

Photosensitivity, Apoptosis, and Cytokines in the Pathogenesis of Lupus Erythematosus: a Critical Review

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Abstract The underlying pathomechanisms of lupus erythematosus (LE), a multifactorial autoimmune disease, remain elusive. Due to the clinical evidence demonstrating a clear relationship between ultraviolet (UV) light exposure and skin lesions of LE, photosensitivity has been proven to be an important factor in the pathogenesis of the disease. Standardised photoprovocation with UVA and UVB irradiation has been shown to be a reliable model for evaluating photosensitivity in patients with cutaneous LE (CLE) and analysing the underlying medical conditions of the disease. In this respect, UV irradiation can cause aberrant induction of apoptosis in keratinocytes and contribute to the appearance of excessive apoptotic cells in the skin of CLE patients. Moreover, apoptotic cells that cannot be cleared by phagocytes may undergo secondary necrosis and release proinflammatory compounds and potential autoantigens, which may contribute to the inflammatory microenvironment that leads to formation of skin lesions in the disease. In addition to UV-mediated induction of apoptosis, the molecular and cellular factors that may cause the abnormal long-lasting photoreactivity in CLE include mediators of inflammation, such as cytokines and chemokines. In particular, interferons (IFNs) are important players in the early activation of the immune system and have a specific role in the immunological interface between the innate and the adaptive immune system. The fact that treatment with recombinant type I IFNs (α and

β) can induce not only systemic organ manifestations but also LE-like skin lesions provides additional evidence for a pathogenetic role of these IFNs in the disease.

Keywords Photosensitivity · Apoptosis · Cytokines · Pathogenesis · Lupus erythematosus · Skin

Introduction

The inflammatory autoimmune disease lupus erythematosus (LE) is characterised by different clinical entities, reaching from a primary cutaneous form (cutaneous lupus erythematosus, CLE) to a severe systemic disease (systemic lupus erythematosus, SLE). The expression of skin lesions in LE shows a great diversity and has led to the subdivision of LE-specific and LE-nonspecific lesions by histological analyses of skin biopsy specimens [1]. The LE-nonspecific cutaneous manifestations are often associated with SLE but may also occur in other diseases and include vascular skin changes, such as periungual telangiectasia, livedo racemosa, thrombophlebitis, Raynaud's phenomenon and acral occlusive vasculopathy [2]. The LE-specific cutaneous manifestations include the different subtypes of CLE, as defined by clinical features, histological changes, laboratory abnormalities and duration of skin lesions: acute CLE (ACLE), subacute CLE (SCLE) and chronic CLE. In addition, a further subtype with characteristic clinical, histological and photobiological features, named LE tumidus (LET), has been defined as a separate entity of CLE [3, 4]. As the course and prognosis in these patients is generally more favourable than in those with other disease subtypes, a revised classification system was suggested in 2004, including LET as the intermittent subtype of CLE [5].

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Photosensitivity

It is commonly accepted that ultraviolet light (UV) exposure can induce and exacerbate skin lesions in patients with all subtypes of CLE, supporting the role of UV light in the pathogenesis of the disease [6, 7]. The described photosensitivity in the different disease subtypes of CLE varies greatly in the literature and has been reported to affect between 27 and 100 % of patients with SCLE, between 25 and 90 % of patients with discoid LE (DLE) and between 43 and 71 % of patients with LET [8, 9]. However, sun exposure can also lead to a significant organ involvement in SLE, such as lupus nephritis [10], requiring the education of patients regarding the avoidance of excessive sun exposure; continuous photoprotection through physical measures, such as protective clothing; and daily application of broad-spectrum sunscreens.

Photosensitivity is listed as one of the American College of Rheumatology (ACR) criteria for the classification of SLE, along with the mucocutaneous manifestations malar rash, discoid rash and oral ulcers [11, 12]. However, the term “photosensitivity” is poorly defined by the ACR as a “skin rash as a result of unusual reaction to sunlight by patient history or physician observation”. As this is a very broad definition, some authors define photosensitivity more specifically as an induction of skin lesions following extensive sun exposure, while other authors also include sunburn or spring and summer aggravation of the disease [13]. In addition, Albrecht and co-workers [14] remarked that “malar rash” is often not distinguishable from photosensitivity in SLE patients. Thus, the ACR criteria are not independent of each other. Moreover, photosensitivity is not specific for SLE, and it has been observed in other conditions, such as polymorphous light eruption (PLE), photoallergic contact dermatitis, dermatomyositis and erythropoietic protoporphyria. Therefore, the ACR criteria often overestimate the occurrence of SLE in patients with primarily cutaneous disease manifestations.

In 2012, the Systemic Lupus Collaborating Clinics (SLICC) revised and validated the ACR criteria to improve the clinical relevance of the criteria and incorporate new knowledge in SLE immunology [15]. The SLICC criteria comprise 17 clinical and immunological criteria; for example, the criteria of “non-scarring alopecia” or “synovitis” were included, whereas “photosensitivity” is no longer listed. However, the SLICC criteria have yet to be evaluated in routine clinical practice, and the degree of impact of these criteria on the representativeness of SLE patients in clinical trials remains to be determined.

Photoprovocation

Experimental photoprovocation with different wavelengths has been developed to better evaluate photosensitivity in LE

patients [16] (Table 1). In 1965, Epstein et al. [17] conducted the first experimental photoprovocation in 25 patients with LE, inventing a repeated exposure technique to objectively define photosensitivity. By applying the repeated UV exposure technique, other investigators used monochromatic light to determine the wavelength [18, 19]. In 1986, a photoprovocation protocol was developed by Lehmann et al. [20] based on the discovery that the action spectrum of LE also reaches into the long-wave UVA region. A subsequent study showed that skin manifestations can be induced by experimental UVA or UVB irradiation in 43 % of patients with the disease [21]. By accounting for multiple factors, such as light source, test area of irradiated skin, dose of UV exposure and frequency of irradiation, this photoprovocation protocol was optimised over the following years [9]. In particular, UVB (290–320 nm) and UVA2 (320–340 nm), but also UVA1 (340–400 nm), can induce or exacerbate skin manifestations in LE [22]. It was further observed that the onset of UV-induced LE-specific lesions was significantly slower and persisted longer than reactions in patients with other photodermatoses, such as PLE. On average, disease-specific reactions in patients with LE did not appear before 1 week after UV irradiation and lasted for up to 2 months. Due to this delayed onset, it might be difficult for LE patients to recognise the association between UV exposure and the induction of skin lesions. Notably, indoor light bulbs and compact fluorescent lamps also emit appreciable levels of UVA and UVB, and several of these light sources even emit UVC [23].

In 2011, the photoprovocation protocol was evaluated in seven European centres and included a total of 47 patients with different subtypes of CLE and 13 healthy volunteers [24]. Of these, 22 (47 %) patients with CLE and none of the healthy volunteers developed cutaneous manifestations according to clinical assessment. Using UVA and UVB irradiation, comparable results were found across the multiple sites. Thus, it is a standardised, safe and reproducible method and may therefore be used for future diagnostic testing and clinical trials. In a recent study, this standardized photoprovocation protocol was evaluated retrospectively in 566 patients [25]. A diagnosis of CLE was clinically and/or histologically confirmed in 431 patients, and 315 patients with PLE were additionally included as controls. A total of 61.7 % of the 431 CLE patients exhibited a positive photoprovocation. Subsequent photoprovocation in 35 patients demonstrated that CLE patients with an initial positive result exhibited a significantly higher frequency of a positive photoprovocation at a later time point, suggesting that the reaction to UV light may change during the course of this heterogeneous disease and that photosensitivity should not be excluded in CLE patients with a negative history of photosensitivity.

Table 1 Photoprovocation in lupus erythematosus: review of the literature since 1986 (modified after [9])

Year	Authors	Country	Tested LE patients	LE patients with a positive photoprovocation	Comments
1986	Lehmann et al. [20]	Germany	67	27 (40 %)	First publication on a photoprovocation protocol for CLE including long-wave UVA irradiation
1989	Wolska et al. [147]	Poland	202	49 (24 %)	Observation of prolonged persistence of erythema after UVB irradiation in CLE compared to healthy controls
1989	Van Weelden et al. [148]	Netherlands	24	20 (83 %)	Photoprovocation of CLE with UVA, UVB and visible light
1990	Lehmann et al. [21]	Germany	128	55 (43 %)	Induction of skin manifestations in CLE with UVA, UVB and combined UVA/UVB irradiation
1991	Beutner et al. [149]	USA	115	90 (78 %)	Persistence of positive reactions in CLE for 10 days
1993	Kind et al. [150]	Germany	150	81 (54 %)	Review on photoprovocation procedures and results, associated with clinical correlations in CLE
1993	Nived et al. [151]	Sweden	23	6 (26 %)	Pathological skin reaction in SLE patients after standardized exposure to UVA and UVB wavelengths
1995	Bensaid [152]	France	19	12 (63 %)	No correlation of photoprovocation results, presence of systemic involvement or anti-Ro/SSA antibodies in CLE
1997	Walchner et al. [153]	Germany	68	29 (43 %)	Overview on photoprovocation and photoprotection in CLE
1997	Hasan et al. [154]	Finland	67	46 (69 %)	Induction of PLE- and LE-like skin lesions in CLE and SLE after UV-irradiation
1999	Leenutaphong [155]	Thailand	15	6 (40 %)	Photoprovocation in oriental LE patients
2001	Kuhn et al. [9]	Germany	323	175 (54 %)	Summary of 15-years experience with photoprovocation in different subtypes of CLE
2001	Kuhn et al. [156]	Germany	60	43 (72 %)	Classification of LET as the most photosensitive subtype of CLE
2003	Sanders et al. [157]	Netherlands	100	93 (93 %)	No correlation of photosensitivity, CLE subtype, presence of antibodies or medical history
2004	Choonhakarn et al. [158]	Thailand	10	5 (50 %)	Assessment of different clinical parameters in LET patients
2011	Kuhn et al. [24]	Germany, Poland, Sweden, UK	47	22 (47 %)	Multicenter study evaluating no significant differences in photoprovocation of CLE patients between seven European study sites
2013	Ruland et al. [25]	Germany	431	266 (62 %)	Study with >400 CLE patients suggesting that the reaction to UV light may change during the course of the disease

Apoptotic Signalling Pathways

Apoptosis, or programmed cell death, represents the most common form of cell death in multicellular organisms during development and adulthood. An estimated number of 100,000 to 1 million cells die per second through apoptosis in the human body, ensuring tissue homeostasis [26, 27]. Morphologically, apoptotic cells are characterised by cellular shrinkage, membrane blebbing and nuclear chromatin condensation. On the molecular level, the hallmarks of apoptotic cells include caspase activation; exposure of negatively charged phospholipids, such as phosphatidylserine (PS), on the outer leaflet of the cell membrane; generation of reactive oxygen species (ROS) and DNA cleavage [28–30]. The final step in the apoptotic process is the removal of apoptotic cells by phagocytes, which—under physiological circumstances—is performed in an immunologically silent fashion, without activation of an immune response against autoantigens

derived from apoptotic cells [31]. In vivo, apoptotic cells are removed within few hours and, therefore, are rarely detected in situ [32]. In contrast to accidental cell death or necrosis, the membrane integrity of apoptotic cells under physiologic conditions remains preserved until engulfment, which prevents immune activation by the unwanted release of intracellular danger-associated molecules (DAMPs) and potential autoantigens, such as HMGB1, heat shock proteins, uric acid and histone–DNA complexes, respectively [33].

The induction of apoptosis is mediated via extrinsic or intrinsic pathways, which ultimately converge on the activation of the executioner caspase 3, caspase 6 and caspase 7. Once activated, these caspases cleave a large panel of cellular substrates, including molecules for membrane rearrangement and blebbing (e.g. ROCK1, XKr8) and DNA cleavage (CAD) [34–36]. The extrinsic pathway is activated following ligation of the so-called death receptors, such as CD95/Fas, tumor necrosis factor receptor 1 (TNF-R1) or TRAIL receptors, all

of which belong to the TNF receptor superfamily and possess an intracellular death domain (DD) [37]. Upon triggering, death receptors assemble through intracellular adaptor proteins (e.g. FADD and TRADD) that interact with pro-caspase 8 in a large multimeric death-inducing signalling complex (DISC) [38]. At the DISC, pro-caspase 8 is activated through auto-proteolytic cleavage and is released as the active caspase 8 heterotetramer to the cytosol, where it cleaves and activates downstream executioner caspases.

The intrinsic apoptosis pathway is initiated by pro-apoptotic intracellular damage sensors of the Bcl-2 family, such as Bax, Bad and Bim [39], which act on the outer mitochondrial membrane and lead to release of cytochrome c into the cytosol. Here, cytochrome c associates with the adaptor molecule, apaf-1, pro-caspase 9 and ATP to form the apoptosis-inducing platform of the intrinsic pathway, the apoptosome. Assembly of the apoptosome leads to the formation of the active caspase 9 heterotetramer which subsequently cleaves and activates downstream executioner caspases. Both extrinsic and intrinsic pathways are connected at multiple levels. For example, active caspase 8 can truncate the cytosolic protein Bid [40]. Truncated Bid then migrates to the mitochondria and acts as a pro-apoptotic stimulus that causes cytochrome c release and amplifies the death receptor-mediated apoptotic response. Conversely, internal cellular stimuli, such as DNA damage, can induce receptor-mediated apoptosis pathways, e.g. by p53-mediated upregulation of CD95/Fas-ligand (CD95L) expression and autocrine induction of CD95/Fas-mediated apoptosis [41].

Clearance of Apoptotic Cells

Clearance of apoptotic cells by phagocytes is the final step of apoptosis [42], and it assures the rapid removal of apoptotic cells that, if not removed, would progress to secondary necrosis or late apoptosis. At this stage, apoptotic cells also lose membrane integrity, release intracellular DAMPS and present a source for activating immunity toward autoantigens, such as histone–DNA complexes [43]. The removal of apoptotic cells involves three distinct steps: (1) recruitment of phagocytes to the site of apoptotic cell death via “find me” signals, (2) engulfment of apoptotic cells by binding of phagocyte receptors to “eat me” signals exposed on or bound to the apoptotic cell surface and (3) initiation of an anti-inflammatory response by the phagocyte that, in addition to mere removal, actively prevents inflammatory responses against antigens derived from apoptotic cells [44].

Several molecules in apoptotic cell supernatants have been identified to serve as chemotactic factors, which recruit phagocytes to the site of apoptotic cell death and are, hence, termed “find me” signals. These include lysophatidylcholine, fractalkine, sphingosine-1-phosphate and nucleotides, such as

ATP and UTP [45–48]. Some of these signals appear to be secreted by specific cell types upon induction of apoptosis (e.g. fractalkine is released from lymphocytes), while others are secreted from various cell types. Currently, the relative contribution of the described find me signals to the overall clearance of apoptotic cells is not clear and in the absence of excess apoptosis resident neighbouring phagocytes, which do not need to be recruited, may be sufficient for the engulfment of all apoptotic cells.

Once at the site of apoptotic cells, phagocytes recognise various specific “eat me” signals that appear on the apoptotic cell surface and distinguish them from healthy cells. A hallmark of apoptotic cells and the paradigm eat me signal is represented by the exposure of negatively charged phospholipids such as PS. In most living cells, PS is strictly confined to the inner leaflet of the plasma membrane by the action of ATP-dependent phospholipid translocases [49]. Following apoptosis induction and caspase 3 activation, PS is rapidly exposed on the outer leaflet of the plasma membrane. The molecular identity of the scramblase activity, which shuttles PS (and other negatively charged phospholipids) to the surface of apoptotic cells, has only recently been identified as Kxr8, a 10-transmembrane protein of the Kx-red blood cell antigen family [36]. At the outer leaflet of the cellular membrane, PS is directly recognised by several receptors [50–54] and additionally serves as a docking site for bridging molecules provided by the cellular environment, including MFG-E8, Gas-6 and protein S [55–57]. Other serum-derived bridging molecules, such as thrombospondin, complement proteins and natural IgM antibodies, also bind to the apoptotic cell surface, although to less well-characterised sites [58–60]. All of these bridging molecules, in turn, mediate phagocytosis through distinct counterpart phagocyte receptors. Surface changes, such as the exposure of PS, altered sugar residues and oxidised lipid moieties [61, 62], together with bridging molecules constitute a vast array of apoptotic eat me signals, which likely serve cell type-specific (regarding both the apoptotic cell type and the phagocyte) and partly redundant functions to assure swift removal under any given circumstance.

In addition to the phagocytosis-related events described above, apoptotic cells have also been known to initiate immunosuppressive, tolerogenic responses in phagocytosing macrophages and dendritic cells (DCs). In vitro, macrophages have been shown to secrete anti-inflammatory cytokines, such as transforming growth factor- β and interleukin-10, after encountering apoptotic cells [63–65]. Likewise, the phagocytosis of apoptotic cells retains DCs in an immature state that is characterised by the low expression of costimulatory surface molecules and inflammatory cytokines, a block in pro-inflammatory signal transduction and resistance to activation [66, 67]. In vivo, several studies have shown that apoptotic cells support the development of peripheral tolerance. For example, injection of antigen-coupled apoptotic cells leads

to antigen-specific tolerance and can prevent experimental autoimmune encephalitis, while induction of limited apoptosis in pancreatic islet β -cells can protect from diabetes [68–70]. In contrast to the numerous eat me signals described above, relatively few anti-inflammatory signals of apoptotic cells have been identified. Apoptotic cells may release anti-inflammatory cytokines [71, 72], but additional signals must exist that mediate anti-inflammatory signalling in phagocytes through direct cell contact, as apoptotic cells isolated in a transwell insert could not induce the suppression of phagocytes, while isolated apoptotic cell membranes did [67, 73]. Knockout and blocking studies have revealed that complement receptor 3, the Gas-6 receptor MerTK and nuclear LX receptors are involved in anti-inflammatory signalling after apoptotic cell phagocytosis [74–76]. On the side of the apoptotic cell, studies with lipid vesicles have identified PS as the main anti-inflammatory mediator of apoptotic cells [65]. However, these experiments did not exclude the possibility that several of the described bridging molecules—derived from serum or from phagocytes themselves—bind to PS vesicles and in turn mediate anti-inflammatory signalling. In this scenario, exposed PS would be a necessary component but not the active agent. Recently, the lipid-binding protein annexin A1 has been identified as an anti-inflammatory signal on the surface of apoptotic cells [67]. Similar to PS, annexin A1 is translocated to the outer membrane of apoptotic cells early after the induction of apoptosis and serves to suppress the adaptive immune response to apoptotic cell-derived antigens by inhibiting DCs. Anti-inflammatory effects of annexin A1 mediated through formyl peptide receptors have long been known and extend to various phagocytes, such as monocytes and macrophages [77–79]. Thus, the exposure of annexin A1 may serve as a “broad spectrum” inhibitory agent associated with apoptotic cell engulfment.

Recruitment of phagocytes, rapid engulfment of apoptotic cells and anti-inflammatory signalling all contribute to the non-phlogistic nature of apoptosis under physiological conditions and, thus, to the maintenance of peripheral tolerance. Defects in either pathway, therefore, may be the underlying cause for the development of several types of autoimmune diseases.

Apoptosis in Lupus Erythematosus

Several lines of evidence support a causative role for the deregulation of apoptosis in autoimmune diseases including LE. Due to rapid engulfment, apoptotic cells are only rarely detectable within the tissues of healthy individuals; however, an increased number of apoptotic cells has been shown to be evident, e.g. in the germinal centres of SLE patients [80] and in the skin of patients with different subtypes of CLE, compared to healthy controls [81]. This distinct accumulation of

apoptotic cells in LE may be the consequence of an abnormally enhanced rate of apoptosis and/or defective engulfment, and experiments illustrate that both processes may be disturbed in the disease. While it has clearly been established that the abnormal survival of self-reactive immune cells due to impaired apoptosis pathways gives rise to LE-like autoimmunity in patients with autoimmune lymphoproliferative syndrome and mice of the *lpr* and *gld* strains, enhanced cell death may also contribute to disease manifestation. Thus, an abnormal activation of apoptosis pathways as well as increased CD95 expression and CD95L serum levels have been found in SLE patients, which may lead to an excess of apoptotic cells [82–84]. In addition, key molecules of death receptor pathways, such as CD95, TRAIL-R1 and caspase 8, have been shown to be upregulated in the skin of CLE patients [85]. Although death receptors are poorly expressed in normal skin [86], their expression can be upregulated following exposure to UV irradiation [87] (Fig. 1).

UV irradiation can directly elicit cell death in keratinocytes as evidenced by the appearance of apoptotic cells, also referred to as “sunburn” cells, with condensed nuclei as early as 8 h after irradiation [88]. In 2006, Kuhn et al. [81] examined whether apoptosis contributes to the pathogenesis of skin lesions in patients with CLE after UV irradiation. Specimens from healthy individuals and patients with PLE were used as controls. A significant increase in apoptotic nuclei was found in the upper epidermal layer of primary and UV light-induced skin lesions of CLE patients compared with the control subjects. In tissue sections obtained from the control subjects at 24 h after a single exposure to UV light, a slight increase in the count of epidermal apoptotic nuclei was present compared with skin tissue from CLE patients obtained under the same conditions before lesion formation. In sections obtained from the control subjects at 72 h after irradiation, a significant decrease in the apoptotic nuclei count was observed, consistent with the proper clearance of apoptotic cells over the period between 24 and 72 h after irradiation. In contrast, the number of apoptotic nuclei increased significantly within this period in tissue sections from patients with CLE. These data support the hypothesis that apoptotic cells accumulate in the skin of patients with CLE after UV irradiation, as a result of impaired or delayed clearance.

Both UVA and UVB irradiation act on several cellular levels to induce apoptotic pathways in the skin. For example, UV-induced direct DNA damage (e.g. cyclobutane pyrimidine dimers [89]) can activate the p53-dependent cell death pathways (e.g. by transactivating pro-apoptotic members of the Bcl-2 family, such as Bax and Noxa) [90]. UVB irradiation has also been shown to trigger CD95-dependent apoptosis independent of CD95L through enhanced receptor aggregation [91], and cleavage of Bid has been shown to enhance keratinocyte apoptosis via the mitochondrial pathway [92]. Another mediator of effects induced by UV irradiation in

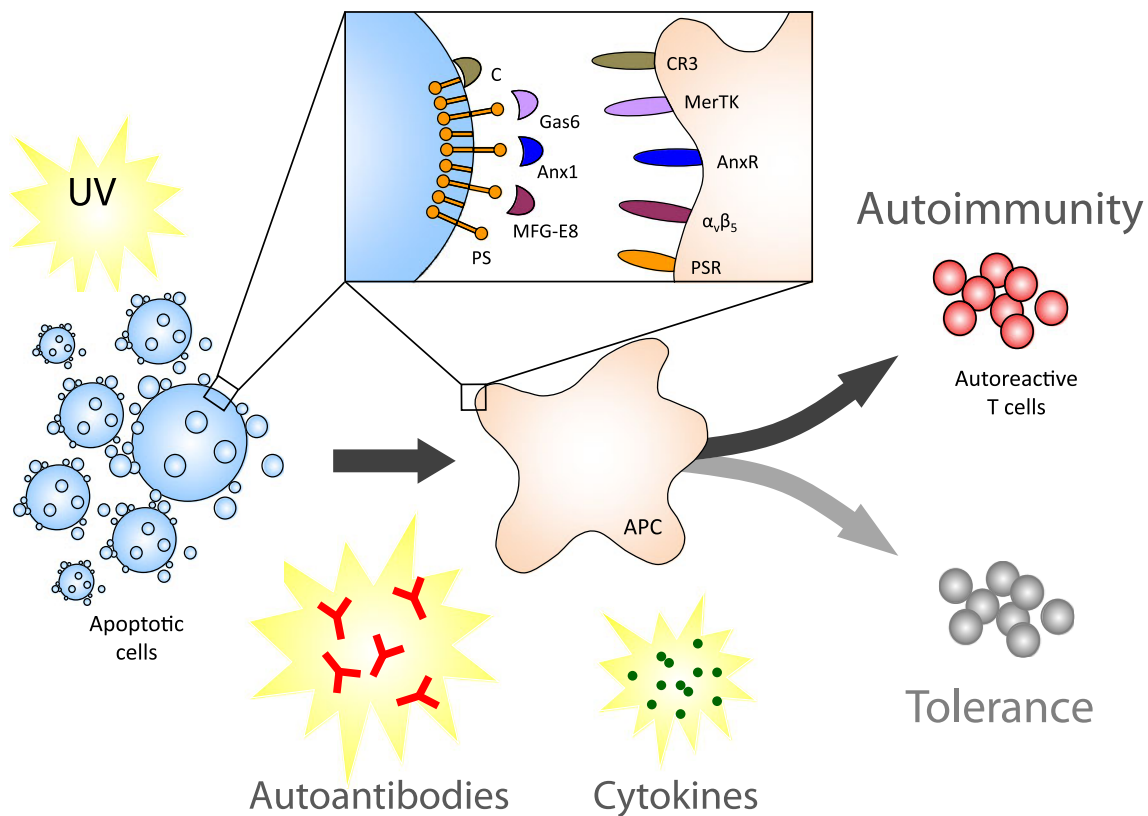


Fig. 1 Clearance of UV-induced apoptotic cells in autoimmunity. Under physiological conditions, apoptotic cells are efficiently cleared by phagocytes such as antigen presenting cells (APC). Various molecules on the surface of apoptotic cells and corresponding receptors on the APC mediate phagocytosis and anti-inflammatory effects of apoptotic cells (see text for details), maintaining tolerance to apoptotic cell-derived self-antigens. At different levels, excessive apoptosis through UV irradiation as well as

autoantibodies and cytokines present in microenvironment can interfere with the anti-inflammatory removal of apoptotic cells and cause autoimmunity. (C, complement proteins; CR3, complement receptor 3; Gas6, growth arrest-specific 6 protein; MerTK, Mer tyrosine kinase; Anx1, annexin 1; AnxR, annexin 1 receptors; MFG-E8, milk fat globule-EGF factor 8; $\alpha_v\beta_5$, $\alpha_v\beta_5$ integrin; PS, phosphatidylserine; PSR, phosphatidylserine receptors)

keratinocytes are ROS, which lead to the oxidative damage of biomolecules, such as DNA, and to the activation of mitogen-activated protein kinases, in particular p38, which induce cell death via the mitochondrial pathway [93]. Thus, exposure to UV irradiation likely contributes to an enhanced rate of apoptosis, as observed in CLE. Recently, Toberer et al. [85] investigated whether CLE and other inflammatory skin diseases differ with regard to the expression of molecules that are crucial for the initiation and regulation of apoptosis. Anti-activated caspase 3 and anti-CD95 antibody staining revealed a significant increase of apoptotic keratinocytes in CLE lesions compared to controls, such as psoriasis and lichen planus. Therefore, accumulation of apoptotic keratinocytes in the disease might be due to increased epidermal expression of CD95, resulting in the increased activity of the extrinsic apoptotic pathway in CLE. In addition to these alterations in apoptotic pathways, Herrmann et al. [94] suggested the hypothesis that the defective clearance of apoptotic cells in SLE patients may reflect phagocyte dysfunction.

In fact, most studies in mice and humans point to a clearance defect in the pathogenesis of LE, as the genetic disruption of several eat me ligand/receptor systems (e.g. MFG-E8, MerTK) lead to the development of LE-like autoimmunity in mice [75, 95]. Additionally, genetic deficiencies for complement proteins, such as C1q, are strongly associated with the development of LE in humans [96]. Several aspects of LE pathogenesis could be attributable to “leftover” late apoptotic remnants, that is, autoantibodies characteristic of SLE are mainly directed against nuclear components, such as DNA and nucleic acid binding proteins (e.g. histones, SSA and SSB), and, intriguingly, these molecules are also found on membrane blebs that are formed upon induction of apoptosis [97]. Hence, defective clearance of these apoptotic blebs may allow access to these autoantigens, which are sequestered under physiological circumstances. In addition, the cytokine signature of LE patients, in particular, high levels of interferon- α (IFN- α), might be a direct result of DNA-containing debris that can activate plasmacytoid DCs to secrete high amounts of this cytokine [98]. Interestingly, a recent

study has indicated that beneficial aspects of glucocorticoid treatment in SLE may not be due to direct immunosuppressive properties of these drugs alone [99]. In addition, glucocorticoid treatment was shown to enhance the expression of the eat me signal MFG-E8 by macrophages and thereby to facilitate apoptotic cell engulfment.

Finally, defects in the anti-inflammatory signals of apoptotic cells may also facilitate the development or exacerbate the course of LE. A recently conducted study assessed the characteristics of apoptotic neutrophils and monocyte-derived DCs of SLE patients, their interaction and the influence of autoantibodies and inflammatory cytokines on this interaction [100]. In this study, apoptotic neutrophils and DCs from SLE patients showed no inherent defects compared to healthy controls, and the suppressive nature of their interaction was not affected. Autoantibodies as well as the inflammatory cytokines IL-17 and IL-1 β had no influence on the interaction in this set-up; however, IFN- α decreased the suppressive effect of apoptotic cells on DCs. Consequently, DCs under the influence of IFN- α continued to secrete high amounts of TNF and IL-6, which could add to the high level of these cytokines found in SLE patients and, in the case of TNF, induce additional apoptosis. These data suggest that aberrant immune reactivity in SLE is not generally due to a cell intrinsic defect, their processing or their interaction with DCs but likely arises from the milieu in which this interaction occurs. Moreover, this study highlights the importance of IFN- α during early stages of SLE and its potential as therapeutic target [101].

High levels IFN- α present in the serum may exert a dual, detrimental role: first, by inducing apoptosis directly or via the upregulation of death receptor pathway-associated molecules in keratinocytes and, second, by abrogating the anti-inflammatory properties of the generated apoptotic cells. Recently, Weyd et al. [67] demonstrated that the early exposure of annexin A1 constitutes a molecular mechanism by which apoptotic cells gain their immunosuppressive phenotype to induce peripheral tolerance and prevent the development of autoimmune diseases. Moreover, exposure of annexin A1 has also been suggested to facilitate the phagocytosis of apoptotic and secondary necrotic cells and to induce the recruitment of phagocytes [78, 102, 103]. It will be interesting to determine the effect of annexin A1 exposure on apoptotic cells with regard to the development of LE-like diseases. Interestingly, autoantibodies directed against eat me signals, such as PS, have been detected in the serum of CLE patients [104]. In a study by Kretz et al. [105], it was observed that levels of anti-annexin A1 antibodies were significantly higher in sera of CLE patients compared to sera of healthy controls and other connective tissue diseases. While anti-PS antibodies might disturb the association of PS with various bridging molecules, anti-annexin A1 antibodies could

directly inhibit an anti-inflammatory signal on apoptotic cells.

Taken together, an increased rate of apoptosis or defective clearance of apoptotic cells can lead to the appearance of excess apoptotic remnants which present a source of autoantigens and stimulate the immune systems by release of intracellular DAMPS. In addition, deficiency or inhibition of anti-inflammatory signals provided by apoptotic cells (e.g. by autoantibodies) might exaggerate these inflammatory stimuli and may, thereby, cause the development of autoimmunity.

“Molecular Mimicry”: Induction of Interferon in Autoimmune Conditions

Type I IFNs (α and β) are important players in the early activation of the immune system and have a specific role in the immunological interface between the innate and the adaptive immune system. In 1957, Isaacs and Lindenmann [106] discovered these cytokines after clinical observations of their anti-viral function and gave them their name because they “interfere” with virus growth in infected cell cultures. Type I IFNs can be produced by each cell via toll-like receptor (TLR) dependent and independent mechanisms, which recognise so-called pathogen-associated molecular patterns (PAMP), e.g. viral nucleic acids [101].

The IFNs also play a central role in the early activation of viral infections. In this case, PAMPs, particularly virus-associated DNA and RNA motives, are recognised either in the endosome (TLR-dependent) or in the cytosol (TLR-independent) [101]. The activation of these receptors induces a complex proinflammatory pathway including the activation of type I IFNs and IFN-associated proinflammatory cytokines (e.g. CXCL9, CXCL10, CXCL11), which orchestrate the recruitment of CXCR3⁺ effector cells (Fig. 2). In LE, this proinflammatory pathway may be inappropriately activated by endogenous nucleic acids, a faulty switching called “molecular mimicry” [107]. Presumably, different mechanisms can lead to the false activation of the IFN system in LE; however, the most studied mechanism is the stimulation of the IFN pathways in pDCs through immune complexes. Here, endogenous DNA or RNA, which is complexed with disease-specific autoantibodies (e.g. anti-dsDNA, anti-U1-RNP), is gathered by circulating pDCs in a CD32-dependent matter. The immune complexes end up in the endosome where their nucleic acids stimulate the innate immune system via TLR3, TLR7 or TLR9 [108, 109]. Importantly, UV-induced changes of the DNA result in modifications, which exponentiate their immunostimulatory capacity. In particular, UV-induced 8-OHG motifs are excellent stimulators of the IFN system and may be important in the pathogenesis of UV-induced LE skin lesions [110].

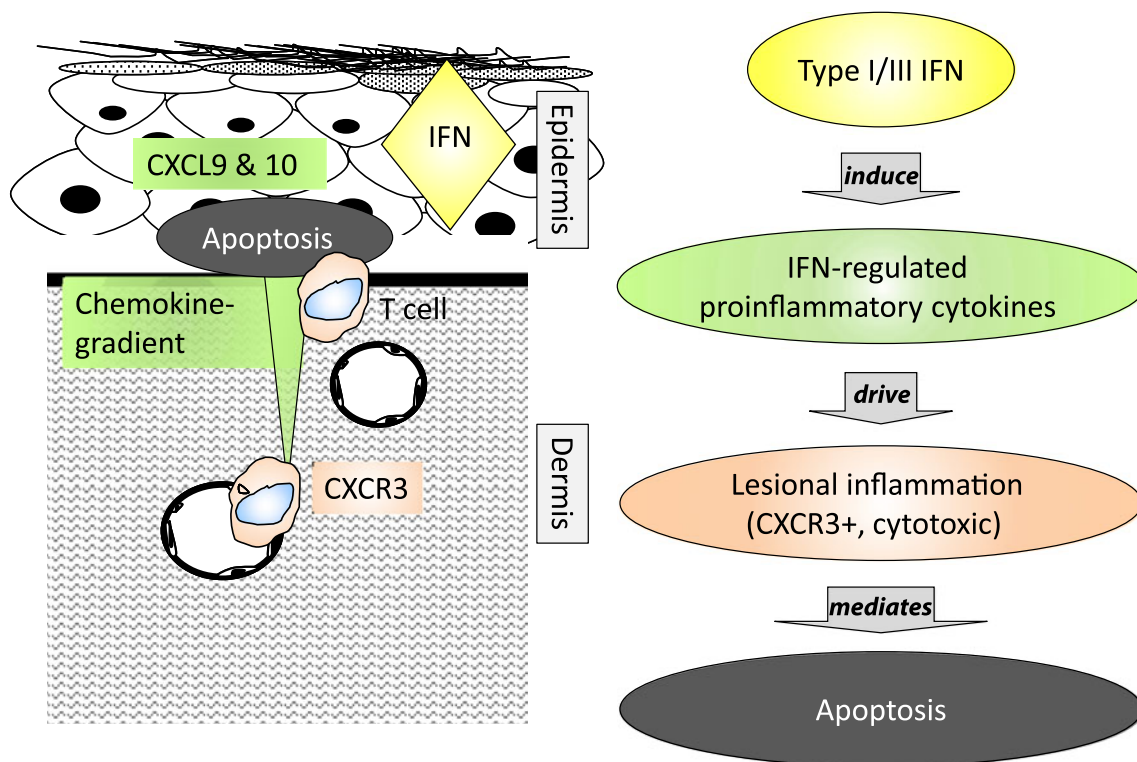


Fig. 2 Functional model of the IFN-driven cytotoxic inflammation in cutaneous lupus erythematosus. Type I/III interferons induce the expression of proinflammatory cytokines, including CXCL9 and CXCL10, in the skin which mediates the recruitment of effector cells along a chemokine gradient (*green wedge*) and also enhance their cytotoxic capacity. These

CXCR3+ effector cells migrate into the skin, infiltrate the basal epidermal layer and induce keratinocyte apoptosis, reflected by colloid bodies and hydropic degeneration in conventional histology of fully developed skin lesions of cutaneous lupus erythematosus

The Role of the Interferon System in Systemic Lupus Erythematosus

Observations that IFNs may also be important in autoimmune disorders, including LE, were made in the 1990s, when first purified, and then recombinant IFNs were used for the treatment of chronic viral infections and malignant tumors. In 1990, Rönnblom et al. [111] reported the induction of SLE by interferon-alpha treatment in a patient with a malignant carcinoid tumor. The patient developed myalgia and low-grade arthritis in multiple joints with a high titre of antinuclear antibodies (ANA) and anti-dsDNA antibodies. After the treatment was stopped, the symptoms subsided, although a moderate ANA titre persisted. Over the following years, several other case reports and smaller case series were published, supporting the hypothesis that type I IFNs may have a direct pathological function in the development of LE [112].

Additional evidence for a pathogenetic role of IFNs in SLE came from clinical studies focusing on the interrelation of IFN serum levels and disease activity in SLE patients. In 2005, Dall'era et al. [113] were able to demonstrate that type I IFN levels correlated positively with disease activity, as measured

by the SLEDAI score and anti-dsDNA levels, and inversely with C3 levels. Moreover, it became clear that flu-like symptoms, such as fever, muscle pain and fatigue, which are typically observed in SLE patients in stages of active disease, are mediated by type I IFNs [114]. This activation of the IFN system is also reflected by the expression of several IFN-regulated genes, the “IFN-signature”, in peripheral blood cells of SLE patients with active disease [115, 116]. These IFNs are mainly produced by plasmacytoid dendritic cells, which are “natural IFN-producing cells” and have the capacity to produce large amounts of these cytokine upon appropriate stimulation [109].

The Interferon System in Cutaneous Lupus Erythematosus

The first evidence for the involvement of the IFN system in CLE lesions was provided in 1995 by Fähr et al. [117], who found the MxA-protein, an antiviral mediator specifically regulated by type I/III IFNs, to be highly expressed within LE-specific skin lesions. At that time, the authors concluded that there was viral involvement or activation of the IFN

system by unknown mechanisms in CLE. Clinically, patients with CLE may present similar IFN-mediated symptoms, such as fever and fatigue. These symptoms are associated with disease activity and elevated expression of the IFN-regulated MxA protein in circulating peripheral blood cells in an amount that is comparable with tumor patients under treatment with recombinant IFN- α [118]. In skin lesions of patients with CLE, several IFN-regulated cytokines, including CXCL9 and CXCL10, have been shown to be highly expressed, similar to the IFN signature described in the peripheral blood of SLE patients [119]. The endogenous induction of the IFN system in autoimmune skin most likely depends on a similar mechanism found in SLE, including activation of plasmacytoid dendritic cells via TLR7 and TLR9 [120]. The fact that treatment with recombinant type I IFNs (α and β) can induce not only SLE but also CLE-like skin lesions provides additional evidence for a pathogenetic role of these IFNs in CLE [121].

Determination of Interferon Expression for the Histological Inflammatory Pattern in Cutaneous Lupus Erythematosus

The most common histological inflammatory patterns observed in skin lesions of CLE patients are the “patchy” pattern, which is typically observed in DLE and LET, and “interface dermatitis”, which is characteristic of all CLE subtypes with an epidermal involvement, including ACLE, DLE and SCLE [122]. IFN-producing plasmacytoid DCs (pDCs) are the cell population responsible for the patchy pattern, which presents histologically with dense perivascular and perifollicular infiltrates of lymphoid cells. pDCs are involved in a number of inflammatory skin diseases, including viral and autoimmune disorders. This cell population forms “clusters” in CLE, representing the main cell population responsible for the dense patchy inflammatory infiltrate [123]. These cells produce large amounts of IFNs, which in turn induce the expression of proinflammatory chemokines, including CXCL9, CXCL10 and CXCL11, driving the recruitment of CXCR3-positive effector cells into the skin lesions. CXCR3 is also expressed by pDCs, which are also recruited into the skin, providing additional resources for IFN production and therefore an ongoing “self-recruitment” of proinflammatory cells [124].

Similar IFN-mediated inflammatory mechanisms are most likely also important for the “interface-dermatitis” at the dermo-epidermal junction. Here, the IFN-mediated chemokine CXCL10 is the most prominent proinflammatory player, which is expressed in the hydropic degenerated parts of the basal epidermis, where CXCR3⁺ cytotoxic lymphocytes invade the basal epidermal layer and induce apoptosis [122]. CXCL10 thus determines the “interface-dermatitis”

pattern in CLE. Importantly, the cytotoxic lymphocytes, which invade the basal epidermal layer, carry IFN-regulated chemokines within their cytotoxic granules and drive the recruitment of CXCR3⁺ lymphocytes as well as IFN-producing pDCs. This presents another proinflammatory feedback loop, which is important to understand the chronic inflammation observed in CLE [124]. The primary source of IFN production in the junctional zone is another matter of intensive study. In addition to the pDCs, which are meanwhile the most established type I IFN producers in the skin, there is increasing evidence that keratinocytes may contribute to the IFN milieu in the skin. Keratinocytes are also highly potent producers of type III IFNs (IFN λ), which bind a receptor that is distinct from the IFN- $\alpha\beta$ -receptor but induce very similar proinflammatory pathways [125].

Type I Interferons Aggravates Cytotoxic Inflammation in Cutaneous Lupus Erythematosus

Type I IFNs drive the lesional inflammation in skin lesions of CLE patients by the induction of proinflammatory IFN-regulated chemokines. However, they also aggravate the effect of the immune response by upregulating cytotoxic proteins and supporting apoptotic pathways mediated by members of the “death-receptor” family. IFN- α is known to upregulate the expression of the cytotoxic molecules perforin and granzyme B [126] as well as the apoptosis receptor CD95 [127]. Moreover, IFN- α upregulates the expression of TRAIL, the TNF-related apoptosis-inducing ligand, on keratinocytes and PBMCs. TRAIL, which binds to certain death receptors, including the pro-apoptotic TRAIL-R1, is highly expressed within skin lesions of CLE patients [128].

Tumor Necrosis Factor-Alpha, Interferon and Lupus Erythematosus: the Yin and Yang of Inflammatory Responses

In addition to IFN-associated proinflammatory mechanisms, other proinflammatory mediators have been demonstrated to be involved in the complex proinflammatory network of CLE, including TNF α . However, the functional role of TNF α in CLE remains a matter of discussion. On one hand, TNF α seems to play a proinflammatory role in at least some CLE subsets. SCLE is associated with the TNF-alpha-308A polymorphism, and TNF has been shown to be highly expressed in refractory skin lesions of patients with SCLE [129]. Moreover, individual case reports demonstrate CLE patients who responded to anti-TNF α treatment [130]. On the other hand, several publications report LE-like skin lesions, including DLE, SCLE and chilblain LE, as side effects of anti-TNF α treatment

in patients with psoriasis and rheumatoid arthritis [131]. The latter observation may be explained by the fact that $\text{TNF}\alpha$ is an inhibitor of the IFN pathways *in vivo*. Iatrogenic $\text{TNF}\alpha$ -inhibition blocks this inhibition and promotes IFN-mediated immune responses. This indirect activation of the IFN pathway can induce lupus-like disease in genetically predisposed patients, a phenomenon that Fiorentino called the “Yin and Yang of TNF -inhibition” [132].

Therapeutic Options for Cutaneous Lupus Erythematosus

Several agents, such as glucocorticoids, chloroquine and hydroxychloroquine, azathioprine, cyclophosphamide and recently belimumab, are licensed for SLE and other immunologic diseases [133]. To date, however, no medication has been specifically approved for the treatment of CLE; therefore, off-label use of topical and systemic agents occurs for most patients with skin manifestations of the disease [134]. Antimalarials, such as chloroquine and hydroxychloroquine, are used as a first-line systemic therapy and are indicated when skin lesions are widespread, disfiguring, scarring or refractory to topical agents [135]. Topical steroids show a high efficacy in the treatment of single CLE lesions; however, due to the well-known side effects of topical steroids (e.g. atrophy, telangiectasia, steroid-induced rosacea-like dermatitis), application of steroids should be time-limited and preferably intermittent [136]. As CLE can be triggered by sun exposure, the prevention of skin lesions by consistent UV protection, such as photoresistant clothing and the application of broad-spectrum sunscreens, is important [137]. A recent randomised controlled trial demonstrated that the application of a broad-spectrum sunscreen prevents UV-induced skin lesions under standardised conditions [137]. In 20 patients with CLE and 10 healthy controls, defined areas of sunscreen-treated and sunscreen-untreated skin on the upper back were irradiated with combined UVA/UVB irradiation. Disease-specific skin lesions were induced by UVA/UVB light in the untreated areas of nine CLE patients; however, no specific eruptions or sun damage were observed in the sunscreen-treated areas in any of the CLE patients or in the control subjects. Histological analysis of skin biopsy specimens confirmed the clinical results, also seen for other sunscreens [136, 137]. These data underline the importance of UV protection to prevent the induction of skin manifestations of CLE.

The recent insights into the role of IFN-driven immune responses in the pathogenesis of LE opens the door to understanding the mechanisms of drugs established in the treatment of the disease and developing new target-oriented therapeutic strategies that focus on inhibiting the IFN pathway. The anti-

malarial drug chloroquine and the IFN and kinase inhibitors are good example of both groups, respectively. Chloroquine was developed during the 1930s as one of the first synthetic antimalarial drugs, as a successor of the “natural” quinine. Quinine had been used traditionally by the native population of South America for the treatment of several “fevers” for centuries and was commercialised by the fraternity of the Jesuits for malaria treatment. In parallel, quinine had been used for the treatment of DLE, as first documented by Payne in 1894. During the 1950s, several clinical studies supported the efficacy of antimalarial agents in the treatment of LE patients, particularly of chloroquine, which was best tolerated at that time, and during the following decades, chloroquine became the “gold standard” for treating the disease. However, the rationale behind this “antimalarial” treatment of an autoimmune disease was unclear until recently, in spite of the similarities between these disorders that occur from different sources. Both diseases, LE and malaria, are characterised by fever, activation of the innate immune system and development of ANA, including anti-dsDNA antibodies [138]. The fever in malaria is known to be induced by the release of nuclear material after the burst of infected body cells, which are mediated by the activation of receptors of the innate immune system; similar mechanisms are also known to be important in LE. The released nucleic acids, particularly haemazoin-bound DNAs, stimulate the innate immune system probably by at least three ways: (1) by TLR9 (CpG-rich DNA), (2) by the cytosolic DNA receptor (AT-rich DNA) and (3) by activation of the inflammasome via AIM2 [139].

Despite the progress in understanding the pathogenesis of both diseases, it remained unclear why antimalarials are effective in malaria-infected patients as well as in LE patients until Kuznik et al. [140] demonstrated that the release of immunostimulatory nucleic acids is a common pathomechanism in both conditions. Antimalarials, particularly chloroquine and quinacrine, highly effectively bind free nucleic acids and thereby inhibit their capacity to stimulate the innate immune system via TLR receptors [140]. Therefore, antimalarials act in LE by targeting the activation as well as the reactivation of the IFN system (through endogenous nucleic acids released after cell death in the proinflammatory vicious circle in LE [124]) but not by a direct “immunosuppressive” mechanism. This finding is in agreement with the clinical observation that treatment with antimalarials is a safe therapeutic strategy that causes only few to no opportunistic infections [141].

Summary

Despite the high frequency of photosensitivity in CLE patients, the exact mechanism by which UV irradiation influences the pathogenic course of the disease remains poorly

understood [9]. Different pathogenetic mechanisms, including the accumulation of apoptotic keratinocytes in the skin of CLE patients have been reported. For example, it has been shown that a higher number of apoptotic keratinocytes is present in the skin of patients after UV irradiation, not only in fully developed skin lesions but also during lesion formation 24 h after UVA and UVB exposure [81, 142]. This observation has led to the hypothesis that after UV irradiation, apoptotic cells accumulate in the skin of patients with CLE due to increased apoptosis or impaired/delayed clearance of apoptotic cells. The non-engulfed cells may undergo secondary necrosis and release proinflammatory compounds and potential autoantigens, which may contribute to the inflammatory micromilieu that leads to formation of skin lesions in the disease.

Identification of the IFN system as an important pathogenetic player in SLE and CLE initiated the development of new treatment strategies in the disease, with a focus on the activation of the innate immune system or downstream pathways. Direct inhibition of the type I IFN system in SLE was the rationale behind the development of the anti-IFN- α antibody sifalimumab (MEDI-545) and the IFN- $\alpha\beta$ -receptor antibody MEDI-546, both of which depress the IFN signature in vivo, although substantial data on their clinical efficacy has not been available until recently [143, 144]. Other strategies have focused on the activation of the IFN system by inhibiting TLR7 and TLR9 (CpG-52364, DV 1179 and IMO-3100) [145]. The JAK pathway also represents a potential therapeutic target in LE. Here, the recent development of the inhibitors CP-690,550 (tofacitinib) and R333 are of specific interest for the future treatment of CLE. Tofacitinib (CP-690,550) is a JAK1/JAK3 inhibitor that suppresses the production of type I IFN and the activation of interferon regulatory factor-7 [146]. R333 was designed as a topical JAK/SYK inhibitor for the skin and is now being tested in patients with DLE and SLE skin lesions (ClinicalTrials.gov, NCT01597050).

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