Evidence for a Pathophysiological Role of Keratinocyte-Derived Type III Interferon (IFNλ) in Cutaneous Lupus Erythematosus

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Type I IFNs (IFNα/β) have been shown to have a central role in the pathophysiology of lupus erythematosus (LE). The recently discovered type III IFNs (IFNλ1/IL29, IFNλ2/IL28a, IFNλ3/IL28b) share several functional similarities with type I IFNs, particularly in antiviral immunity. As IFNλs act primarily on epithelial cells, we investigated whether type III IFNs might also have a role in the pathogenesis of cutaneous LE (CLE). Our investigations demonstrate that IFNλ and the IFNλ receptor were strongly expressed in the epidermis of CLE skin lesions and related autoimmune diseases (lichen planus and dermatomyositis). Significantly enhanced IFNλ1 could be measured in the serum of CLE patients with active skin lesions. Functional analyses revealed that human keratinocytes are able to produce high levels of IFNλ1 but only low amounts of IFNα/β/γ in response to immunostimulatory nuclear acids, suggesting that IFNλ is a major IFN produced by these cells. Exposure of human keratinocytes to IFNλ1 induced the expression of several proinflammatory cytokines, including CXCL9 (CXC-motiv ligand 9), which drive the recruitment of immune cells and are associated with the formation of CLE skin lesions. Our results provide evidence for a role of type III IFNs in not only antiviral immunity but also autoimmune diseases of the skin.

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INTRODUCTION

Inappropriate activation of the type I IFN system (IFNα/β) is a key feature of lupus erythematosus (LE). Patients with active systemic LE (SLE) present with flu-like symptoms including fever and myalgia, which reflect enhanced IFNα serum levels and correlate positively with SLE disease activity (Dall'era *et al*., 2005). Additionally, an exacerbation or aggravation of SLE has been reported following therapeutic administration of recombinant IFNα (Ronnblom and Alm, 2003). In cutaneous LE (CLE), large numbers of IFN-producing plasmacytoid dendritic cells (pDC) can be detected within LE skin lesions (Blomberg *et al*., 2001; Farkas *et al*., 2001). Type I IFN-inducible chemokines are strongly expressed in CLE skin lesions and their expression pattern reflects the typical histological pattern found in the different CLE subsets (Wenzel *et al*., 2007). IFN-regulated proinflammatory cytokines, including CXCL9 (CXC-motiv ligand 9) and CXCL10 (CXC-motiv ligand 10), have been shown to drive CLE skin inflammation (Meller *et al*., 2005; Wenzel *et al*., 2005b; Sontheimer, 2009). It is assumed that apoptotic material containing immunostimulatory nucleic acids is released during cell stress reactions and initiates the inflammatory response in LE. Complexed nucleic acids are internalized by specialized DCs and stimulate type I IFN-production via Toll-like receptor (TLR)-dependent and TLR-independent mechanisms (Ronnblom and Pascual, 2008; Meyer, 2009).

Recently, a new class of IFNs, the type III or "λ" IFNs, has been discovered as a unique cytokine family with antiviral function (Kotenko *et al*., 2003; Sheppard *et al*., 2003). IFNλ1 (IL29), IFNλ2 (IL28a), and IFNλ3 (IL28b) genetically and structurally belong to the IL10 family of cytokines. The IFNλs share many functions and mechanisms with type I IFNs, but they differ substantially with respect to their targeted cells, with IFNλ mainly acting on epithelial cells (Sommereyns *et al*., 2008). Accordingly, it has been reported that type III IFNs are essential for the antiviral response following viral infection of epithelial cells (e.g., intranasal influenza A and vaginal herpes simplex virus type 2), whereas intraperitoneal infection with hepatotropic viruses evoke an antiviral response depending on type I IFNs but not on type III IFNs (Ank *et al*., 2008).

The type III IFNs bind to a heterodimeric receptor composed of the IFNλ-specific IFNλ receptor (IFNλR) selectively expressed in certain cell types and the ubiquitously expressed IL10Rβ, which is also a part of the receptors for IL10, IL22, and IL26. However, although type I and type III IFNs bind unrelated heterodimeric receptors, type III IFNs

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Abbreviations: CDLE, chronic discoid lupus erythematosus; CLE, cutaneous lupus erythematosus; CXCL9, CXC-motiv ligand 9; DC, dendritic cell; LE, lupus erythematosus; PBMC, peripheral blood mononuclear cell; SCLE, subacute CLE; SLE, systemic LE; TLR, Toll-like receptor

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display type I IFN-like activity. They also signal through JAK1/TYK2 (Janus kinase 1/tyrosine kinase 2) tyrosine kinases and activate STAT1 and STAT2 (signal transducers and activator of transcription 1 and 2) (Sommereyns *et al.*, 2008), leading to the expression of ''IFN-stimulated genes'' such as myxovirus resistance 1, oligoadenylate synthetase, and interferon-inducible protein 27 (IFI27) that mediate antiviral effects of both IFN classes (Zhou *et al.*, 2007; Ank *et al.*, 2008).

Because of the similarity between the type I and the type III IFN system and the fact that IFNλ particularly acts on epithelial cells, we hypothesized that an activation of the type III IFN system in keratinocytes might contribute to the proinflammatory network of CLE.

RESULTS

Expression of IFNλ and the IFNλR is enhanced in CLE skin lesions

Initially, we analyzed skin biopsies taken from untreated chronic discoid LE (CDLE) and subacute CLE (SCLE) skin lesions ($n=16$) and healthy controls ($n=6$) for the expression of IFNλ and the IFNλR by immunohistochemistry. As shown in Figure 1, a significant expression of IFNλ was detectable in the lesional epidermis of both CDLE and SCLE patients. In the dermis, IFNλ was expressed by some mononuclear cells and weakly by endothelial cells. The strongest IFNλ expression was seen in active skin lesions taken from sun-exposed areas from patients with SCLE. Little expression was detected in healthy controls. The IFNλR was also strongly expressed in the epidermis of CLE lesions. Constitutive weak expression was found in the upper epidermis of healthy controls. Epidermal expression of IFNλ and the IFNλR was not enhanced in atopic dermatitis and only weakly in psoriasis, but significantly elevated in dermatomyostis and lichen planus skin samples.

Elevated IFNλ1 serum levels are found in patients with active CLE

We next investigated IFNλ1-serum levels in patients with active CLE (CDLE and SCLE) by ELISA. As shown in Figure 2a,

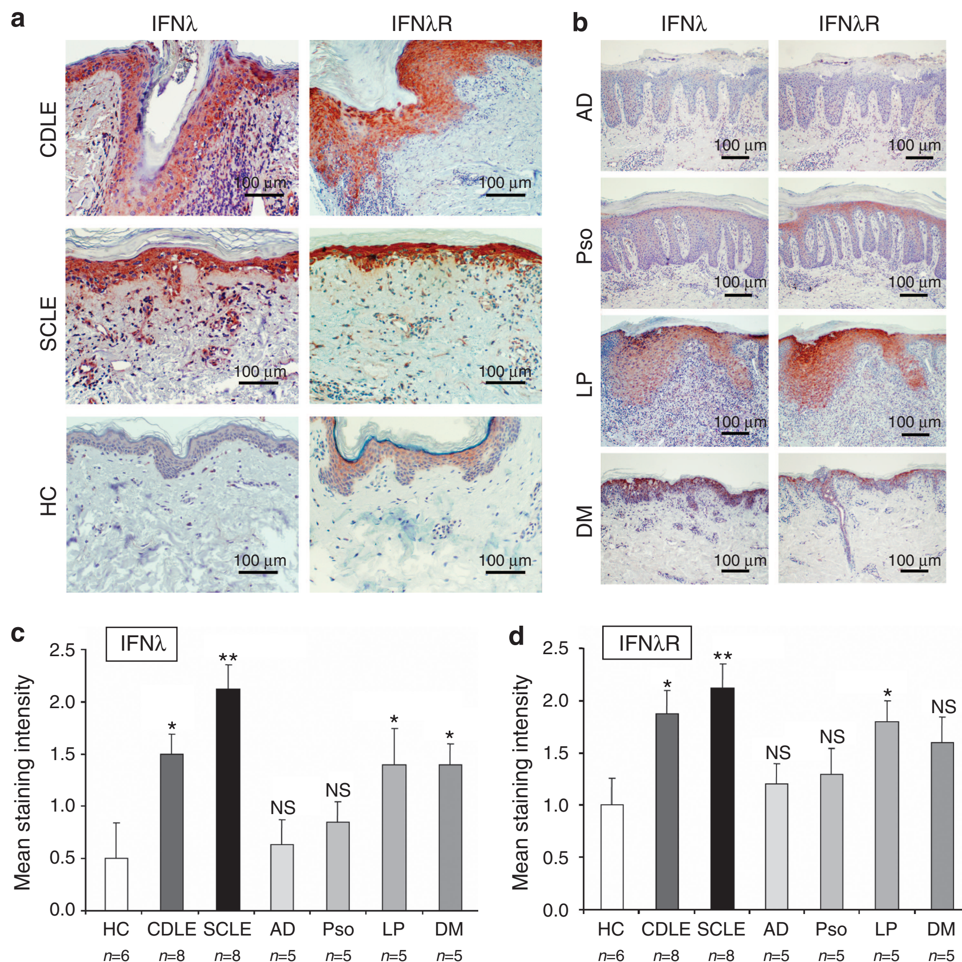

Figure 1. Expression of IFNλ and the IFNλ receptor (IFNλR) in cutaneous lupus erythematosus (CLE) and other inflammatory skin disorders. (**a**) Representative immunohistological micrographs of skin specimens of patients with chronic discoid LE (CDLE), subacute CLE (SCLE), and healthy controls (HCs) stained for the lesional expression of IFNλ and the IFNλR (scale bar = 100 μm). (**b**) *In vivo* expression of IFNλ and the IFNλR in additional inflammatory skin diseases, including atopic dermatitis (AD), psoriasis (Pso), lichen planus (LP), and dermatomyositis (DM) (scale bar = 100 μm). (**c, d**) Semiquantitative analysis of the expression of (**c**) IFNλ and the (**d**) IFNλR in healthy controls, CDLE, SCLE, and other inflammatory skin diseases. Shown is the mean staining intensity within the subsets ± SEM. Statistical analyses were performed using the Mann–Whitney *U*-test (*$P<0.05$, **$P<0.01$; NS, not significant).

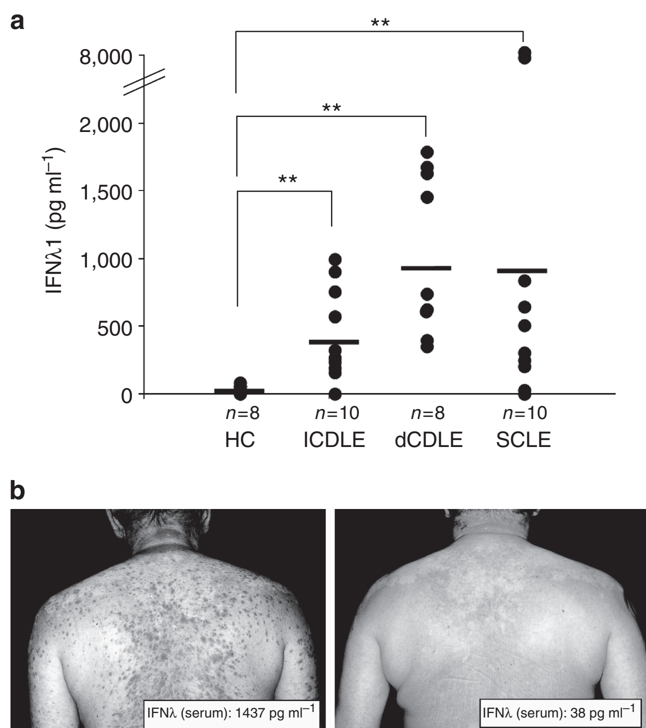

Figure 2. Serum levels of IFNλ1 in patients with cutaneous lupus erythematosus (LE). (**a**) Shown are IFNλ1 serum levels in individual patients with localized chronic discoid LE (lCDLE), disseminated chronic discoid LE (dCDLE), subacute cutaneous LE (SCLE), and healthy controls (HCs). The bars indicate the mean value of the different subsets ($^{**}P<0.01$; Mann–Whitney *U*-test). (**b**) Clinical findings and IFNλ1 serum levels in a representative patient with dCDLE before and after treatment.

the majority of patients with active CLE showed elevated IFNλ1 serum levels. The highest levels were found in patients with widespread skin lesions. A comparison of patients with disseminated CDLE lesions and patients where the disease was limited to one body region (localized CDLE) supported the notion that the extent of the disease correlates with the IFNλ1 serum levels. In one representative patient with exacerbated disseminated CDLE, we followed the IFNλ1 serum levels over a time period of 1 year (Figure 2b). These levels declined parallel to the clinical remission following systemic treatment with corticosteroids and hydroxychloroquine.

Production of IFNλ1 by human keratinocytes following TLR stimulation

Our immunohistological analyses demonstrated IFNλ expression in CLE particularly within the epidermis, suggesting that keratinocytes might be the producers of this cytokine *in vivo*. To verify this hypothesis, we investigated IFNλ expression in epidermal explants *in vitro*. Because immunostimulatory nuclear acids are thought to induce IFN expression in LE through TLR activation (Ronnblom and Pascual, 2008) and as TLR stimulation also induces type III IFNs (Ank *et al.*, 2008), we exposed epidermal explants to synthetic immunostimulatory nucleic acids (double-stranded RNA/polyriboinosinic polyribocytidylic acid (polyIC) and double-stranded DNA/CpG-oligodeoxynucleotide). polyIC induces expression of the IFNλ1 protein in the explanted epidermal sheets (Figure 3a). The immunohistochemical studies were confirmed by ELISA analyses, which revealed a significantly enhanced concentration of IFNλ1 ($P<0.01$) in the culture supernatant (Figure 3b). Interestingly, polyIC treatment of the epidermal explants did not induce detectable amounts of IFNα, IFNβ, and IFNγ.

Further evidence was obtained with cultured human keratinocytes. As shown in Figure 3c, IFNλ1 was secreted into the culture supernatant following stimulation with polyIC. Again, only very low levels of IFNα and IFNβ were detected. Analyses of vesicular stomatitis virus-infected keratinocytes and detection of CXCL10 levels served as positive controls (Lebre *et al.*, 2007; Ank *et al.*, 2008). Additional semiquantitative reverse transcriptase-PCR analyses demonstrated increased expression levels of both IFNλ1 and the IFNλR in polyIC-treated human keratinocytes, and also in vesicular stomatitis virus-infected cells (Figure 3d). In accordance with our immunohistological analyses, we found that IFNλR mRNA was constitutively expressed at low levels in unstimulated keratinocytes.

IFNλ treatment of keratinocytes induces the expression of proinflammatory cytokines

It has been reported that keratinocytes express the IFNλR and respond to IFNλ stimulation by phosphorylation of STAT1 and upregulation of the major histocompatibility complex I (Witte *et al.*, 2009). Therefore, we hypothesized that IFNλ might have an autocrine proinflammatory effect on keratinocytes. Indeed, exposure of human keratinocytes to recombinant IFNλ1 significantly enhanced the secretion of IL6, IL8, CCL3, and CXCL9 into the culture supernatant (Figure 4a). However, IFNλ1 treatment had no effect on CXCL10 production by cultured keratinocytes.

Culture supernatants of IFNλ1-stimulated keratinocytes drive the recruitment of peripheral blood mononuclear cells (PBMCs)

To investigate the relevance of type III IFNs on the recruitment of immune cells, we subsequently tested the functional effect of culture supernatants harvested from stimulated and unstimulated keratinocytes on the migration of PBMCs in an *in vitro* transwell system. As demonstrated in Figure 4b, supernatants of IFNλ1-treated keratinocytes had a significantly higher capacity to recruit PBMCs than untreated controls. Supernatants of polyIC-stimulated keratinocytes served as positive controls.

IFNλ and the IFNλ-inducible proteins CXCL9, MxA and, IFI27 show a similar epidermal expression pattern in CLE skin lesions

We previously reported differences in the epidermal expression pattern of the chemokines CXCL9 and CXCL10 in CLE skin lesions (Wenzel *et al.*, 2007). CXCL9 is typically expressed within the whole epidermis, whereas CXCL10 is predominantly found in basal epidermal areas in CLE skin lesions with an extensive interface dermatitis. Based on our observation that CXCL9 but not CXCL10 was produced by IFNλ1-treated keratinocytes *in vitro*, we hypothesized that

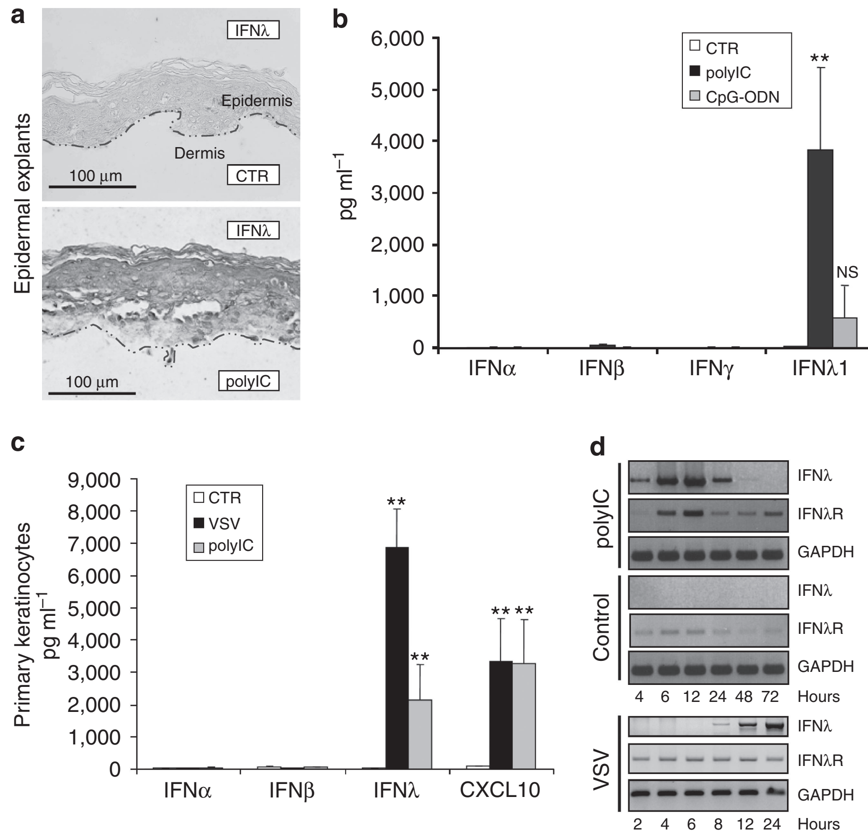

Figure 3. Induction of IFNλ1 expression in explanted epidermal sheets and cultured primary human keratinocytes. (**a**, **b**) Explanted epidermal sheets ($n=5$) were stimulated with polyIC and CpG-oligodeoxynucleotide (CpG-ODN). (**a**) Depicted are representative immunohistological micrographs of epidermal IFNλ expression in untreated (top) and polyIC-treated (bottom) specimens (original magnification ×400, scale bar = 100 μm). (**b**) Shown is the mean concentration (±SEM) of IFNα, IFNβ, IFNγ, and IFNλ1 in the supernatants of stimulated and unstimulated (CTR) cultures after 24 hours ($n=5$, $^{**}P<0.01$). (**c**, **d**) Primary human keratinocytes were stimulated with polyIC ($n=6$), or infected with vesicular stomatitis virus (VSV) ($n=5$). (**c**) Shown is the mean concentration (±SEM) of IFNα, IFNβ, IFNλ1, and CXCL10 in the supernatants of stimulated and unstimulated cultures ($^{**}P<0.01$; Mann–Whitney *U*-test). (**d**) Representative result of a reverse transcriptase-PCR (RT-PCR) analysis of IFNλ1 and IFNλ receptor (IFNλR) mRNA expression in untreated, polyIC-stimulated, and VSV-infected keratinocyte cultures over time.

the individual expression pattern of these chemokines might correlate with that of IFNλ and IFNα. In support of this idea, we analyzed the CLE skin samples by immunohistochemistry for the expression pattern of IFN-inducible proteins, including MxA, IFI27, CXCL9, and CXCL10, and the expression of type I and type III IFNs. As depicted in Figure 5, CXCL9 and IFNλ were coexpressed in the whole epidermis. The same was true for MxA and IFI27, the antiviral proteins that are induced by both type I and type III IFNs (Doyle *et al.*, 2006; Mordstein *et al.*, 2008). CXCL10, which is not induced by type III IFNs, was only found along the dermo-epidermal junctional zone. In these areas, CD3+ effector T cells, which have been shown to carry CXCL10 within their cytotoxic granules (Wenzel *et al.*, 2007), as well as blood dendritic cell antigen 2 (BDCA2)+ pDCs were found along with an expression of IFNα. Thus, the expression pattern of CXCL9 correlates with that of IFNλ, and the pattern of CXCL10 with that of IFNα.

DISCUSSION

Our observations provide several lines of evidence that keratinocyte-derived IFNλ has a role in the pathophysiology of CLE. First, IFNλ was strongly expressed in the epidermis of CLE skin lesions. Second, enhanced IFNλ1 levels could be measured in the serum of patients with active disease. Third, the lesional expression pattern of IFNλ correlates with that of the IFNλ-inducible chemokine CXCL9, compatible with the notion that IFNλ drives the inflammatory recruitment of immune cells. Based on our *in vitro* studies with explanted epidermal sheets, cultured keratinocytes, and cell migration analyses, it is highly plausible that the IFNλ1 in CLE patients derives from keratinocytes and supports the formation of inflammatory skin lesions.

Originally, the type III IFNs were discovered because of their antiviral function. The observation that the loss of antiviral protection in IFNαβ-receptor-deficient mice is variable for different viruses prompted a search for complementary antiviral systems (Kotenko *et al.*, 2003). This led to the identification of the type III IFN system consisting of IFNλs and the IFNλR that share many antiviral functions with the type I IFN system. Importantly, IFNλ was found to mainly act on epithelial cells and appears to have a critical role in epithelial antiviral defense (Sommereyns *et al.*, 2008). For

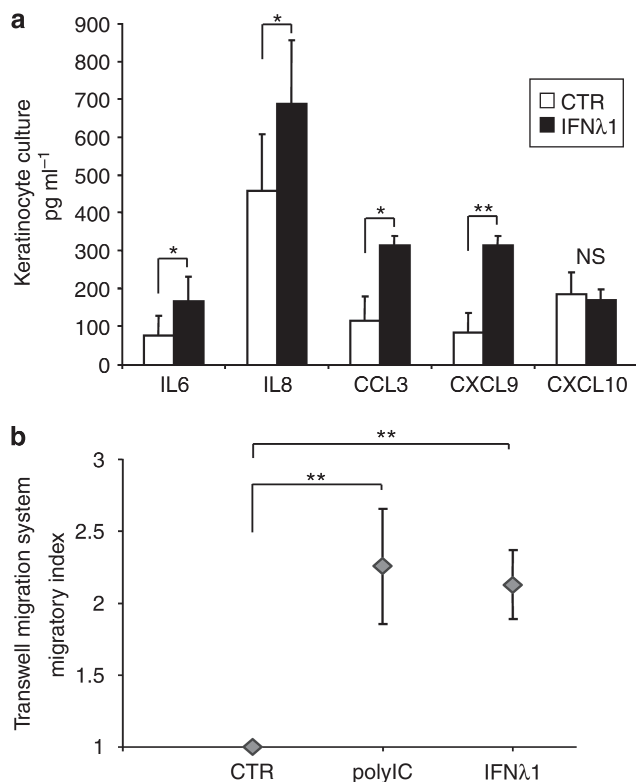

Figure 4. IFNλ1 stimulation of human keratinocytes induces the secretion of proinflammatory cytokines and mediates immune cell migration. (**a**) Primary human keratinocytes were stimulated with recombinant IFNλ1. Shown is the mean concentration (±SEM) of IL6, IL8, CCL3, CXCL9, and CXCL10 (chemokine (C-X-C motif) ligands 9 and 10) in supernatants of IFNλ1-treated and untreated cultures after 24 hours ($n=5$, $*P<0.05$, $**P<0.01$; NS, not significant). (**b**) The migratory effect of supernatants harvested from IFNλ1- and polyIC-stimulated keratinocytes on the recruitment of peripheral blood mononuclear cells (PBMCs) was analyzed in an *in vitro* transwell system. Depicted is the mean migratory index (=specifically migrated/unspecifically migrated cells)±SEM of five independent biological replicates.

example, IFNλ treatment prevents herpes simplex virus type 2 replication in the vaginal mucosa more effectively than IFNα (Ank *et al.*, 2008). In accordance with these observations, we found that keratinocytes are able to produce considerable amounts of IFNλ1 after infection with vesicular stomatitis virus and also following incubation with polyIC, which imitates a viral infection and activates the innate immune system through TLR3 and melanoma differentiation-associated gene 5 (MDA5; Gitlin *et al.*, 2006). Interestingly, we observed that stimulated keratinocytes produce high levels of IFNλ1, but only low levels of IFNα, IFNβ, and IFNγ, suggesting that IFNλ is a major IFN of human epidermal cells in direct response to viral stimuli.

In recent years, it has been demonstrated that type I IFNs, the major antiviral defense system of the body, have an important role in the pathophysiology of both SLE and CLE (Ronnblom *et al.*, 2006; Wenzel and Tuting, 2007). High IFNα serum levels, which are characteristic for acute viral infections, have also been observed in acute SLE. Evidence has accumulated that the activation of the type I IFN system in SLE involves the stimulation of TLR7 and 9 through DNA/RNA-autoantibody complexes, thereby imitating the process that alerts the immune system to the presence of viral nucleic acids (Ronnblom and Pascual, 2008). Our results provide evidence that activation of the type III IFN system in the epidermis not only provides tissue-specific antiviral defense but also supports the lesional inflammation in CLE. A role for the type III IFNs in this autoimmune disease is further supported by our findings in related skin disorders. We also found IFNλ to be expressed in lichen planus and dermatomyositis skin samples, two autoimmune diseases that share several features with CLE, particularly the histological ''interface-pattern'' (Wenzel and Tuting, 2008), but not in other inflammatory dermatoses such as atopic dermatitis and psoriasis.

We hypothesize that IFNλ induction might represent an early step in the complex proinflammatory network in CLE. Our results demonstrate that IFNλ induces the epithelial expression of CXCL9 but not CXCL10, and importantly CXCL9 has been shown to be the functionally most relevant CXCR3 ligand for the induction of SLE in an experimental mouse model (Menke *et al.*, 2008). IFNλ-mediated CXCL9 expression might represent an early-step LE skin disease by supporting the recruitment of CXCR3+ ''natural type I IFN-producing'' pDCs and cytotoxic T cells toward the epidermis (Yoneyama *et al.*, 2004). pDCs produce IFNα and cytotoxic T cells IFNγ at the dermo-epidermal junction, which both induce the expression of CXCL10 in keratinocytes (Meller *et al.*, 2005; Wenzel *et al.*, 2005b), which further drives the typical interface dermatitis (Wenzel and Tuting, 2008). In this way, the type III IFNs may interconnect with the type I IFNs in the pathophysiology of CLE. It remains tempting to speculate which signals are responsible for the induction of IFNλ expression in CLE *in vivo*. As we detected the most extensive expression of IFNλ and the IFNλR in the sun-exposed skin of SCLE patients, it is conceivable that UV-induced DNA damage may be involved. Uncleared apoptotic nuclear material, which is also released following cytotoxic destruction of keratinocytes, might lead to IFN activation and fuel the lesional inflammation in CLE (Kuhn and Bijl, 2008).

In conclusion, our results provide evidence that activation of the type III IFN system in the epidermis supports the lesional inflammation not only in acute viral infection (Ank *et al.*, 2008), but also in specific autoimmune diseases, particularly in CLE.

PATIENTS AND METHODS

Patients and healthy donors

Punch biopsies (4 mm) were taken in stages of active skin disease from 16 patients with CLE. From the two major disease subsets, CDLE and SCLE, eight patients each were selected. Additionally, skin biopsies of other chronic inflammatory skin diseases (atopic dermatitis, psoriasis, lichen planus, and dermatomyositis, $n=5$, respectively) were analyzed for control purposes. Healthy control specimens were obtained from the unaffected skin of six patients undergoing cosmetic surgery. The remaining control skin

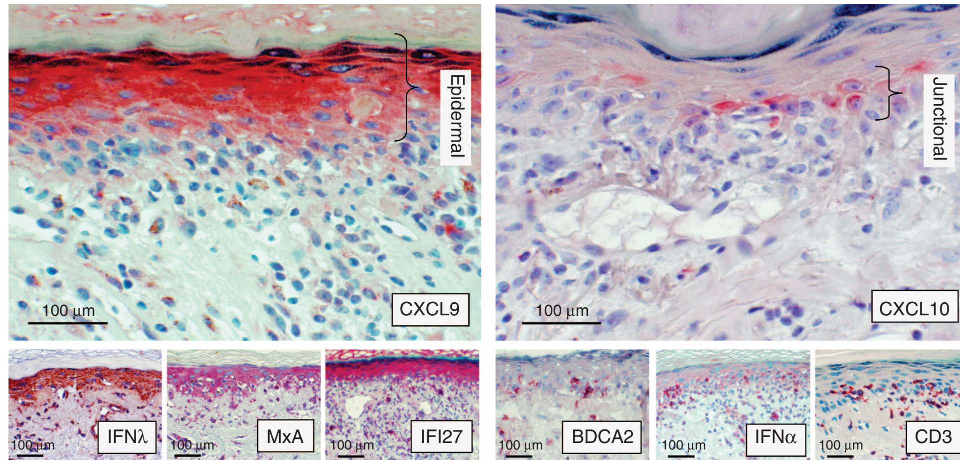

Figure 5. *In vivo* expression pattern of type I and type III IFN-inducible proteins in cutaneous lupus erythematosus (CLE) skin lesions. Depicted are representative immunohistological micrographs of an active chronic discoid lupus erythematosus (CDLE) skin lesion stained for CXCL9 and CXCL10, IFNλ, MxA, IFI27, BDCA2, IFNα, and CD3 (original magnification ×200, scale bar = 100 μm). Similar results were detected in tissue samples from eight different patients with CDLE and subacute CLE (SCLE).

material was used to isolate primary human keratinocytes. Additionally, blood serum samples taken from patients with active CDLE ($n=18$) or SCLE ($n=10$) and healthy donors ($n=8$) were included. The study was performed in accordance with the Declaration of Helsinki Principles after approval by the local regulatory committee (BN 09004). All patients signed an informed consent.

Histology and immunohistology

Sections were prepared from formalin-fixed and paraffin-embedded skin biopsies. Standard hematoxylin and eosin and periodic acid schiff staining were performed for diagnostic purposes. The lesional expression of IFNλ and the IFNλR was analyzed by immunohistochemistry using antibodies specific for IL28/29 (sc-66933; Santa Cruz, Santa Cruz, CA) and the IFNλR (HPA017319; Sigma, Steinheim, Germany). Additionally, the following monoclonal antibodies were used: MxA (M143, Professor Haller, University of Freiburg, dilution 1:100), CXCL9 (MAB392; R&D Systems, Minneapolis, MN), CXCL10 (33 036, R&D), BDCA2 (DDX0043; Dendritics, Lyon, France), CD3 (F7238; Dako, Hamburg, Germany), and IFNα (MMHA-2; R&D Systems). Visualization was performed using the CSA II or the LSAB2 staining kit with fast red as chromogen (both from DAKO), following the manufacture's instructions. Appropriate isotype-specific control antibodies were included. Staining intensity was graded on a scale from 0 (no staining) to 3 (intense staining) as described before (Wenzel *et al.*, 2005b).

Preparation of explanted epidermal sheets and primary keratinocyte cultures

To obtain epidermal explants skin specimen, a 4-mm diameter punch biopsy was taken from skin samples of healthy patients undergoing plastic surgery. After incubation for 24 hours at 4 °C in 2.4 U dispase (Becton Dickinson, Heidelberg, Germany), the epidermis was carefully separated from the dermis. The explanted epidermal sheets were cultured and stimulated in keratinocyte serum-free growth medium (KGM2; Promocell, Heidelberg, Germany) supplemented with 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin using 96-well plates. Alternatively, epidermal sheets were incubated for 15 minutes at 37 °C in 0.2% trypsin and 0.01% EDTA in phosphate-buffered saline to obtain a single-cell suspension. The trypsin-digest was stopped with RPMI 10% fetal calf serum and cells were seeded in primary culture at 1.5×10^6 cells per 75 cm^2 tissue culture flask. Subsequently, cells were subcultured at a density of 5×10^4 cells per 75 cm^2 flask and grown until 80% confluence. All cells were cultivated at 37 °C and 5% CO_2 atmosphere.

Reagents for immune activation

Recombinant IFNα2b (1,000 U ml^{-1}; Intron A, Schering, Berlin, Germany), recombinant IFNλ1 (100 ng ml^{-1}; R&D), polyIC (20 μg ml^{-1}; Sigma), and CpG-oligodeoxynucleotide 2216 (3 μg ml^{-1}, MWG-BIOTECH AG, Ebersberg, Germany) were used to stimulate explanted epidermal sheets and cultured keratinocytes *in vitro*.

Virus infection

Keratinocytes were infected with vesicular stomatitis virus (RNA virus of the family Rhabdoviridae) for 1 hour (multiplicity of infection = 1), washed twice, and then incubated in keratinocyte medium (KGM2). Total cellular RNA was isolated at different time points (2–24 hours) after infection for reverse transcriptase-PCR analyses, and the culture supernatant was collected for ELISA (24 hours).

Quantitative reverse transcriptase-PCR

Explanted epidermal sheets and cultured keratinocytes were harvested at 4h–72 hours after stimulation and total RNA was isolated using the NucleoSpin RNA XS according to the instructions of the manufacturer (Macherey-Nagel, Düren, Germany). Reverse transcription was performed using the SSIII system (Invitrogen, Karlsruhe, Germany) and random primers. Semiquantitative reverse transcriptase-PCR was performed with the following primers: IFNα (F 5′-GAT

GGTTTCAGCCTTTTGGA-3′; R 5′-GCTCACCCATTTCAACC AGT-3′), IFNβ (F 5′-TCATGAGTTTTCCCCTGGTG-3′, R 5′-G ATGCTCCAGAACATCTTTGC-3′), IFNλ1 (F 5′-GCCATGGCT GCAGCTTGGAC-3′; R 5′-GGTGGACTCAGGGTGGGTTGA C-3′), IFNλR (F 5′-ACCTATTTTGTGGCCTATCAGAGCT-3′; R 5′-CGGCTCCACTTCAAAAAGGTAAT-3′), and GAPDH (F 5′-CCACATCGCTCAGACACCAT-3′; R 5′-GGCAACAATATCCA CTTTACCAGAGT-3′) as a housekeeping gene. Each analysis was performed with at least four biological replicates.

Cytokine measurement

IFNα, IFNβ, IFNγ, IFNλ, and CXCL10 proteins were measured in cultured supernatants using commercially available sandwich ELISAs following the instructions provided by the manufacturers (R&D Systems and PBL, Piscataway, NJ). IL6, IL8, and CXCL9 were detected using the Flow Cytomix bead assay according to the manufacturer's instructions (Bender MedSytems, Vienna, Austria), including data analysis with FlowCytomix Pro2.2 software (Bender MedSystems, Vienna, Austria).

Immune cell migration assays

Cell migration assays were performed with PBMCs in 24-well plates with transwell inserts of 5 μm pore size as described before (Wenzel *et al.*, 2005a). 5×10^5 cells were plated on the cover membrane in RPMI-1640 medium containing 0.4% fetal bovine serum. Culture supernatants of stimulated (IFNλ1: 100 ng ml^{-1}; polyIC: 20 μg ml^{-1}) and unstimulated keratinocytes were placed in the bottom chamber. For determination of the migratory index, 50 μl of the cells that had migrated to the lower well after 2 hours at 37 °C were collected in TruCOUNT tubes (Becton Dickinson, Heidelberg, Germany) and analyzed by flow cytometry. The absolute number of investigated cells in the samples was determined by comparing the relative count of investigated PBMCs by the relative count of fluorescence beads, using the following equation: (number of events in region containing cells/number of events in absolute count bead region) × (absolute number of beads per test/test volume) = absolute count of cells per volume. The migratory index was evaluated by dividing specifically migrated cells (''stimulation'') by unspecifically migrated cells (''control''). Five independent biological replicates were performed.

Statistical procedures

All statistical analyses were performed with SPSS software (Chicago, IL), (version 17) using the nonparametrical Mann–Whitney *U*-test. Probabilities of <0.05 were considered to be significant (*), and *P*-values of <0.01 as highly significant (**).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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