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Keratinocytes – amplifiers of immune responses in systemic lupus erythematosus

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Abstract

Purpose of Review: Epithelial cells have been acknowledged as important players in autoimmune diseases by directing and enhancing inflammatory responses. Here, we summarize recent publications that examine keratinocyte (KC) dysfunction and its contribution to cutaneous and systemic disease in systemic lupus erythematosus patients.

Recent Findings: Chronic upregulation of type I interferon (IFN) in KCs is a feature of both lesional and nonlesional lupus skin. This IFN rich environment modulates epidermal cell death responses and promotes inflammatory responses to UV light exposure. In addition, newer technologies such as single cell RNA-seq are informing our understanding of lupus-specific intercellular crosstalk and how this contributes to disease.

Summary: Recent discoveries in KC dysfunction in lupus skin include aberrant IFN responses to environmental stress, enhanced cytokine and chemokine secretion and epigenetic changes leading to increased cell death. Further research will enable precision therapies for lupus treatment.

Keywords

Cutaneous lupus erythematosus; skin; keratinocyte; interferon

Introduction

For decades, immune cells have been appreciated as critical mediators of tissue damage and inflammation in systemic autoimmune diseases. In systemic lupus erythematosus (SLE), discovery of dysregulation of myeloid cells, T cells and B cells has led to better understanding of pathophysiology, patient stratification and drug development (1). More recently, understanding of changes within each involved organ is identifying further mechanisms that contribute to disease. For cutaneous lupus erythematosus (CLE), one of the most common organ manifestations in SLE patients, recent data support that the cells that

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comprise the skin itself may also play a critical role in disease. Specifically, epithelial and stromal cells can initiate, promote and amplify inflammatory responses. In this review, we highlight recent studies underpinning the idea that keratinocytes (KCs) and other stromal cells potentiate inflammatory responses in lupus skin by interacting with each other and with immune and non-immune cells. We will address KC dysfunction and mechanisms of crosstalk between KCs and surrounding immune and non-immune cells that have recently been discovered.

The skin as an immune competent organ

Our outmost barrier of the human body comprises the epidermis, dermis and subcutis that is protective against chemical, biological, physical and microbial hazards. The epidermis contains multiple layers of keratinocytes which can be divided into the basal, spinous and granular layer terminating with the stratum corneum (2). Other cells that can be found in the epidermis are Langerhans cells, tissue residing memory T cells, and melanocytes. The dermis mostly contains fibroblasts and can be divided into the papillary dermis with vessels to nurture the epidermis and the reticular dermis which contains the hair apparatus. Furthermore, macrophages, dendritic cells, NKT cells, mast cells, tissue resident T cells, regulatory T cells and plasma cells are found in the dermal compartment(3). Tight regulation between dermal and epidermal cell populations and interaction with the skin microbiome maintains skin homeostasis. Importantly, disturbances in each of these processes and cell types can affect skin integrity and health.

Epidermal KCs act as sentinels for multiple environmental triggers (4, 5). Therefore, KCs are equipped with a wide range of signaling molecules, including pathogen-associated molecular pattern (PAMP) receptors and cytokine and chemokine receptors. In addition, KCs produce many of these signaling molecules themselves (6). In recent years, KCs have been described as ‘cytokinocytes’, thereby promoting the concept of epithelial cells as critical mediators to environmental stressors.

Cytokines and chemokines made by keratinocytes

Pathogen recognition receptors represent the most effective tool to detect potential danger signals outside and inside of a cell. KCs express multiple of these receptors, including surface and endosomal TLRs (TLR1–6, TLR9 and TLR10) (7), as well as intracellular receptors against nucleic acids (RNA sensors such as RIG-1 and MDA5 and the DNA sensors cGAS, ZBP1, STING, AIM2) and other damage-associated stimuli (NOD1/2, NLRP1/3/10) (6, 8–14). This range of receptors allows KCs to respond quickly to external stressors, to mount efficient innate immune responses, to switch towards different modes of cell death, and to communicate with other non-immune and immune cells. After pattern recognition, an arsenal of cytokines can be released by KCs, including type I and III interferons (IFN) as well as cytokines from various families such as IL-1 (IL-1A, IL1-B, IL-18), TNF, IL-6, IL-31, TGFB (TGFB1, TGFB2, TGFB3), and IL-17(e.g. IL-17, IL-36). Chemokines released by KCs include members of the CC (e.g. CCL2, CCL5, CCL20), CX3C (e.g. CX3CL1) and CXC (CXCL9, CXCL10, CXCL11)-family (6) that recruit cognate chemokine-receptor positive cells into the skin.

The role of keratinocyte-derived IFNs in lupus skin

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with a variety of clinical manifestations including CLE, arthritis, nephritis and CNS involvement. Lupus pathogenesis involves interactions between genetic predisposition, environmental factors such as UV light and smoking, and aberrant cell behavior leading to NETosis, nucleic acid recognition, cell death and autoantibody production. These alterations lead to organ infiltration by immune cells and tissue damage. In SLE and CLE, type I IFNs have emerged as critical mediators in pathogenesis. Their effects include education of myeloid cells, T cell activation, loss of tolerance, and sensitization of tissues against environmental stimuli (15). This is reflected by a persistent upregulation of IFN-stimulated genes in affected tissues, which has been dubbed the type I IFN signature. KCs express a broad range of type I IFNs including *IFNB, IFNK, IFNE* and type III IFNs (*IFNL*) (6). In SLE, both nonlesional and lesional skin KCs have been shown exhibit robust IFN production, especially IFNκ (16–18). Type I IFNs enhance UV-driven cytokine production and cell death, underlining their pathophysiologic role in photosensitivity seen in lupus (16, 17). Furthermore, IFNs contribute to autocrine loops including IL-6 secreted by KCs, resulting in a proinflammatory environment of the skin(17) (Figure 1A). The importance of type I IFN in CLE pathogenesis is further highlighted by striking beneficial effects of drugs targeting IFN signaling, which have shown excellent responses in cutaneous lupus, including refractory cases (19, 20).

Initiation of IFN production in KCs comes from several sources. Several pattern recognition receptors induce type I IFN in KCs. Endosomal TLR3, 8 and 9 induce type I IFN via IRF3 (21). In the cytoplasm, RNA recognition by MDA5 and RIG1 leads to IFN induction via MAVS. Double-stranded DNA is recognized by cGAS, which produces the second messenger cGAMP, leading to conformational change of STING at the ER, leading to a downstream signaling response that includes phosphorylation and activation of TBK1 and IRF3/7 to promote IFN expression and secretion (22, 23). Specific DNA ligands for cGAS include viral dsDNA and mitochondrial DNA (mtDNA) (24, 25). In SLE, mtDNA has also been identified as a critical activator of IFN responses in neutrophils and monocytes (26–28). cGAS-dependent IFN induction in the skin has been described in monogenic chilblain lupus driven by deficiency in three prime exonuclease 1 (TREX1) (29). Recently, it was discovered that the transformation of dsDNA into Z-conformation may provide enhanced cGAS activation through stabilization of Z-DNA via ZBP1 (30) (Figure 1A).

Upon type I IFN binding to its receptor (IFNAR), interferon-stimulated genes (ISGs) are produced conferring an antiviral state in the target cell (21). Some interferons behave as ISGs. Indeed, the chronic expression of KC-specific *IFNK* depends on initial IFN-β and IFNAR signaling, such as after nucleic acid sensing or UV exposure (31). Importantly, IFNκ is substantially reduced by anti-IFNAR treatment in KCs. Moreover, IFN-β regulates type III IFNs (IFN λ), which have been shown to also be upregulated in CLE lesions (31) and drive myeloid cell expansion and T cell activation in TLR7-mediated lupus (32). Hence, the interplay of increased IFNs culminates in chronic expression of other IFNs and ISGs, resulting in an IFN-rich, proinflammatory environment in lupus skin (Figure 1A).

Given the effects of IFN signaling, regulation of this response is critical. One important regulator is TRIM21 (encoding Ro52), a common autoantigen in lupus that is associated with photosensitivity and development of CLE(33). TRIM21 is a negative regulator of type I IFN downstream of nucleic acid sensing pathways as it ubiquitinates DDX41 and several IFN transcription factors (IRF3, 5 and 7) which then get degraded by the proteasome (34–36). In mice, systemic knockout of TRIM21 results in splenomegaly, enhanced total IgG levels and IFN-stimulated genes (ISG) in the blood and spleen (37). After UV exposure, TRIM21$^{-/-}$ mice exhibited enhanced cGAS-STING-dependent IFN expression in dermal fibroblasts and bone-marrow derived macrophages, suggesting TRIM21 is regulating systemic IFN responses after cutaneous UV exposure (37).

The IFN-inducible Z-DNA sensor ZBP1 has emerged as another cytoplasmic regulator of type I IFN responses and cell death upon viral infection (38, 39). ZBP1 has been shown to interact with cGAS upon mitochondrial damage in cardiomyocytes (30). In KCs, UV-light promotes B-DNA to Z-DNA transition through oxidative damage and release of mitochondrial DNA, which is then stabilized by ZBP1 to further drive IFN-responses in the skin (40) (Figure 1A). Notably, ZBP1 is upregulated in nonlesional and lesional lupus skin, correlates with ISG expression, and is critical for enhanced UV-induced IFN responses. Thus, in an IFN-rich environment as seen in SLE, the stabilization of mitochondrial Z-DNA through ZBP1 explains sustained IFN responses and cytokine secretion observed in patients with autoimmune photosensitivity (40). Intriguingly, mitochondrial DNA release is inhibited by TRIM21, which suggests that there could be an interplay of Z-DNA stabilization by ZBP1 and TRIM21-mediated inhibition of IFN signaling in lupus patients (41).

A hallmark of SLE is the production of autoantibodies against dsDNA. Recently, it was shown that SLE patients also harbor antibodies against Z-DNA- (42). The process by which Z-DNA serves as an autoantigen is currently unknown. Further studies will elucidate whether epidermal Z-DNA induces autoantibody production, further linking photosensitivity to systemic manifestations in SLE or whether other sources of Z-DNA drive autoantibody production in SLE.

Increased chemokine production in lupus keratinocytes

Another consequence of the chronic IFN-rich environment in lupus is the enhanced release of multiple chemokines that lead to immune cell skin infiltration. KC-produced CXCL9, CXCl10 and CXCL11 recruit CXCR3^{+} immune cells including macrophages, plasmacytoid dendritic cells (pDCs) and effector T cells into lesional skin (43). Cytotoxic CD8^{+} T cells contribute to epidermal damage through killing of keratinocytes. Recent work using a suction blister model investigated the contribution of chemokines in interface dermatitis using spectral flow cytometry, spatial transcriptomics and spatial proteomics (44). In this model, production of IFN-induced CXCR3 ligands was confirmed. In addition, KCs proximal to CLE lesions exhibited higher levels of AIM2, Caspase 8 and IL-18, which may contribute to induction of CCL8 and CXCL6 that may recruit myeloid cells to the skin. Specifically, CD14^{+}CD16^{+} APCs respond to CXCL6 via CXCR1, while CD14^{+} monocytes are responsive to CCL8 through CCR2(44) (Figure 1B).

Recent developments on the role of epidermal apoptosis and Hippo signaling in lupus skin

Responses to UV light are pro-inflammatory in lupus patient skin. Under a physiological dose, UVB triggers oxidative and genotoxic stress, mainly in the proliferative basal cells in the epidermis of the skin, resulting in “sunburn” or apoptotic keratinocytes (45). In lupus epidermis, exposure to UVB results in an increased immune cell infiltration associated with accumulated apoptosis-mediated cell death (46). The importance of cell death is emphasized by histologic features of CLE lesions showing dying KCs in the basal layer and recruitment of immune cells towards the dermo-epidermal junction (interface dermatitis).

Specific cell death responses in KCs have been investigated, particularly in response to UV light. Early studies on photosensitivity in lupus identified translocation of Ro52 to the outer cell membrane, which is mediated by oxidative stress after UV (47–50). This expression on the surface facilitates binding by autoantibodies (48, 49). Additionally, La/SSB antigens undergo redox-dependent conformational change, which promotes shuttling from the nuclear compartment into the cytoplasm(51). Furthermore, nuclear antigens and apoptotic bodies are found in the lupus band in 50% of patients, suggesting a deposition of debris in the basal membrane zone (52). Mechanistically, the type I IFN-rich environment found in lupus skin heightens UVB-induced apoptotic keratinocytes both *in vitro* and in murine models (53). Immortalized human keratinocytes (N/TERT cells) primed with IFN-α exhibit enhanced UVB-mediated apoptosis without changing other forms of cell death. Indeed, small-molecule inhibitors blocking pyroptosis, necroptosis and ferroptosis revealed that IFNα priming sensitizes keratinocytes to UVB-induced caspase-8 and IRF1-dependent apoptosis (53) (Figure 1A). This is also noted *in vivo as* overexpression of epidermal *Ifnk* in murine skin results in increased UVB-induced apoptotic keratinocytes (53). The propensity for apoptotic cell death may be linked to epigenetic dysregulation of Hippo pathway signaling in SLE KCs. This pathway is activated secondary to hypomethylation of *WWC1,* which leads to chronic phosphorylation of Yap in SLE lesional and non-lesional skin. This results in increased apoptotic gene expression and increased cell death in SLE KCs (54) (Figure 1A). Besides apoptosis, components involved in Hippo signaling also regulate cellular senescence (55). The accumulation of senescent cells has been described in epidermal progenitor cells of lupus lesions with a higher rate of senescence markers p16 and p21 in the basal layer (56). Senescence promotes inflammatory reactions through secretion of various proinflammatory cytokines such as IL-6, commonly termed as senescence associated secretory phenotype (SASP)(57). In TREX1-deficient lupus fibroblasts, higher rates of senescent cells are induced by UV exposure (29). Whether senescence is an early event in KCs that is involved in the pathogenesis of CLE/SLE or is a consequence of chronic environmental stress remains to be elucidated.

Keratinocyte crosstalk with immune cells

Epidermal Langerhans cells (LCs) serve a protective role for KCs as they release EGFR ligands, leading to keratinocyte differentiation, thereby protecting against UV-driven skin damage (58). In lupus, LCs are reduced and produce less EGFR ligands,

resulting in enhanced photosensitivity. Recent work identified that the type I IFN-rich environment reduces ADAM17 sheddase activity, which is required for production of EGFR ligands (Figure 1B). Inhibition of type I IFN restores ADAM17 function and reduces photosensitivity in lupus prone mice (59). Hence, IFNs released by KCs might influence surrounding cell such as LCs and the protective effects of IFNAR inhibitors in human lupus might be partially related to restoration of ADAM17 activity in LCs.

Newer ways to assess the immune infiltrates of tissues, including single cell RNA-sequencing, have provided us with more information on the transformation of immune cells in affected organs and the crosstalk that exists between stromal and epidermal cells with immune cells. Using these modalities, it was noted that that non-lesional SLE skin primes various immune cells towards pro-inflammatory phenotypes upon entry into the skin (18). Ligand-receptor analysis among all major skin populations revealed that nonlesional SLE skin exhibits more interactions than healthy controls, with KCs interacting more with myeloid cells and endothelial cells. Importantly, $CD16^+$ dendritic cells, which likely arise from non-classical monocytes in peripheral blood, undergo type I IFN education in the skin leading to inflammatory phenotypes. Indeed, lupus-prone NZM2328 mice exhibit increased cutaneous myeloid-derived dendritic cell infiltration in a type I IFN-dependent manner after UV light exposure(60). The IFN-rich environment also affects T cells, which exhibit high ISG expression in nonlesional skin compared to healthy controls. Type I IFNs promote Treg dysfunction, thereby contributing to dysregulated immune homeostasis, including after UV light exposure (18, 61). Thus, immune responses initiated by SLE KCs in healthy-appearing skin contribute to systemic immune cell education.

The spectrum of cutaneous lupus lesions is very broad including acute, subacute (SCLE) and chronic discoid (DLE) lesions. A recent study assessed the immune cell composition in SCLE versus DLE by imaging mass cytometry(62) : Distribution and percentages of immune cells in SCLE and DLE did not differ. However, DLE exhibited higher levels of TBK1, indicating potential for increased IFN signaling in DLE. Furthermore, individuals who smoke exhibited enhanced lesional neutrophil recruitment and increased Granzyme B expression in endothelial cells, providing a mechanism by which smoking enhances propensity for treatment refractoriness in CLE(62). Importantly, plasmacytoid dendritic cells (pDCs), thought to be an important producer of type I IFN in lesional skin, lacked pSTING and *IFNB* expression. In contrast, other leucocytes including classical monocytes, intermediate monocytes and macrophages exhibited higher type I IFN production(62). Comparison of IFNκ production by KCs and dendritic cells found similar levels of IFNκ production in both cell types(63). These data highlight the role of KCs as cytokinocytes in CLE and suggest that treatment should target both epidermal and immune sources of inflammatory mediators.

The role of B cells in CLE has been highlighted in studies with enrichment of B cell signatures in DLE compared to SCLE(64, 65). Upon transfection of immunostimulatory DNA, KCs release B lymphocyte stimulator (BAFF) which could lead to survival of B cells(66). However, direct crosstalk between KCs and B cells needs to be further explored. The data examining immune cell → KC crosstalk is less robust yet suggests a role for bidirectional contributions to disease. For instance, interface dermatitis is promoted by

destruction of KCs through $CD8^+$ T cells and a type I immune response through IFNγ (67, 68). Moreover, CLE harbors significantly higher amounts of neutrophil extracellular traps, which may be associated with increased tissue damage and a scarring phenotype. Neutrophils were identified as ROS producers in CLE, which might further promote tissue damage (69). However, the precise role of neutrophils in the pathogenesis of CLE has to be further studied. Also, future studies should investigate crosstalk between different immune cells as well as between immune cells and epidermal cells.

Keratinocyte crosstalk with non-immune cells in the skin

The interactions of keratinocytes with other skin cells contribute to the diverse clinical manifestations of skin immune responses. This has been understudied in the context of SLE and other autoimmune skin diseases.

Keratinocyte-fibroblast interaction in SLE

Studies using skin equivalents depict the importance of fibroblasts, as there is disturbed keratinocyte maturation, differentiation, significant epidermal thinning, and compromised skin barrier function in the absence of fibroblasts. (70). Methods of communication include direct cell-cell contact, paracrine, and vesicle-mediated signaling. For example, extracellular vesicles (EVs) from keratinocytes contain integrins and accessory molecules that mediate communication with fibroblasts during wound healing to influence tissue repair and regeneration (71). In addition, keratinocyte-derived microRNA-21-containing microvesicles (miR-21-MVs) induce a proinflammatory response, promote fibroblast migration, and enhance fibroblast-mediated angiogenesis (72). In SLE and CLE patients, we are only just beginning to understand stromal interactions in the skin. Fibroblasts in non-lesional and lesional skin of SLE patients with CLE exhibit pro-inflammatory phenotypes and an IFN-educated transcriptional signature (Fig 1B). These IFN-educated fibroblasts are primarily localized to the dermal-epidermal junction indicting they may be primarily educated by basal, IFN-secreting inflammatory KC.(18) Importantly, the fibroblasts isolated from SLE patients exhibit a hyper-inflammatory response when compared to healthy control fibroblasts, indicating that SLE fibroblasts are primed to over-respond when exposed to KC-derived cytokines and chemokines(73). Importantly in patients who develop discoid lupus erythematous lesions, the response to mediators such as TGFβ is a pro-fibrotic response, indicating fibroblasts from SLE patients with DLE may misinterpret signals from KCs to lead to scar formation(73).

Keratinocyte melanocyte interaction in SLE

Melanocytes and KC also participate in bi-directional communication via cell-cell contact resulting in increased melanization of KC after exposures such as UV light (74, 75). EVs also contribute to this communication pathway as keratinocyte-derived small EVs (sEVs) enhance melanin transfer from melanocytes to keratinocytes by increasing dendrite formation and activating MITF(74), carry signaling micro-RNAs (76) and enhance melanocyte proliferation, tyrosinase activity, and melanin synthesis(77). When KC/melanocyte crosstalk was examined in SLE patients with active skin disease, no change in KC → melanocyte crosstalk was noted (18, 78, 79). However, increased predicted

melanocyte → KC crosstalk was noted in DLE patient lesions. Specific ligands were not provided to better understand the skewing of this crosstalk (Figure 1B). These data indicate that there could be DLE-specific melanocyte/KC interactions that result in phenotypic differences from other types of CLE lesions. This is intriguing given the depigmentation seen in SLE patients. EV-mediated KC/melanocyte crosstalk in SLE has not yet been examined. Thus, the significance of melanocytes and their interaction with keratinocytes in SLE remains incompletely understood.

Conclusions

Understanding KC dysfunction and crosstalk to immune and non-immune cells has broadened our understanding of lupus pathogenesis (Figure 1A, B) and provided targets to more precisely and effectively treat lupus. Continued research, including utilization of newer single cell technologies, will continue to expand our understanding of lupus pathogenesis and how non-immune cells contribute to disease.

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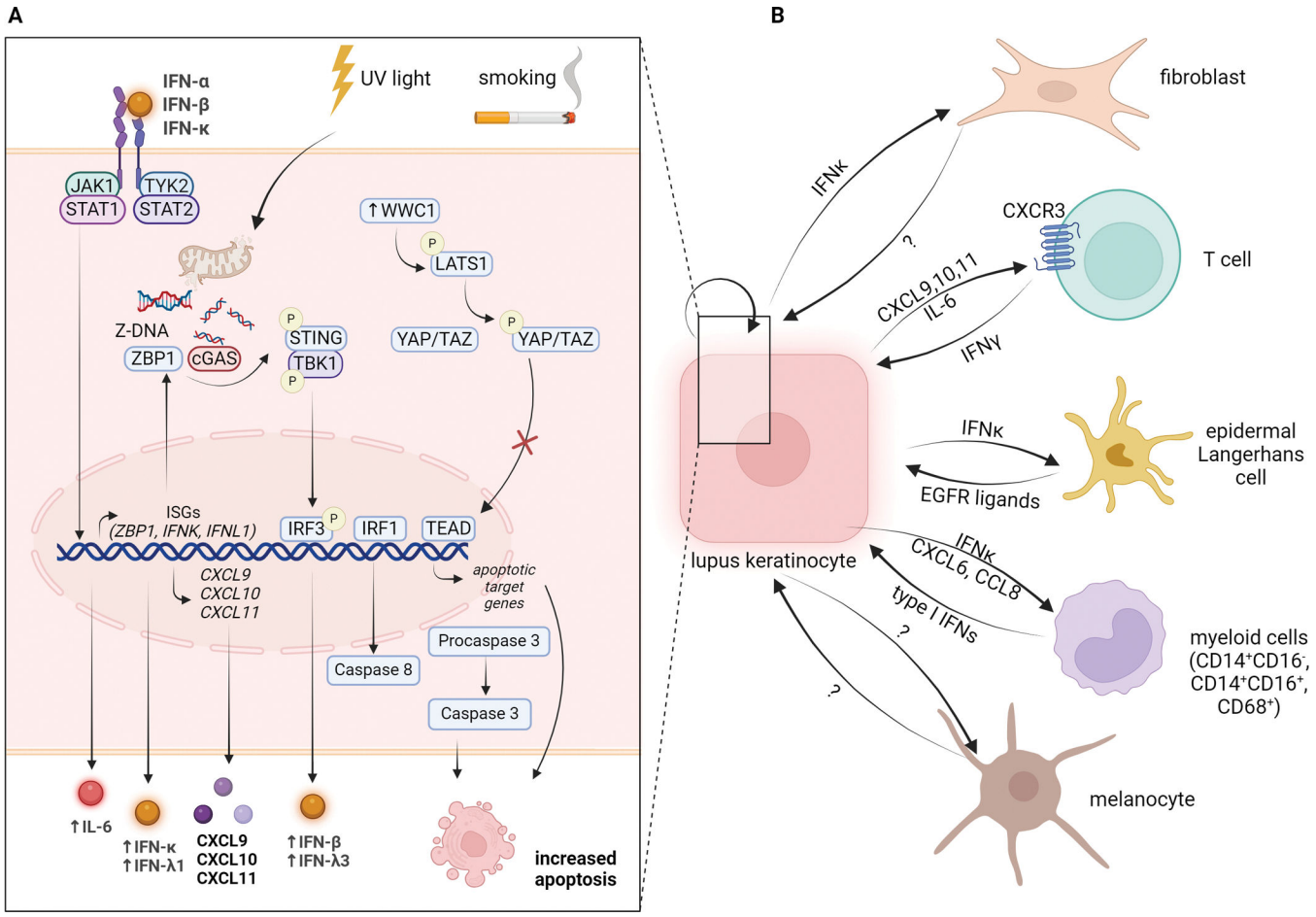

Figure 1. Dysfunction and crosstalk of lupus keratinocytes.

A. Recently identified pathways contributing to KC dysfunction in lupus skin. Chronic autocrine type I IFN signaling mediates expression of ISGs, notably *IFNK, IFNL1* and *ZBP1 and drives* a chronic loop of IL-6 and chemokine (*CXCL9, CXCL10, CXCL11*) secretion. Environmental stress such as UV light contributes to accumulation of cytosolic DNA, which is recognized by cGAS (B-DNA) and ZBP1 (Z-DNA). These interactions induce a signaling cascade that includes STING, TBK1 and IRF3, resulting in heightened IFN gene expression (*IFNB1, IFNL3*). Chronic IFN signaling also leads via IRF1 to upregulation of caspase 8, which promotes apoptotic cell death through cleaved caspase 3. The Hippo signaling pathway also contributes to cell death, where increased WWC1leads to LATS1 kinase phosphorylation of YAP and cytosolic retention of YAP/TAZ. thus, YAP/TAZ binding to TEAD is reduced, and apoptotic gene expression is promoted.

B. Selected mediators implicated in crosstalk between lupus KCs and immune/non-immune cells in the skin. KC-derived IFNK leads to ISG-high fibroblasts and recruitment of IFN-rich myeloid cells into the skin. Chemokines such as CXCL9,10 and 11 attract CXCR3+ T cells which contribute to tissue damage by secretion of IFNγ. Epidermal Langerhans cells protect KCs upon UV exposure but are reduced in lupus skin. The KC-

derived chemokines CXCL6 and CCL8 further recruit myeloid cells into the skin. Crosstalk between KCs and melanocytes has not yet been studied.