considering both studies, it is noticeable that PD-L1 and sPD-L1 were both lower in DLE than in SCLE. In light of this information, it appears plausible that the inadequate inhibitory effect of PD-L1 and sPD-L1 on T-cell activity might promote the chronic form of CLE rather than the subacute form. The slight differences observed regarding the PD-1/PD-L1 axis in DLE and SCLE could possibly explain the clinical differences between the two forms.

Our results contribute to a complicated yet uncovered field of autoimmunity and immunopathology, specifically concerning the skin. The diagnosis and treatment of CLE are still a challenge in many cases in the clinical practice, and the burden of therapy-resistant CLE is substantial. In recent years, novel findings in the pathogenesis of CLE have led to the exploration of many new potential target molecules and cells in the treatment of CLE.[26] Our research aims to uncover a yet-hidden aspect of CLE pathophysiology and potentially identify distinct features between clinical subtypes. These subtle components of the pathomechanism may contribute to the therapy refractoriness observed in cases of DLE.

Currently, there is limited data regarding the PD-1/PD-L1 axis in LE. In our studies, some alterations of the PD-1/PD-L1 pathway have been demonstrated, and the potential role of the PD-1/PD-L1 axis in CLE pathogenesis has been suggested. The immunohistochemical differences between DLE and SCLE regarding KC PD-L1 indicate the potential for different involvement of this axis in the clinical forms of CLE. These differences might elucidate the clinical and prognostic distinctions between DLE and SCLE; however, further extensive studies are required to establish these characteristics.

The roles of sPD-1 and sPD-L1 in regulating immune response and autoimmunity have gained significance in recent years. Based on our research and previous measurements by other groups, it is suggested that sPD-1 and sPD-L1 could potentially influence the PD-1/PD-L1 axis and may play a role in triggering some form of autoimmunity.

It is difficult to study individual components of the PD-1/PD-L1 axis due to its intricate mechanism, which is entangled with several cellular signaling pathways and various cell types. Enhancing our comprehension of this pathway within the cascade of other cell signaling pathways would enrich our knowledge regarding immunopathogenesis and immunopathology.

In the future, more studies on this subject might uncover other parts of this complex regulatory mechanism. Gaining insights into the PD-1/PD-L1 axis in various forms of CLE may provide a foundation for better comprehending the fundamental distinctions in the pathogenesis, prognosis and therapy responsiveness of DLE and SCLE.

6. Conclusions

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

- In our study, we first investigated the potential role of the PD-1/PD-L1 axis in CLE.
- Alterations of the PD-1/PD-L1 pathway appear to play a role in CLE pathogenesis.
- Lower KC PD-L1 expression was observed in the chronic form of CLE (DLE) compared to the subacute form (SCLE).
- 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.

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

- This is the first study investigating the sPD-1 and sPD-L1 in the context of CLE.
- The serum levels of sPD-L1 were notably lower in the DLE group compared to the HCs, SCLE, and SLE groups.
- 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.

7. Summary

The various clinical presentations of CLE are well-recognized in clinical practice. However, finding an accurate diagnosis can be challenging in many cases, and the resistance of skin symptoms to therapy adds to the burden of CLE. The clinical presentation, development of systemic symptoms, and disease course differ between the two primary forms of CLE, namely, SCLE and a chronic form, DLE. Another important distinction between the two forms is that PD-1/PD-L1 inhibitor therapy can induce SCLE, which is unusual in the case of DLE. Based on this clinical observation, the possibility of different involvement of the PD-1/PD-L1 pathway in DLE and SCLE was raised.

In our pioneering studies, we investigated the PD-1/PD-L1 axis in CLE. First, CLE skin samples were analyzed by immunohistochemistry. A markedly lower KC PD-L1 expression was found in DLE compared to SCLE, while the number of PD-1+ lymphocytes was greater in DLE than in SCLE. In this study, no notable differences were found between non-PD1-SCLE and PD1-SCLE. In an alternative approach, the soluble forms of PD-1 and PD-L1 were investigated in individuals with CLE and some cases of SLE. The study revealed a significant increase in sPD-1 levels in SLE compared to DLE, SCLE, and HC groups. Additionally, the analysis found a significantly lower serum level of sPD-L1 in the DLE group compared to SLE, SCLE, and HC groups, with no significant differences observed in other comparisons.

Drawing upon our findings and existing literature, it appears plausible that the PD-1/PD-L1 pathway undergoes modulation in CLE. Nevertheless, it is important to note that the involvement of this pathway may exhibit distinct variations between DLE and SCLE. In our studies, the PD-L1 and sPD-L1 were both lower in DLE than in SCLE. According to this, it might be plausible that the inadequate inhibitory effect of PD-L1 and sPD-L1 on T-cell activity could promote the chronic form of CLE. These subtle differences might explain the variances of clinical presentations of DLE and SCLE.

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9. Bibliography of the candidate's publications

9.1. Publications directly related to this thesis

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

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

9.2. Publications not directly related to this thesis

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

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

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
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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: -

9.3. Conference presentations and posters

Z. Király, A. Kovács, E. Kuroli, M. Marschalkó, B. Hidvégi. Clinicopathologic study of 14 patients with lupus eyrthematosus panniculitis (presentation) PhD Scientific Days, Budapest 2021

Z. Király, A. Kovács, Á. Szepesi, E. Kuroli, M. Marschalkó, B. Hidvégi. Clinicopathologic study of 14 patients with lupus eyrthematosus panniculitis (e-poster) Lupus CORA 2021

Z. Kiraly, A. Szepesi, A. Sebestyen, E. Kuroli, F. Rencz, B. Toth, L. Bokor, J. Szakonyi, M. Medvecz, B. Hidvegi. Immunohistochemical study of the PD-1/PD-L1 pathway in cutaneous lupus erythematosus (e-poster) ESDR 2022

Z. Király, A. Szepesi, A. Sebestyén, E. Kuroli, F. Rencz, B. Tóth, L. Bokor, J. Szakonyi, B. Hidvégi. Immunohistochemical study of the PD-1/PD-L1 pathway in cutaneous lupus erythematosus (presentation) PhD Scientific Days, Budapest 2022

Z. Király, A. Szepesi, A. Sebestyén, E. Kuroli, F. Rencz, B. Tóth, L. Bokor, J. Szakonyi, B. Hidvégi. Immunohistochemical study of the PD-1/PD-L1 pathway in cutaneous lupus erythematosus (presentation) Hungarian Dermatological Society Annual Meeting 2022

Z. Király, K. Lőrincz, E. Kuroli, A. Mohos, M. Medvecz, M. Marschalkó, M. Sárdy, P. Holló, B. Hidvégi. Il-17 gátló jótékony hatása fényérzékeny I. típusú pityriasis rubra pilaris esetében (presentation) Hungarian Dermatological Society Annual Meeting 2022.

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