Skin Barrier and Inflammation Genes Associated with Atopic Dermatitis are Regulated by Interleukin-13 and Modulated by Tralokinumab *In vitro*

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Atopic dermatitis (AD) is a chronic, pruritic skin disease characterized by type 2 immune-mediated inflammation and skin barrier dysfunction (1). The impaired skin barrier in AD is related to decreased expression of epidermal barrier proteins and to changes in lipid composition in the stratum corneum. AD lesions are characterized by increased expression of pro-inflammatory mediators, such as chemokines, leading to recruitment of immune cells, which may further exacerbate the inflammation.

Interleukin (IL)-13 and IL-4 are the signature type 2 cytokines involved in AD. These cytokines both signal through the type II receptor, composed of IL-4Rα and IL-13Rα1, and display functional redundancy in cells harbouring this receptor, including keratinocytes and fibroblasts. This is in contrast to cells, such as T lymphocytes, containing the type I receptor (IL-4Rα combined with the common gamma chain), which is only used by IL-4 for signalling. Monoclonal antibodies targeting IL-4Rα (dupilumab) or IL-13 (tralokinumab and lebrikizumab) have demonstrated clinical efficacy in AD (2–4). Interestingly, protein levels of IL-13, but not IL-4, are consistently detected and shown to be increased in AD skin across studies (5, 6). Furthermore, 3 recently published studies using RNA-sequencing of AD biopsies found increased levels of *IL13* in lesional and non-lesional AD skin, whereas expression of *IL4* was undetectable or very low (7–9). Independent studies also found a correlation between expression levels of IL-13 at mRNA or protein level in lesional AD skin and disease severity (6, 7).

The aim of the current study is to further investigate effects of IL-13 on AD-associated genes in human skin cells and to provide molecular insights into the mechanism of action of tralokinumab, a fully human IgG4 monoclonal antibody that specifically neutralizes IL-13.

MATERIALS AND METHODS

Detailed descriptions of cell cultures, cytokine stimulation, gene expression analysis, protein analysis and data analysis are shown in Appendix S1[1].

RESULTS AND DISCUSSION

Correlation of *IL13* expression with genes expressed in skin biopsies from patients with moderate-severe AD was evaluated by combining data from 3 recent transcriptomic studies (7–9). The combined datasets include RNA-seq data from chronic lesions from each patient ($n=89$) with paired non-lesional samples ($n=87$), and, in addition, acute lesions from a small subgroup of the patients ($n=11$). A positive correlation was found in lesional AD skin between expression of *IL13* and several pro-inflammatory mediators as well as *IL13RA2* (**Table I** and Fig. S1[1]). In contrast, a negative correlation was seen in lesional AD skin between expression of *IL13* and genes related to skin barrier function. For most of the genes, the correlation with *IL13* was even more pronounced when paired lesional and non-lesional samples were included in the analysis (Table I).

Using cultures of human skin cells, IL-13-mediated regulation of several of these genes was then explored, as well as their modulation by tralokinumab. Primary human epidermal keratinocytes (HEK) and human dermal fibroblasts (HDF) were stimulated with 10 ng/ml (0.8 nM) and 2 ng/ml (0.16 nM) IL-13, respectively, each corresponding to ~80% of maximal gene responses, in the presence of a broad range of concentrations (0.003–30 nM) of tralokinumab or isotype control antibody for 24 h. Using qPCR analysis, the effects of IL-13 and tralokinumab were evaluated on gene expression of *CCL2*, *CCL17*, *CCL22*, *CCL26*, *NTRK1* and *IL13RA2* in HEKs, and of *CCL2*, *CCL11*, *CCL17*, *CCL22* and *POSTN* in HDFs.

In line with the correlation analysis, IL-13 markedly induced gene expression of *CCL2*, *CCL26*, *NTRK1* and *IL13RA2* in keratinocytes (**Fig. 1**a) and *CCL2*, *CCL11* and *POSTN* in dermal fibroblasts (Fig. 1c). Tralokinumab, but not the isotype control antibody, potently neutralized IL-13, resulting in a dose-dependent and full inhibition of the inflammatory markers, with sub-nanomolar IC_{50} values (Fig. 1a, c and Table SI[1]). As *IL13RA2* is directly induced by IL-13 in keratinocytes, levels of *IL13RA2* are expected to be decreased in AD skin upon inhibition of the IL-13 signalling axis,

Table I. Expression of *IL13* correlates with several inflammation and epidermal barrier associated genes in skin biopsies from patients with moderate-severe atopic dermatitis

Inflammation			Epidermal barrier function		
Gene	r (L)	r (L+NL)	Gene	r (L)	r (L+NL)
MMP12	0.80	0.77	*ELOVL3*	−0.23	−0.34
ALOX15	0.77	0.76	*KRT10*	−0.32	−0.30
NTRK1	0.76	0.76	*KLK5*	−0.32	−0.36
IL22	0.71	0.67	*FLG*	−0.32	−0.51
CCL22	0.70	0.75	*FLG2*	−0.34	−0.57
CCL17	0.65	0.74	*LOR*	−0.36	−0.53
CCL2	0.64	0.72	*ALOX12*	−0.45	−0.50
CCL1	0.61	0.55	*ELOVL6*	−0.46	−0.62
POSTN	0.55	0.30			
IL13RA2	0.53	0.58			
CCL11	0.50	0.51			
CCL26	0.36	0.44			

Meta-analysis from 3 individual RNA-seq studies showing the correlation of gene expression to *IL13* in lesional biopsies (L) or paired lesional and non-lesional biopsies (L + NL). Analysis was performed by linear regression and Pearson correlations (r) are shown in the Table. Detailed correlation plots, including *p*-values, are shown in Fig. S1[1].

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as is also shown by the IL-4Rα-blocking antibody dupilumab (10). In addition to gene expression analysis, secreted levels of CCL-2/MCP-1 were determined by enzyme-linked immunoassay (ELISA). Here, it was confirmed that IL-13 induced secretion of CCL-2/MCP-1 from HEKs and HDFs, which was potently and dose-dependently inhibited by tralokinumab, with IC_{50} values of 217 and 336 pM, respectively (Fig. 1b, d and Table SI[1]). IL-13 did not induce expression of *CCL17* and *CCL22* in HEKs and HDFs. However, IL-13 induced secretion of CCL-17/TARC and CCL-22/MDC from human peripheral blood mononuclear cells (PBMCs), which was potently and dose-dependently inhibited by tralokinumab (Fig. S2[1] and Table SI[1]).

In addition, the effects of IL-13 and tralokinumab were investigated on 5 skin barrier related genes, *FLG, FLG2, LOR, ELOVL3* and *ELOVL6*, that had shown a negative correlation with *IL13* (Table I and Fig. S1[1]). Differentiated primary human epidermal keratinocytes were stimulated with 50 ng/ml (4.2 nM) IL-13 in the presence of a broad concentration range (0.78–200 nM) of tralokinumab or isotype control antibody for 24 h. In alignment with the correlation analysis and with previous studies (reviewed in (6)), IL-13 clearly downregulated expression of the skin barrier related genes (Fig. 1e), with the exception of *ELOVL6*, of which expression levels remained unchanged upon stimulation with IL-13. Treatment with tralokinumab potently neutralized IL-13, leading to a dose-dependent and full normalization of *FLG*, *FLG2*, *LOR*, and *ELOVL3* expression, with low nanomolar IC_{50} values (Table SI[1]).

In summary, skin barrier- and inflammation-associated genes in AD skin correlate with *IL13* expression, and a subset of these genes were investigated and shown to be regulated by IL-13 and fully normalized by tralokinumab *in vitro*. Compared with estimated steady-state concentrations for tralokinumab of approximately 30–35 nM in skin of patients with AD after 300 mg once every 2 weeks (Q2W) dosing, the IC_{50} values from the *in vitro* studies are at clinically relevant concentrations and the potency of tralokinumab may probably be underestimated due to the high concentrations of IL-13 used *in vitro*. These data provide molecular insights and may explain the clinical effects of tralokinumab, including skin barrier restoration and reduction in skin inflammation.

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Conflicts of interest. MT, TL and HN are employees of LEO Pharma. SW is a speaker, advisory board member and/or investiga-

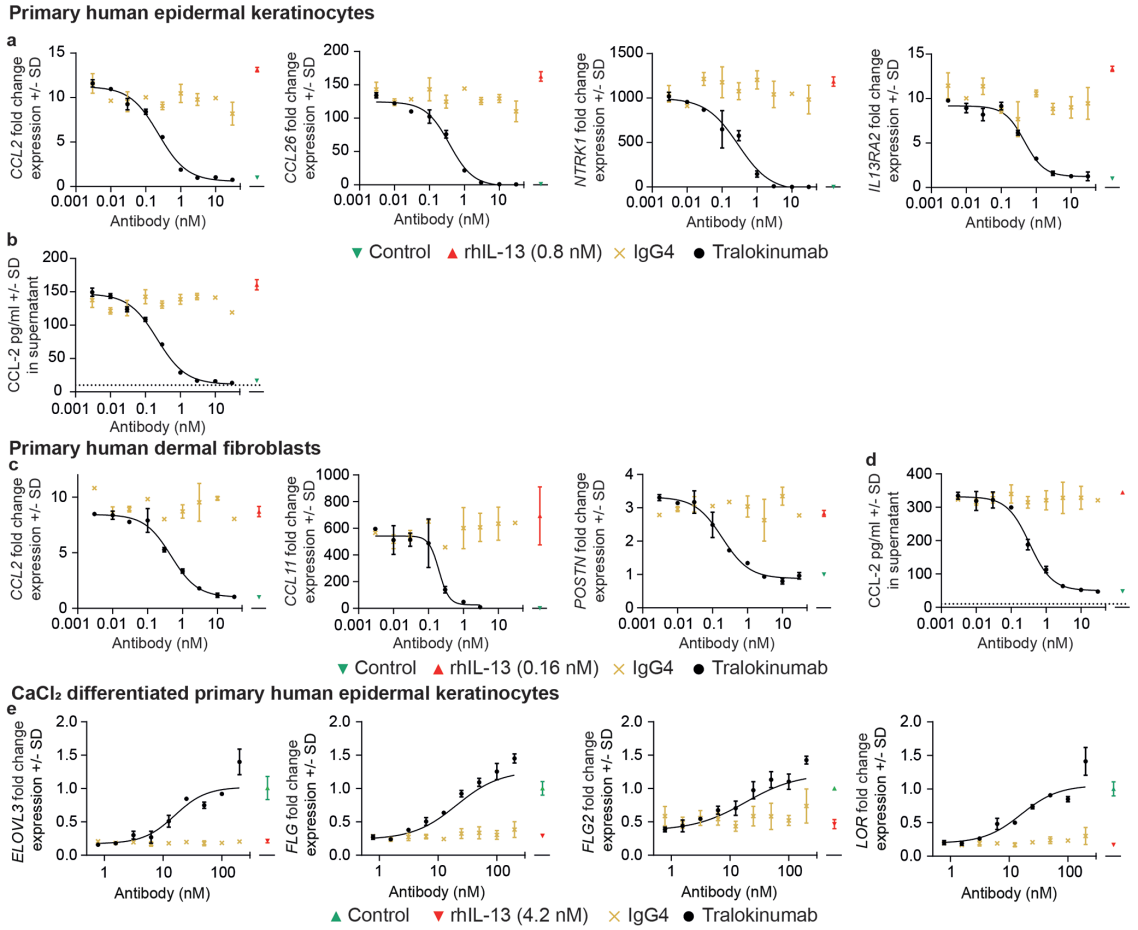

Fig. 1. Atopic dermatitis (AD)-associated genes are regulated by interleukin (IL)-13 and modulated by tralokinumab in cultures of human skin cells. Cells were stimulated with IL-13 in absence or presence of a broad range of tralokinumab or IgG4 isotype control antibody for 24 h and subjected to gene expression analysis by qPCR (a, c and e) and protein analysis of CCL-2 in supernatants by enzyme-linked immunoassay (ELISA) (b and d). (a) Gene expression analysis on primary human epidermal keratinocytes (HEK). (b) CCL-2 levels (pg/ml) in supernatants from HEK cells. *Dashed line* indicates the lower limit of detection. (c) Gene expression analysis on primary human dermal fibroblasts (HDF). (d) CCL-2 levels (pg/ml) in supernatants from HDF cells. (e) As in (a), however, using $CaCl_2$ differentiated HEK cells. Gene expression data is shown as fold change compared with the untreated controls (*green symbol*).

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