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SEB-induced IL-13 production in CLA$^+$ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis

To the Editor,

Staphylococcus aureus, memory skin-homing cutaneous lymphocyte-associated antigen (CLA)$^+$ T cells and IL-13 constitute relevant players in atopic dermatitis (AD) pathogenesis.[1] Since circulating CLA$^+$ T cells reflect cutaneous abnormalities present in human inflammatory skin diseases,[2] an *ex vivo* coculture model made of purified circulating CLA$^{+/-}$ effector and central memory T cells and autologous lesional epidermal cells was established. We show a CLA-dependent production of IL-13 upon activation with staphylococcal enterotoxin B (SEB) that allows the differentiation of the Th2 high and Th2 low groups, with distinct clinical correlations between both groups, within a clinically homogeneous population of adult non-treated moderate-to-severe AD patients.

Our results showed that IL-13, together with IL-4, IL-17A, IL-22, CCL17, and CCL22, was preferentially produced by circulating memory CLA$^+$ T cells upon activation with SEB in the presence of autologous lesional epidermal cells (Figure 1A). Interestingly, SEB activation of the CLA$^+$/Epi cocultures resulted in a predominant IL-13 production among the Th2 cytokines (IL-13, IL-4, IL-5) (Figure 1B). The amount of IL-5 and IFN-γ produced by SEB-activated CLA$^-$ T cells was higher or similar than that by CLA$^+$ T cells, respectively, suggesting their relationship to extracutaneous sites. This model is stimulus-specific since polyclonal activators such as PMA/Ionomycin and CD3/CD28 are not CLA-specific (Figure S1A), and epidermal cells contributed to the T-cell activation (Figure S1B).

Patients were stratified based on the median of the IL-13 response in the SEB-induced CLA$^+$ T-cell AD cocultures (Figure 1C), and we found differentiated T-cell responses to SEB between the Th2 high and the Th2 low groups (Figure 1D). Although both groups were clinically homogeneous (Figure S1C), this stratification suggested differential immunological mechanisms between both groups, since they not only differed in terms of *in vitro* stimulation, but also in terms of severity, plasma markers, IgE levels against *S. aureus* and mRNA expression from cutaneous lesions.

In the Th2 high group, in contrast to the Th2 low, the IL-13 response by SEB CLA$^+$ T cells directly correlated with EASI score and plasma levels of CCL17 and sIL-2R (Figure 2A-C). This group also showed a direct correlation between anti-*S. aureus* IgE levels and SEB-induced CLA$^+$ T-cell-mediated IL-13 response *in vitro* (Figure 2D). The mRNA expression from lesional skin biopsies was similar between both groups (Figure S2A), but the IL-13 produced by SEB-stimulated CLA$^+$/Epi cocultures directly correlated with CCL26 (Figure 2E) and inversely correlated with LCN2 mRNA expression in the Th2 high group (Figure 2F). Additionally, the IL-13/IL-17A and IL-13/IFN-γ ratios in the SEB-stimulated CLA$^+$ T-cell cocultures were higher in the Th2 high than the Th2 low group (Figure S2B), supporting the type 2 signature and the lowered type 17 and type 1 immunity in the Th2 high group, which may facilitate *S. aureus* infection.[3]

The study has a few limitations. We did not study the presence of *S. aureus* in the skin of the AD patients and the number of patients was not very high but we found consistent significant results on the relationship between the SEB-induced CLA$^+$ T-cell IL-13 response and clinical features of the patients.

The novelty of our results relies on the separation of the Th2 high and low populations, corresponding with disease activity, based on the CLA$^+$ T-cell IL-13 response to SEB, which are key mediators in AD pathogenesis.[4] Interestingly, the existence of Th2 high and low groups in non-treated moderate-to-severe AD patients has been shown by serum proteomic profiling.[5] In conclusion, we consider that this new and translational approach allows obtaining readouts on cytokine production that complement current studies based on transcriptomics and flow cytometry and may help to explore the complex heterogeneity of AD pathophysiology from a more functionally point of view.

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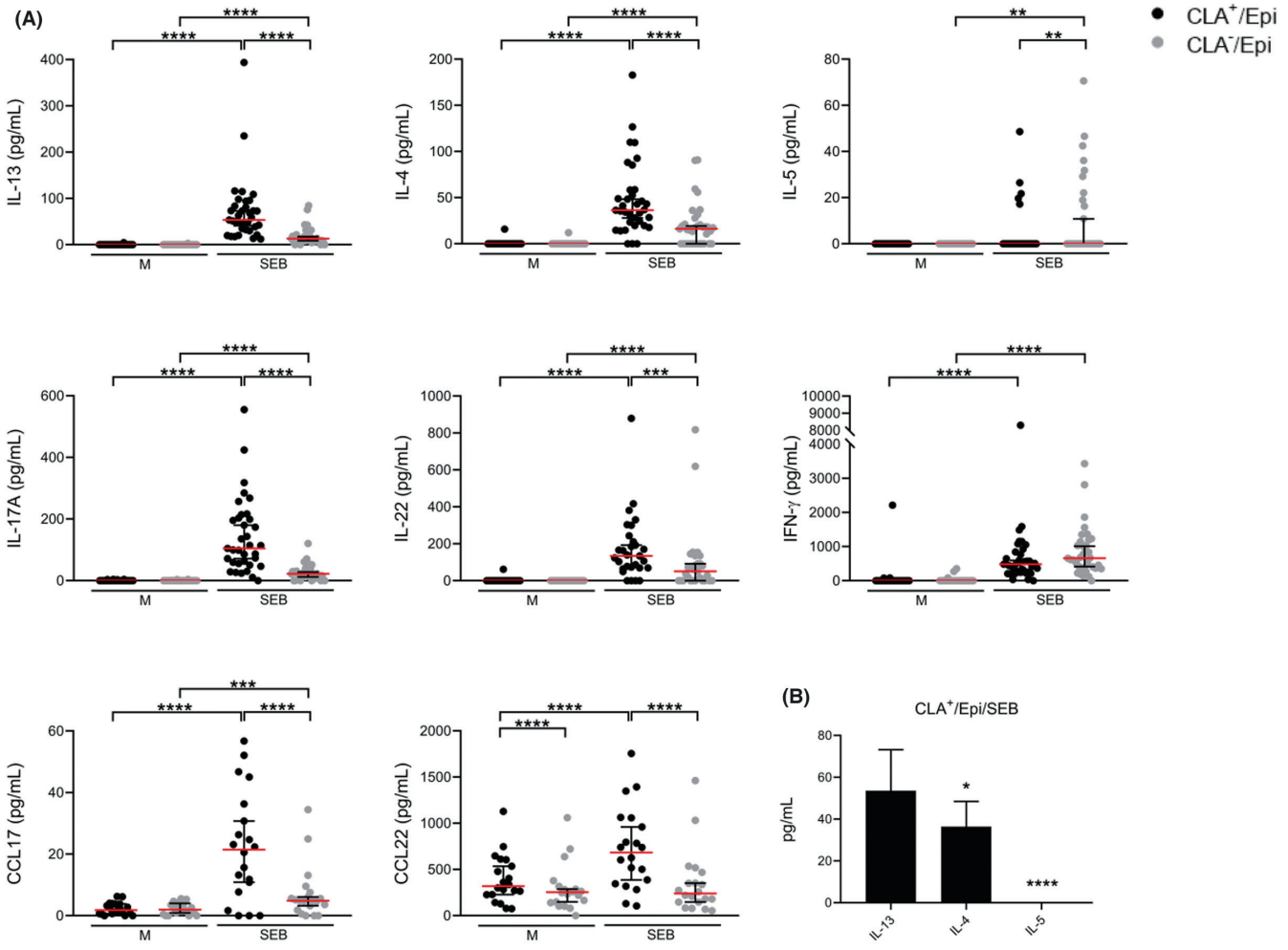

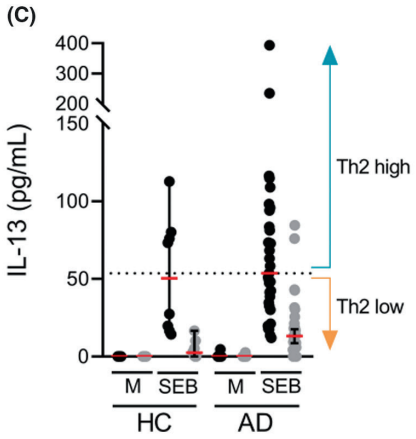

(D)

(pg/mL)	Th2 high Median (25th-75th percentiles)	N	Th2 low Median (25th-75th percentiles)	N	P-value
IL-13	**82.40 (71.70-110.5)**	**18**	**32.57 (18.81-44.87)**	**17**	**<.0001**
IL-4	**49.46 (32.89-96.95)**	**18**	**24.66 (14.43-42.21)**	**17**	**.003**
IL-5	0.00 (0.00-4.92)	14	0.00 (0.00-0.00)	16	.46
IL-17A	86.19 (49.62-180.0)	18	143.8 (72.87-241.2)	17	.23
IL-22	115.1 (69.78-174.3)	14	166.4 (49.75-300.7)	15	.60
IFN-γ	454.0 (332.2-787.3)	18	509.3 (223.5-1069)	17	.99

FIGURE 1 Production of AD-associated mediators by SEB-activated cocultures of CLA^+ T cells and lesional epidermal cells and stratification into the Th2 high and Th2 low groups. (A) Quantification (pg/ml) of IL-13, IL-4, IL-5, IL-17A, IL-22, IFN-γ, CCL17, and CCL22 in 24-hour cocultures in basal conditions or stimulated with SEB ($n = 35$ for IL-13/4/17A and IFN-γ, $n = 30$ for IL-5, $n = 29$ for IL-22, and $n = 20$ for CCL17/22). (B) Th2 cytokines produced by SEB-induced CLA^+ T-cell cocultures presented by column bars and the median ± 95% CI. (C) IL-13 levels in AD ($n = 35$) and control subjects ($n = 8$). Dotted line indicates the median of SEB-induced CLA^+ T-cell IL-13 response in AD. (D) Cytokine response (pg/ml) by SEB-activated CLA^+ T-cell cocultures was compared between the Th2 high and the Th2 low groups. Abbreviations: AD, atopic dermatitis; CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HC, healthy controls; M, untreated; SEB, staphylococcal enterotoxin B. **: $p < .01$; ***: $p < .001$; ****: $p < .0001$

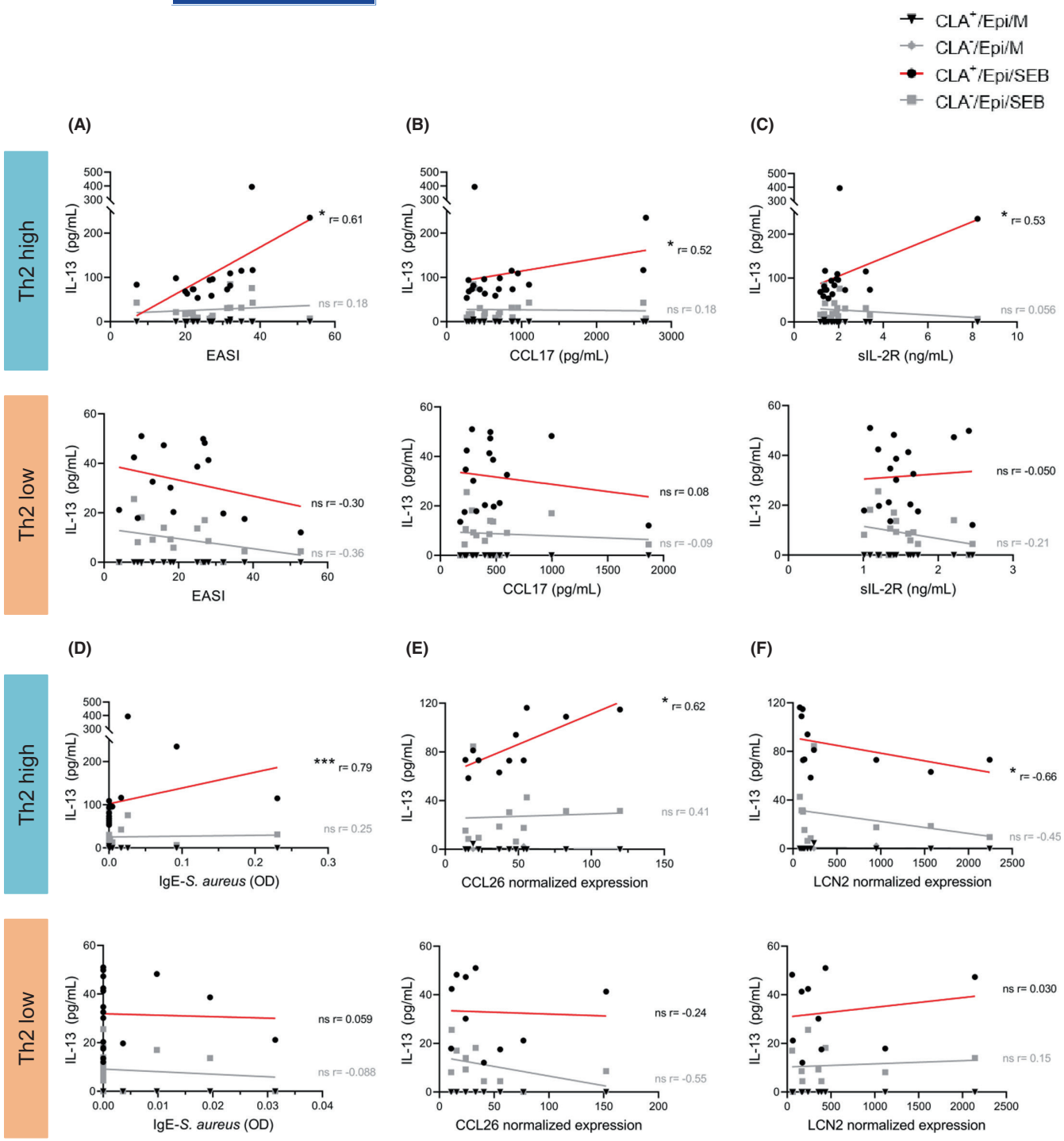

FIGURE 2 In the Th2 high group, SEB-triggered CLA$^+$/Epi IL-13 response directly correlates with EASI, CCL17, sIL-2R, and anti-*S. aureus* IgE plasma levels and CCL26 mRNA expression in cutaneous lesions and inversely correlates with LCN2 mRNA expression in cutaneous lesions. IL-13 (pg/mL) from 24-hour cocultures was correlated with (A) EASI ($n = 17$ for Th2 high and $n = 15$ for Th2 low), (B) plasma CCL17 ($n = 18$ for Th2 high and $n = 17$ for Th2 low), (C) plasma sIL-2R ($n = 18$ for Th2 high and $n = 17$ for Th2 low), (D) anti-*S. aureus* IgE plasma levels ($n = 17$ for Th2 high and $n = 17$ for Th2 low), (E) CCL26 ($n = 11$ for Th2 high and $n = 10$ for Th2 low), and (F) LCN2 mRNA expression in lesional skin biopsies ($n = 11$ for Th2 high and $n = 10$ for Th2 low). Abbreviations: CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; M, untreated; SEB, staphylococcal enterotoxin B. ns: $p > .05$; *: $p < .05$; ***: $p < .001$

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CONFLICT OF INTEREST

Antonio Guilabert is a consultant for Sanofi, Almirall, and AbbVie. Laia Curto-Barredo is a consultant for Sanofi, AbbVie, Leo Pharma, and Lilly. Esther Serra-Baldrich is a consultant for Sanofi, Almirall, Leo Pharma, Pfizer, Galderma, and Lilly. Michael D. Howell is an employee and shareholder of DermTech. The rest of authors declare no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.