Epigallocatechin-3-gallate exhibits anti-inflammatory effects in a human interface dermatitis model—implications for therapy

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Abstract

Background Epigallocatechin-3-gallate (EGCG) has been proven effective in treating viral warts. Since anticarcinogenic as well as anti-inflammatory properties are ascribed to the substance, its use has been evaluated in the context of different dermatoses. The effect of EGCG on interface dermatitis (ID), however, has not yet been explored.

Objectives In this study, we investigated the effect of EGCG on an epidermal human in vitro model of ID.

Methods Via immunohistochemistry, lesional skin of lichen planus patients and healthy skin were analysed concerning the intensity of interferon-associated mediators, CXCL10 and MxA. Epidermal equivalents were stained analogously upon ID-like stimulation and EGCG treatment. Monolayer keratinocytes were treated likewise and supernatants were analysed via ELISA while cells were processed for vitality assay or transcriptomic analysis.

Results CXCL10 and MxA are strongly expressed in lichen planus lesions and induced in keratinocytes upon ID-like stimulation. EGCG reduces CXCL10 and MxA staining intensity in epidermis equivalents and CXCL10 secretion by keratinocytes upon stimulation. It furthermore minimizes the cytotoxic effect of the stimulus and downregulates a magnitude of typical pro-inflammatory cytokines that are crucial for the perpetuation of ID.

Conclusions We provide evidence concerning anti-inflammatory effects of EGCG within a human in vitro model of ID. The capacity to suppress mediators that are centrally involved in disease perpetuation suggests EGCG as a potential topical therapeutic in lichen planus and other autoimmune skin diseases associated with ID.

Received: 30 June 2021; Accepted: 15 September 2021

Conflict of interest

The authors declare that they have no conflicts of interest relevant to the presented data.

Funding source

CB received a scholarship from the Medical Faculty of the University of Bonn (clinician scientist ‘Gerok-scholarship of the BONFOR program’).

Introduction

Interface dermatitis (ID), also referred to as lichenoid tissue reaction, describes the histopathological correlate of a characteristic immune reaction pattern that is associated with type I immunity.1 It is defined by a pronounced inflammatory infiltrate of the upper dermis and perishing keratinocytes of the basal epidermal layer that are labelled vacuolar or hydropic colloid bodies.2 ID underlies clinically heterogeneous dermatoses, amongst which are autoimmune skin diseases such as: cutaneous lupus erythematosus (CLE), dermatomyositis (DM), lichen planus (LP) and lichen sclerosus (LS),3 of which LP is considered prototypic.2 Several subtypes of LP have been described depending on the site of involvement and the morphology of respective lesions.2 Quality of life is often severely impaired in affected patients4–8 and especially mucosal and hypertrophic LP bear the risk of malignant transformation.9–11 There is no drug approved by the U.S. Food and Drug Administration or the European Medicine Agency; thus, patients and physicians are dependent on off-label therapies which is an apparent unmet medical need. Regardless of clinical heterogeneity and diverging initial triggers, common final path in all diseases featuring ID is an interferon-associated, anti-epidermal attack by cytotoxic T lymphocytes.3,12–14 These effector cells are recruited to the site of inflammation by respective keratinocytes producing large amounts of C-X-C Motif Chemokine

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Ligands 9/10/11 (CXCL9/10/11).15 Our working group has recently reviewed pathomechanistic hallmarks of ID and the particular role of interferon gamma (IFNy) and endogenous nucleic acid (ENA) sensing by keratinocytes in perpetuating a pro-inflammatory 'vicious circle'.16 We have translated those pathogenic fundamentals into an epidermal human in vitro model enabling analysis of therapeutic interventions.16

Epigallocatechin-3-gallate (EGCG) is the major polyphenol in green tea leaves of Camellia sinensis. A standardized green tea preparation, of which EGCG is the major component, is approved for the treatment of condylomata acuminata.17 Aside from efficacy in treating viral warts, anticarcinogenic as well as anti-inflammatory properties are ascribed to the substance. A recent meta-analysis demonstrated beneficial effects of topical anti-inflammatory properties are ascribed to the substance. A

In the present study, we investigate the anti-inflammatory effectiveness of EGCG in the human in vitro model established by our working group and thus evaluate it as an innovative topically applicable therapeutic for patients suffering from LP and other autoimmune skin diseases featuring ID.31–33

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Materials and methods

Immunohistochemistry

Sections from LP lesions and healthy control (HC) skin were prepared from formalin-fixed, paraffin-embedded skin biopsies. Immunohistochemistry was performed using the following antibodies: CXCL10 antibody (1 : 200, ab9807; Abcam, Cambridge, UK); MxA antibody (1 : 2500, M143, Prof. Otto Haller, Freiburg, Germany). Staining intensity was scored semiquantitatively (0 = no, 1 = weak, 2 = fair, 3 = strong intensity) as described previously.34

Cell culture and stimulation

Three-dimensional human epidermis equivalents (epiCS) and the appropriate media were purchased from CellSystems (Trossdorf, Germany). EpiCS were stimulated at day 14 after air-lift. Endogenous nucleic acids (ENA) were isolated from unstimulated normal human epidermal keratinocytes (HEK) using the 'Genomic DNA from tissue’ kit from Macherey-Nagel (Dueren, Germany) without RNA digestion. ENAs were applied in a concentration of 5 µg DNA/mL. Lipofectamine 2000 served as transfectant (2.5 µL/mL, Invitrogen, Carlsbad, CA, USA). 1 × 10^5 U/mL IFNy (PeproTech GmbH, Hamburg, Germany) was applied. epiCS were preincubated with EGCG 100 µmol/L for 20 min before addition of the stimulus. (−)-Epigallocatechin gallate [≥95%, E4143, (SLBZ2865)] was purchased from Sigma-Aldrich, St. Louis, MO, USA. Twenty-four hours after stimulation, epiCS were formalin-fixed for 1 h, paraffin embedded and then processed for immunohistochemistry.

HEKs (FC-0025) were purchased from CellSystems (Trossdorf, Germany) and cultured in Growth Medium 2 (PromoCell, Heidelberg, Germany). One day prior to stimulation, cells were seeded into 24 well plates at a density of 1.5 × 10^3/well. Stimulus was administered as described for epiCS. Concerning HEKs, FuGENE (10 µL/mL, Promega Corporation, Fitchburg, MA, USA) functioning as transfection reagent and analogous preincubation was carried out with 40 µmol/L EGCG containing medium. 6.5 hours after stimulation, supernatants were harvested and prepared for protein analysis via ELISA, whereas cells were either processed for vitality assay or RNA isolation. The experimental approach is depicted in Fig. 2a.

Read out supernatant

 Supernatants were analysed for CXCL10 concentration via the human CXCL10/IP-10 DuoSet ELISA from R&D systems (Minneapolis, MN, USA) and using Bio-tek SynergyTM HT Multi-Detection Microplate Reader (BioTek, Winooski, VT, USA) and the Gen5 (V.11.5) software (BioTek, Winooski, VT, USA).

Vitality assay

Cells were exposed to 10% MTT-reagent-containing medium for 4 h [Thiazolylblau, Carl Roth GmbH + Co. KG, Karlsruhe, Germany, dissolved in PBS (5 mg/mL)]. Afterwards, formazan precipitates were solubilized by DMSO addition and absorbance was measured via the aforementioned Microplate Reader.

RNA isolation and gene expression analysis

RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel, Dueren, Germany) according to the manufacturer’s instructions. Samples were further processed by the next generation sequencing (NGS) Core Facility of the Medical Faculty of the University of Bonn.

Statistics

GraphPad PRISM 8 software (GraphPad Software, San Diego, CA, USA) was used for statistic analysis of all experiments except for gene expression data. Mann–Whitney test was used. P-value <0.05 was considered ‘significant’ ( ), ≤P < 0.01 ‘highly significant’ ( *** ), Gene expression results were analysed via Partek® Flow® (Partek Incorporated, St. Louis, MO, USA). Results were filtered as follows: P-value ≤ 0.01; fold change <−2 or >2, FDR step-up ≤0.02.

Ethical approval

The study was approved by the local ethics committee (No. 090/04. Bonn, Germany) and complies with the Declaration of Helsinki Principles.
Results

CXCL10 and MxA are strongly expressed in lesions of LP patients
First, we performed immunohistochemistry concerning the pro-inflammatory markers, MxA and CXCL10, on lesional biopsies of LP patients and healthy skin. In accordance with previous findings from our working group and others,3,16,35 LP skin lesions showed a significantly higher epidermal staining score for both CXCL10 and MxA in the epidermis (Fig. 1).

EGCG reduces CXCL10 and MxA levels in 3D epidermis equivalents and CXCL10 secretion by keratinocytes upon ID-like stimulation
As expected, the ID-like stimulus (IFNy, eNA) induced CXCL10 as well as MxA in human epidermis equivalents, showing a pattern comparable to the one observed in actual ID of LP. This effect was significantly attenuated by EGCG-pretreatment prior to stimulation (Fig. 2b,c). The CXCL10-inhibitory capacity of EGCG could be confirmed in cultured HEKs: stimulation of HEKs with IFNy and eNA resulted in CXCL10 levels higher than 2500 pg/mL. Administration of EGCG, in turn, suppressed the levels of CXCL10 to a non-detectable concentration (Fig. 2d).

EGCG minimizes the cytotoxic effect upon ID-like stimulation
Vitality assay revealed that the pro-inflammatory stimulus significantly decreases the cells’ viability. Keratinocytes treated with EGCG prior to ID-like stimulation, however, did not show significant reduction in vitality when compared to untreated cells (Fig. 2e).

EGCG downregulates typical pro-inflammatory cytokines and pathways that are crucial for the perpetuation of interface dermatitis
When comparing ID-like stimulated HEKs with those treated with EGCG prior to stimulation, we found 2131 genes to be differentially expressed (P-value < 0.01; fold change < -2 or >2, FDR step-up ≤ 0.02; Fig. 3a, Table S1, Supporting Information). Sixty-four per cent of the top 25 downregulated genes sorted by

Figure 1 (a) Representative histological findings in healthy skin and lichen planus. Representative findings of CXCL10 and MxA immunostaining in healthy skin (HC) and lichen planus (LP) lesions. Original magnification × 200. Scale bars indicate 100 µm. (b) Semi-quantitative scoring of staining intensity in healthy skin and lichen planus. Epidermal staining score of the respective immunohistochemical staining with anti-CXCL10- and anti-MxA-antibody rated as 0 = no, 1 = weak, 2 = fair, 3 = strong staining intensity in HC skin (n = 5) and LP (n = 7). Given are respective mean values. Standard deviations are indicated by error bars. ** indicates high significance (P-value <0.01).
fold change could be found in the list of the top 500 genes differentially regulated in LP and lupus erythematosus (Fig. 3b, compare Lauffer et al.\(^1\)). The top three upregulated genes in LP and lupus erythematosus, CXCL9, CXCL10 and CXCL11, in turn, were amongst the top 25 downregulated genes upon EGCG treatment of keratinocytes [Fig. 3b (marked by red asterisks); compare Lauffer et al.\(^1\)]. As demonstrated in Fig. 3c, EGCG treatment significantly downregulated multiple genes encoding key drivers of innate inflammatory pathways. Mediators that are involved in nucleic acid sensing were suppressed (cGAS, STING, LGP2, IFI16, MDA5, RIG-I, MYD88, TLR3). Furthermore, a downregulation of cell death promoting factors could be observed (TNF, TRAIL, NFKB1, RELA, RIPK1, RIPK3, FAS, CASP1, CASP8, CASP10). Moreover, IFNs and their receptors (IFNk, IFNLR1, IFNAR2) were directly downregulated by EGCG treatment as well as a multitude of associated mediators (IRF1, IRF2, IRF7, MX1, OAS1, JAK2, STAT1, STAT2, USP18, CXCL9, CXCL10, CXCL11, CX3CL1, CCL3). EGCG additionally suppressed factors mediating crosstalk to the adaptive immune system: MHC class I antigens were downregulated (HLA-J, HLA-F, HLA-E, HLA-B), as well as MHC class II antigens (HLA-DOB, CD74). Furthermore, expression of mediators of interaction with B cells (BLyS) and T cells (ICAM1) was reduced within keratinocytes treated with EGCG compared to untreated pro-inflammatoryly stimulated cells.

**Discussion**

Epigallocatechin-3-gallate has previously been investigated in various inflammatory dermatoses.\(^{19,23,26,28}\) Green tea consumption has even been proposed as an alternative approach to managing oral LP.\(^36\) The plasma concentration of EGCG detected upon intake of green tea extraction in 350 mL water,\(^37\) however, is far from the concentration needed to achieve anti-inflammatory effects. Consumption of EGCG dietary supplements, resulting in much higher plasma and tissue concentrations of EGCG than upon intake of tea beverages, has been shown to be associated with hepatotoxicity.\(^38\) Topical application can thus be considered a much safer and more effective delivery approach than oral intake.\(^39,40\) We herein present data on the effect of EGCG on keratinocytes within a human in vitro model of ID. As recently described,\(^16\) this model is based on stimulation with IFN\(\gamma\) and eNAs. Type II IFN, IFN\(\gamma\), is of significant importance in LP\(^29\) and, in vivo, predominantly derives from natural killer cells, group 1 innate lymphoid cells, \(\gamma\)\(\delta\) T cells and CD8\(^{+}\) cytotoxic T cells as well as CD4\(^{+}\) Th1 cells [reviewed in 41]. Sensing of eNAs is a well-established mechanism in autoimmunity\(^42\) and specifically in ID.\(^16,43\) In vitro, stimulation of keratinocytes with eNAs induces type I and type III IFNs,\(^16\) which both play a crucial role in dermatoses featuring ID.\(^34-47\)

Epigallocatechin-3-gallate interferes with nucleic acid sensing pathways: it has been shown to inhibit self-DNA-induced cGAS activation in murine and human macrophages\(^24\) and controlled inflammation in mesangial cells in lupus-like mice via inhibition of the PI3K/Akt/mTOR pathway.\(^48\) Our data particularly reveal downregulation of important nucleic acid sensors in ID-like stimulated keratinocytes upon EGCG treatment. cGAS, STING, LGP2, IFI16, MDA5, RIG-I, MYD88 and TLR3 are affected accordingly. Besides interferon responses, nucleic acid sensing also mediates inflammatory cell death cascades\(^49\) which are considered a hallmark of ID.\(^1\) Receptor-interacting-protein-kinase 3 (RIPK3), a key protein of necroptosis, is expressed in the epidermis of LP and CLE skin samples.\(^2\) Likewise, RIPK1 drives cell death and NF-\(\kappa\)B signalling and has been proposed as a therapeutic target, accordingly.\(^50\) A current Phase 2 proof-of-concept clinical study (NCT04781816) evaluates the effectiveness of the RIPK1 inhibitor, SAR443122, in cutaneous lupus. Our data show downregulation of RIPK1 and RIPK3 in keratinocytes upon EGCG treatment. Moreover, we detected downregulation of FAS (Cell Surface Death Receptor), whose ligand is upregulated in ID.\(^3\) CASP1 which is of major importance in the induction of pro-inflammatory pyroptosis\(^51\) is downregulated by EGCG in our approach as well as CASP10 which has been associated with systemic LE\(^52\) and has been shown to be involved in FAS signalling and responses of other death receptors.\(^53\) Likewise, CASP8 is downregulated; Puviani \(\text{et al.}\)^\(54\) have demonstrated that its activation by increased levels of FASL induces apoptosis in human keratinocytes in pemphigus patients. As reviewed by Kesavardhana \(\text{et al.}\),\(^55\) the role of FASL is complex. Inhibition of CASP8 supposedly shifts extrinsic apoptosis pathways to TNF-induced necroptosis. TNF expression, in turn, is also downregulated in keratinocytes by EGCG treatment. In keratinocytes, dose-dependent cleavage of CASP3 and CASP8 as well as direct cytotoxic effects upon exposure to TRAIL have been described.\(^36\) Diminished expression of TRAIL by EGCG treatment might contribute to the observed capacity of EGCG to reverse the direct cytotoxic effects of ID-like stimulation. Nuclear NFkB is overexpressed in LP and CLE skin.\(^1,57\) The dimeric transcription factor formed by different subunits mediates variable downstream cascades. Both of its subunits known to activate pro-inflammatory pathways, NFRB1 and RELA,\(^58\) are downregulated in ID-like stimulated keratinocytes upon EGCG treatment.

Interferon cascades upon eNA-sensing are prevented by EGCG treatment in our approach: ID-like stimulated keratinocytes show reduced expression of type I IFN, IFN\(\kappa\), under the influence of the catechin. Likewise, USP18, which is strongly induced by type I and type III IFNs\(^49\) and is upregulated in LP and LE,\(^1\) is downregulated. All type-I IFNs bind to IFNAR,\(^50\) while type III IFN, IFN\(\gamma\), signals via the corresponding receptor IFNLR.\(^51\) In August 2021, the FDA has approved anifrolumab, an IFNAR antagonist, for adults with systemic LE who are receiving standard therapy.\(^62\) We reveal downregulation of both, IFNLR1 and IFNAR2, by EGCG. Their downstream signalling is quite congruent.\(^61\) It includes phosphorylation of JAK1 and TYK2\(^63\) and subsequent
phosphorylation of receptor-bound STATs (STAT1 and STAT2) enabling their heterodimerization and complex formation with Interferon Regulatory Factor (IRF) 9. Upon translocation to the nucleus, this complex induces transcription of OAS, MxA and a magnitude of transcription factors including further IRFs. IFNγ signals via the JAK1/JAK2 and STAT1/STAT2 pathway upon interaction with its receptor (IFNγR). Activation of JAK-STAT pathways has been described in LP and other autoimmune skin diseases featuring ID. The results of a recent study on topical JAK1/2 inhibition with ruxolitinib in LP patients...
EGCG in interface dermatitis

Figure 2 (a) Depiction of the experimental approach. 3D-epidermis equivalents (epiCS) and human epidermal keratinocytes (HEK) are exposed to either medium control (Co) or ID-like stimulation (IFNγ_eNA) with or without EGCG pretreatment. epiCS are analysed via immunohistochemistry, while HEKs are processed for vitality assay (MTT) or gene expression analysis. Supernatants are analysed via ELISA. (b) Representative histological findings in untreated epiCS as well as ID-like stimulated epiCS and epiCS treated with EGCG prior to ID-like stimulation. Representative findings of CXCL10 and MxA immunostaining in untreated epiCS (Co) as well as ID-like stimulated epiCS (IFNγ_eNA) and epiCS treated with EGCG prior to ID-like stimulation. (c) Semi-quantitative scoring of staining intensity findings in untreated epiCS as well as ID-like stimulated epiCS and epiCS treated with EGCG prior to ID-like stimulation. Keratinocytic staining score of the respective immunohistochemical staining with anti-CXCL10- and anti-MxA-antibody rated as 0 = no, 1 = weak, 2 = fair, 3 = strong staining intensity in epiCS treated as described above (Co/IFNγ_eNA + EGCG, n = 3 per treatment group). (d) CXCL10 levels within the supernatant of unstimulated HEKs, HEKs treated with EGCG as well as ID-like stimulated HEKs and HEKs treated with EGCG prior to ID-like stimulation. Given are the means of the, respectively, measured CXCL10 levels in the supernatants of untreated HEKs (Co), HEKs treated with EGCG (Co + EGCG), ID-like stimulated HEKs (IFNγ_eNA) and ID-like stimulated HEKs treated with EGCG (IFNγ_eNA + EGCG) in pg/mL. Standard deviations are indicated by error bars, ** indicates high significance (P-value < 0.01), and * indicates significance (P-value < 0.05). (e) Vitality (MTT) assay results from untreated HEKs, HEKs treated with EGCG as well as ID-like stimulated HEKs and HEKs treated with EGCG prior to ID-like stimulation. Mean of unstimulated and untreated HEKs (Co) defined as 100%. Given are means of relative vitality in comparison with the mean of controls. Standard deviations are indicated by error bars, * indicates significance (P-value < 0.05), and ns indicates no significance (n = 4 per treatment group).

(NCT03697460) are not yet available. Interestingly, inhibitory capacity of EGCG on IFNγ-induced phosphorylation of JAK2 and STAT1 has been previously described in human melanocytes and analogous effects were observed in HaCat and Jurkat cells as well as PBMCs of alopecia areata patients. Our gene expression data add downregulation of gene expression of JAK2 as well as STAT1 and STAT2 in ID-like stimulated keratinocytes upon EGCG treatment. EGCG treatment, moreover, reduces expression of OASL and MxA expression as well as of IRF1, IRF2, IRF7 within our approach.

Activation of JAK-STAT pathways results in expression of CXCR3 ligands in keratinocytes. In vivo, the ligands, CXCL9, −10 and −11, constitute the three most abundantly upregulated genes in LP and LE and are produced by lesional keratinocytes. Hereby, cells carrying corresponding CXCR3 receptors are attracted to the site of inflammation; amongst them are macrophages, activated pDCs and T cells [Th1-type CD4 T cells and cytotoxic CD8 T cells]. A previous murine study revealed direct binding of EGCG to CXCL9/10/11 and thereby limitation of the chemokines' biological activity. Our data support significant effects of EGCG on the protein level of CXCL10 in stimulated keratinocytes and furthermore demonstrates downregulation of gene expression of the ligands in keratinocytes upon EGCG treatment. Moreover, CX3CL1, also called fractalkine, is downregulated in keratinocytes upon EGCG treatment. It is expressed by epithelial cells in inflammatory conditions, specifically in keratinocytes of LP lesions and many rheumatic diseases, amongst them lupus erythematosus. CX3CL1 functions as a chemokine recruiting cells carrying the corresponding CX3C receptor 1 (CX3CR1). CX3CR1 is expressed by Th1 cells as well as cytotoxic effector lymphocytes including NK cells, γδ T cells and CD8 T cells, that express perforin and granzyme B. Granzyme B in turn has been detected within the ID shared gene signature and is linked to necroptosis of keratinocytes in ID. In view of the emerging understanding of CX3CL1, it has been suggested as a potential therapeutic target in rheumatic and related inflammatory disorders. CCL5 which is amongst the top genes upregulated in LP and LE is markedly downregulated in ID-like stimulated keratinocytes upon EGCG treatment. Its signalling is closely related to the inflammatory infiltration of T cells in oral LP and inhibition of its receptor appears to be a promising therapeutic approach in the autoimmune skin disorder alopecia areata.

In LP, susceptibility of keratinocytes to T cell-mediated cytotoxic responses is increased via MHC class I induction by IFNγ in a JAK2-dependent manner. Interestingly, EGCG downregulates the expression of different MHC class I molecules: HLA-B, HLA-E, HLA-F, HLA-J as well as the HLA class II antigen HLA-DOB and CD74, also known as HLA-DR antigen-associated invariant chain. In line with our findings, Hamed et al. detected downregulation of the expression of both HLA-B and HLA-DR antigens in HaCat cells treated with EGCG. The potential of human keratinocytes to act as antigen-presenting cells has recently been further underlined by Orlik et al., who demonstrated the ability of IFNγ-pretreated human keratinocytes to activate co-cultured naïve T cells and polarize them towards a Th1/Th17 phenotype. The phenomenon is described as dependent on costimulatory receptors induced by IFNγ. Amongst them is CD54, also known as ICAM1, which is known to be overexpressed in diseases featuring ID. Its experimental blockade results in strongly decreased capacity of keratinocytes to activate co-cultured naïve T cells via disruption of cell-cell contact formation suggesting ICAM1 as a potential therapeutic target in inflammatory skin disorders. Interestingly, EGCG strongly downregulates ICAM1 expression in ID-like stimulated keratinocytes in our approach.
promoting B cell survival, is also downregulated by EGCG. In vivo, it is strongly expressed by keratinocytes in CLE86 as well as lichen planopilaris. A monoclonal antibody against BlyS (Belimumab) is approved by the U.S. Food and Drug Administration as well as the European Medicines Agency for the treatment of systemic LE and its specific effect on the skin is being evaluated in a clinical trial for LE patients with therapy-resistant skin manifestations (EudraCT Number: 2017-003051-35).

**Figure 3** (a) Entirety of significantly altered gene regulation in HEKs treated with EGCG prior to ID-like stimulation vs. ID-like stimulated HEKs without treatment. Upregulated genes are represented by red dots, whereas downregulated genes are depicted by blue dots. Filters in Partek® Flow® (Partek Incorporated) were set to P-value ≤ 0.01; fold change < -2 or >2, FDR step-up ≤ 0.02 (n = 4 per treatment group). (b) Selection of the 25 most downregulated genes in HEKs treated with EGCG prior to ID-like stimulation vs. ID-like stimulated HEKs without treatment. Given are the 25 most downregulated genes in HEKs treated with EGCG prior to ID-like stimulation vs. ID-like stimulated HEKs without treatment sorted by fold change. Genes that are amongst the top 500 genes differentially regulated in lichen planus and lupus erythematosus (according to Lauffer et al.) are highlighted in blue. The red asterisks mark the top 3 upregulated genes detected in LP and CLE by Lauffer et al. (c) Heat map depiction of relevant genes differentially expressed in HEKs treated with EGCG prior to ID-like stimulation vs. ID-like stimulated HEKs without treatment. Heat map presentation of relevant genes differentially expressed in HEKs treated with EGCG prior to ID-like stimulation vs. ID-like stimulated HEKs without treatment. Left column: mediators of nucleic acid sensing and cell death cascades. Centric column: IFN signalling, JAK-STAT pathways, chemokines. Right column: mediators of crosstalk to immune cells.

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In conclusion, our data suggest EGCG as a potential, topically applicable active compound for LP and other inflammatory skin disorders featuring ID. New formulations of topical EGCG, as currently strived for,\(^1,8,22\) will hopefully overcome current limitations due to its lack of stability\(^{40,88}\) and might thus help EGCG to acquire a significant role in the therapeutic armamentarium.

**Acknowledgements**

The authors thank all employees of the Next Generation Sequencing core facility and the Core Unit for Bioinformatics Data Analysis from the University of Bonn for their most valuable support. CB is specifically thankful for having been granted a scholarship from the Medical Faculty of the University of Bonn (physician-scientist ‘Gerok-scholarship’ in the BONFOR program) which has capacitated her to conduct the research. Figures were created with BioRender.com, GraphPad Prism 8.4.3 and Microsoft PowerPoint 2010. Open access funding enabled and organized by ProjektDEAL.

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**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Differentially regulated genes in keratinocytes treated with EGCG prior to ID-like stimulation in comparison to ID-like stimulated cells without pre-treatment.