Anifrolumab has a direct immunoregulatory effect on inflamed keratinocytes: Implications for the treatment of lupus erythematosus skin lesions

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Introduction: Cutaneous lupus erythematosus (CLE) is an autoimmune skin disease characterized by a type I interferon (IFN)-driven interface dermatitis in which cytotoxic lymphocytes invade the basal layer of the epidermis and induce the keratinocytic cell death. Anifrolumab is a monoclonal antibody targeting the type I interferon receptor (IFNAR1) approved for the therapy of systemic lupus erythematosus (SLE). Recent clinical observations indicated that anifrolumab might be particularly effective in the treatment of lupus erythematosus (LE) skin manifestations. We hypothesize that anifrolumab does not only inhibit interferons circulating in the blood but also has a direct impact on keratinocytes.

Materials & Methods:

- **Patients and skin samples**

Punch biopsies from 20 patients with various inflammatory skin diseases and 5 healthy controls were collected and fixed for histology and immunohistochemistry. Additionally, gene expression data from 19 CLE lesions and 8 controls were analyzed from a public database.

- **Histology & Immunohistochemistry**

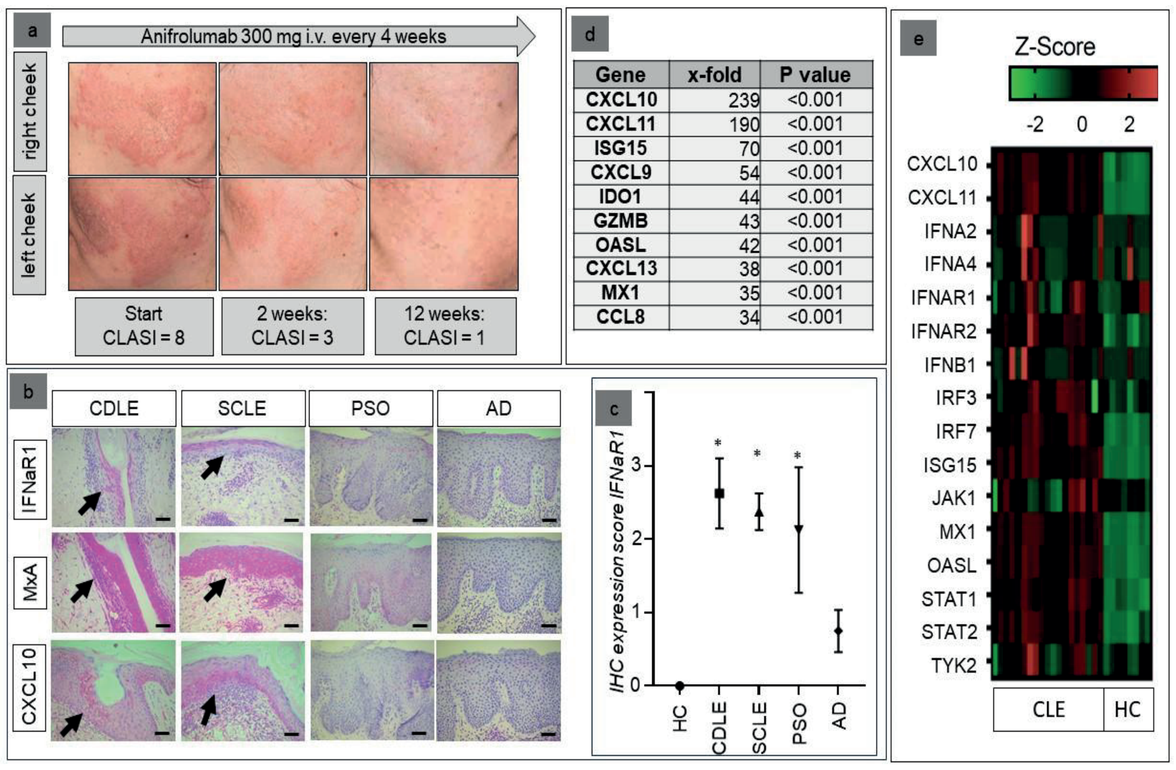
Biopsies and 3D epidermis models were stained with H&E and evaluated by a dermatopathologist. Immunohistochemistry was performed using antibodies against IFNAR1, CXCL10, and MxA, with staining intensity scored from 0 to 3.

- **Cell culture experiments**

Various keratinocyte cell lines and a 3D epidermis model were cultured under standard conditions and pre-treated with the IFN-α receptor blocker anifrolumab. Cells were then stimulated, incubated for 24 hours, and CXCL10 production was measured by ELISA.

- **Next Generation Sequencing and statistical analyses**

RNA was prepared using a 3'-mRNA library kit and sequenced on an Illumina HiSeq platform. Data were statistically analyzed with Welch's t-test and other methods, with significance set at $p < 0.05$.



Gene	x-fold	P value
CXCL10	239	<0.001
CXCL11	190	<0.001
ISG15	70	<0.001
CXCL9	54	<0.001
IDO1	44	<0.001
GZMB	43	<0.001
OASL	42	<0.001
CXCL13	38	<0.001
MX1	35	<0.001
CCL8	34	<0.001

Figure1: 1a) Clinical example of the response of the skin lesions of a 36-year old female patient to anifrolumab, resistant to earlier systemic treatment with mycophenolate mofetil (2g/d) and methotrexate (15mg/week). 1b) Examples of the expression patterns of IFNAR1, MxA, and CXCL10 in healthy individuals (HC=healthy control) and patients with different inflammatory skin disorders (AD = atopic dermatitis, PSO = psoriasis, CDLE = chronic discoid lupus erythematosus, SCLE = subacute cutaneous lupus erythematosus; immunohistochemical staining in red, original magnification: x200). 1c) Mean immunohistochemical staining expression of the IFNαβ-receptor (IFNAR1) in patients with chronic discoid lupus erythematosus (CDLE), subacute cutaneous lupus erythematosus (SCLE), atopic dermatitis (AD) and psoriasis (PSO) compared to healthy controls (HC) (+/- standard deviation, * = p<0.05) 1d) Top 10 upregulated genes within CLE skin lesions compared to healthy skin. 1e) Individual expression of genes within the Reactome™ pathway "IFNαβ-signaling" in the skin of CLE patients and healthy controls.

Results: Our results show that IFNAR1 is expressed in lesional keratinocytes in CLE patients in immunohistochemistry. Gene expression analyses confirmed a strong activation of the interferon signaling pathway in CLE lesions. In vitro experiments with HaCaT cells, NTERT cells and 3D-epidermis models demonstrated that anifrolumab inhibits the expression of CLE-typical IFN-mediated proteins, including MxA and CXCL10 expression after stimulation with IFNα and synthetic and endogenous immunogenic nucleic acids.

Conclusion: This study demonstrates that anifrolumab not only suppresses the type I IFN effect, but also inhibits other pathways of keratinocyte stimulation including cytosolic pattern recognition receptor (PRR)-activation, which is a crucial player in the autoamplification of the proinflammatory vicious circle in CLE. These results suggest that the direct effect of anifrolumab on keratinocytes may be an important factor in its clinical efficacy in LE skin lesions and may explain the beneficial clinical effects of anifrolumab specifically in LE skin lesions.

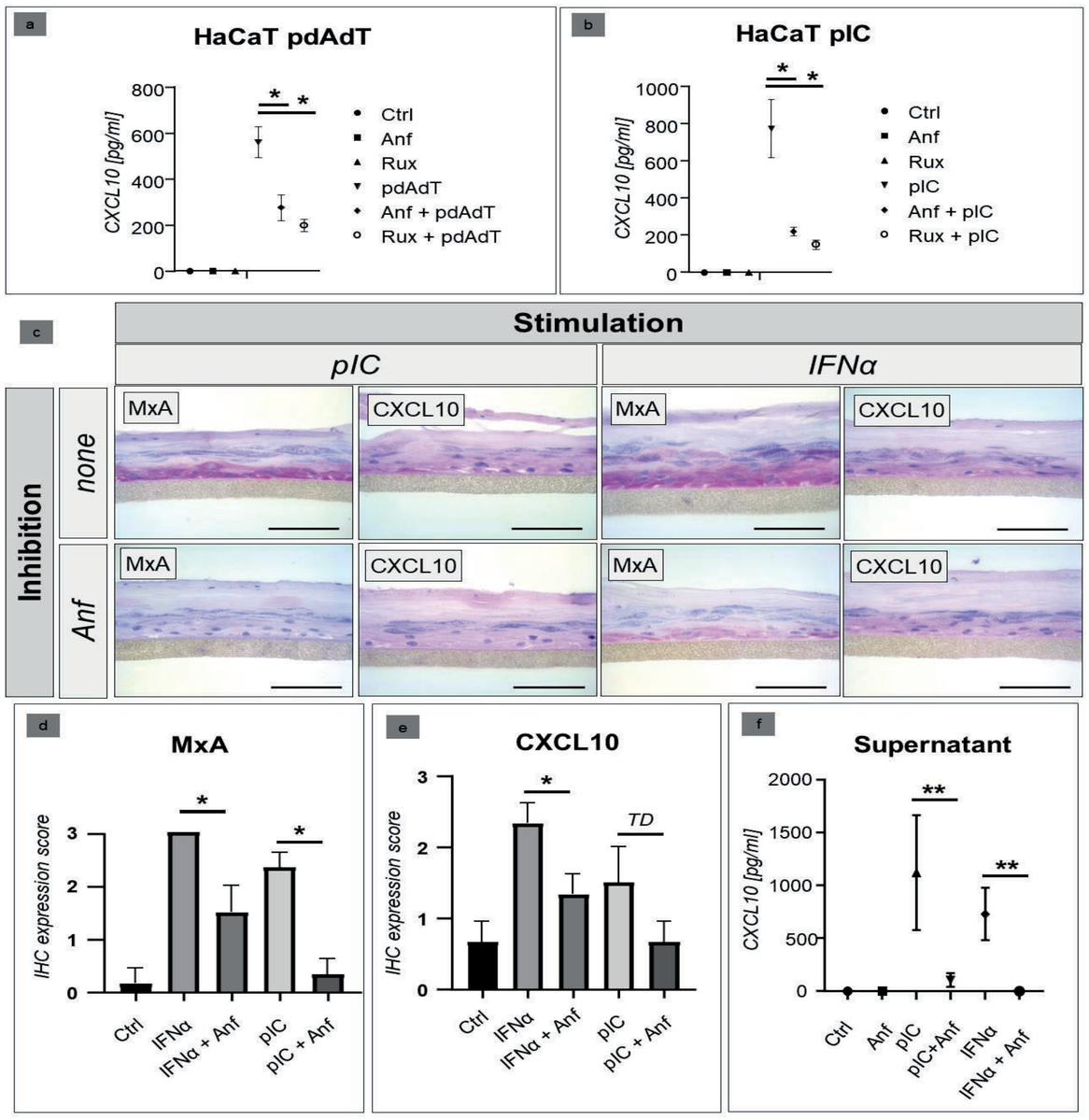


Figure 2: 2a&b) Comparison of the inhibitory effect of anifrolumab (Anf) with the JAK inhibitor ruxolitinib (Rux) in HaCaT cell culture after stimulation with synthetic RNA (poly I:C/ PIC) and DNA (poly(dA:dT)/PdAdT analogues. The figures show the expression of CXCL10 in the supernatant, measured by ELISA (Ctrl = negative control, +/- standard deviation, * = <0.05). 2c,d&e) Inhibitory effect of anifrolumab (Anf) on the lesional type I/III IFN-signature (visualized by MxA and CXCL10 expression using immunohistochemistry, in red) in a 3D epidermis model, stimulated with poly I:C (PIC) and recombinant IFNα (+/- standard error of mean, * = p<0.05). 2f) Inhibitory effect of anifrolumab (Anf) on the CXCL10 expression in the supernatant of a 3D epidemis model with NHEK cells (normal human epidermal keratinocytes; +/- standard deviation, ** = p<0.01).

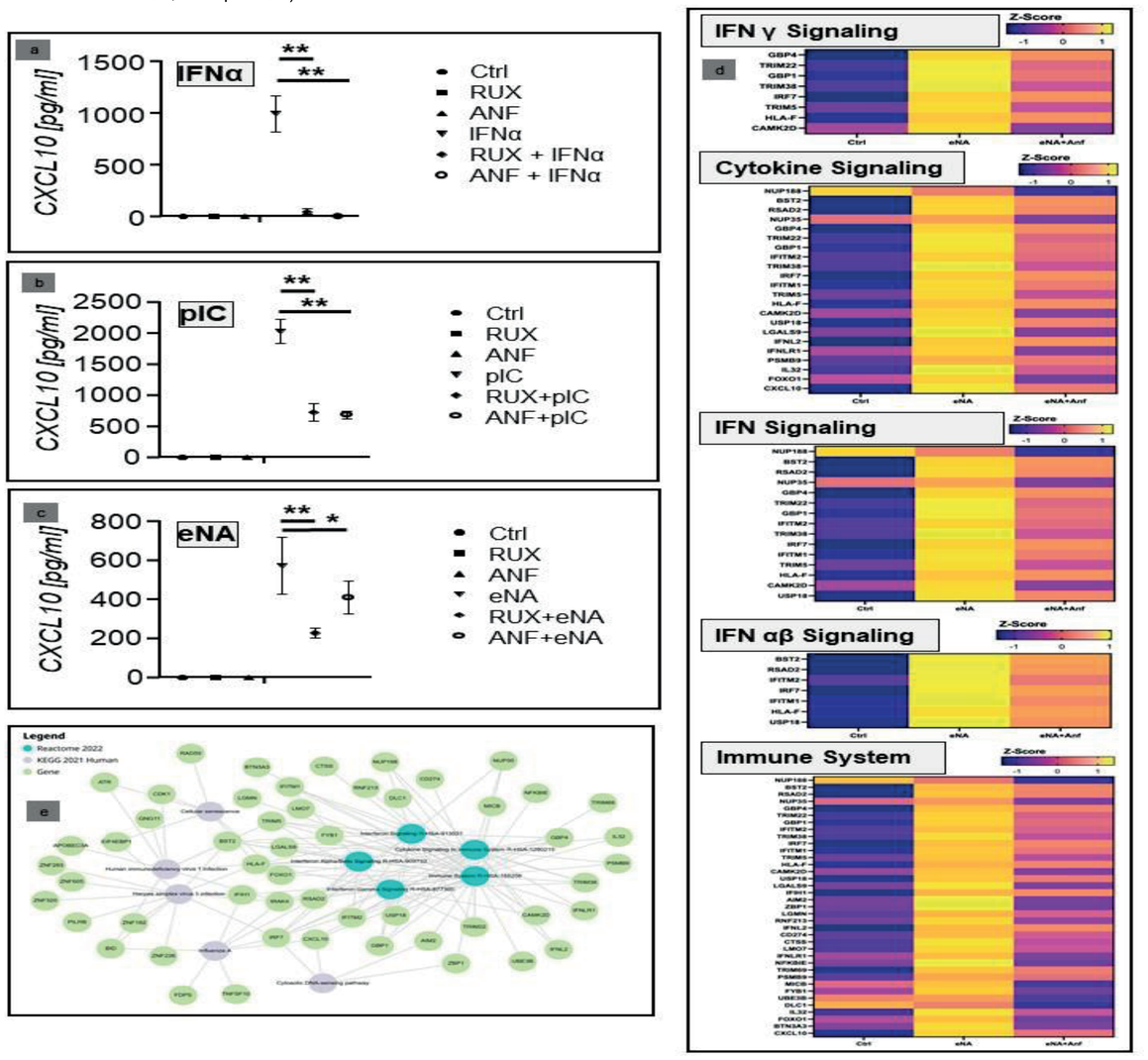


Figure 3: 3a,b&c) Inhibitory effect of anifrolumab (Anf) in comparison to ruxolitinib (Rux) in cultured keratinocytes (N/TERT-cell) after stimulation with two synthetic immunostimulatory nucleic acids (PIC; pdAdT) and a physiolological stimulus (extracted nucleic acids, eNA). Depicted is the expression of CXCL10 in the supernatant, measured by ELISA (Ctrl = negative control; +/- standard deviation, * = p<0.05, ** = p<0.01). 3d) Next generation sequencing analyses of the effect of anifrolumab on the expression of the mRNA of IFN-associated inflammatory pathway molecules. Depicted is the effect of anifrolumab on eNA-stimulated cells in comparison to negative and positive control within the given Reactome pathways as Z-Score. 3e) Network of the top 5 dysregulated KEGG and Reactome pathways and their associated individual genes affected by the treatment of stimulated N/TERT cells by anifrolumab.