Circulating CLA+ T cell effector function allows patient stratification for IL-13 and IL-31 production in adult non-treated moderate-to-severe atopic dermatitis

Lídia Sans-De San Nicolàs[1], Ignasi Figueras-Nart[2], Irene García-Jiménez[1], Montserrat Bonfill-Ortí[2], Antonio Guilabert[3], Laia Curto-Barredo[4], Marta Bertolín-Colilla[4], Marta Ferran[4], Esther Serra-Baldrich[5], Ramon M. Pujol[4] and Luis F. Santamaria-Babí[1]

[1]Immunologia Translacional, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona (UB), Parc Científic de Barcelona (PCB), Barcelona, Spain; [2]Departament de Dermatologia, Hospital de Bellvitge, Universitat de Barcelona (UB), L'Hospitalet de Llobregat, Spain; [3]Departament de Dermatologia, Hospital General de Granollers, Granollers, Spain; [4]Departament de Dermatologia, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; [5]Departament de Dermatologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

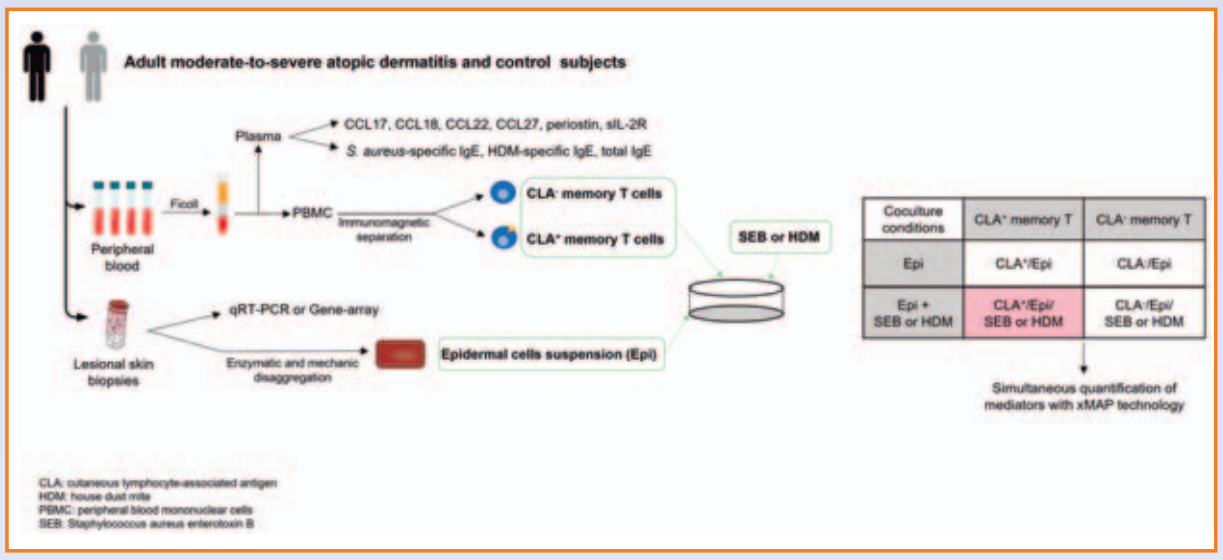
Background

Atopic dermatitis (AD) is a chronic heterogeneous T-cell mediated skin disease. Circulating skin-homing cutaneous lymphocyte-associated antigen (CLA)+ memory T cells reflect the cutaneous abnormalities in AD[1,2]. There is currently no clear *in vitro* functional immunological approach to explore endotypes for Th2 cytokines.

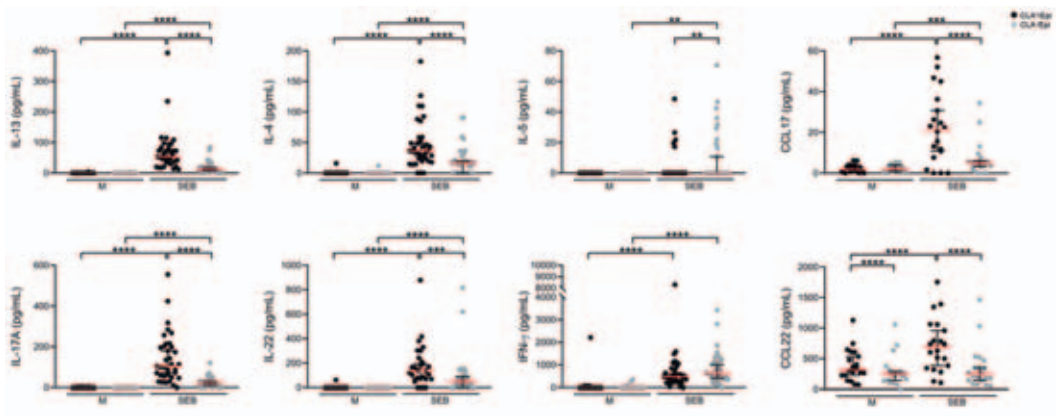
Staphylococcus aureus, CLA+ memory T cells and IL-13 are relevant players in AD. *S. aureus* colonizes AD skin and is associated to flare ups, disease severity and enhanced Th2 immune response. Application of *S. aureus* enterotoxin B (SEB) to intact AD skin induces T cells activation. We aimed at understanding AD functional immune Th2 response heterogeneity through SEB activation of CLA+ memory T cells.

IL-31 is produced by dendritic cells, mast cells, basophils, eosinophils, type 2 innate lymphoid cells, M2 macrophages, and preferentially CD4+ T cells with a Th2 phenotype. It is involved in inflammation, pruritus, epidermal barrier disruption and tissue remodelling. The relationship between IL-31 and allergens has been poorly characterized and mainly limited to canine models. IL-31 production by T cells is mostly studied through qRT-PCR and intracellular flow cytometry of peripheral blood mononuclear cells in a low number of patients, and without association with the clinic. We aimed at deciphering the role of allergen in IL-31 production by memory T cells and its possible relationship with clinical features in AD patients.

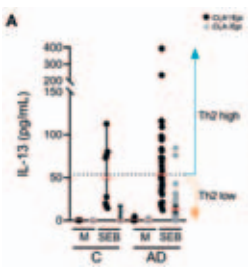
Methods



SEB-induced IL-13 production in CLA+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis[3]



IL-13, IL-4, IL-17A, IL-22, CCL17 and CCL22 are preferentially produced by circulating CLA+ memory T cells activated with SEB. 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 (M) 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). Statistics are represented as median with 95% confidence interval. Wilcoxon test was used for comparisons. **: p<.01; ***: p<.001; ****: p<.0001



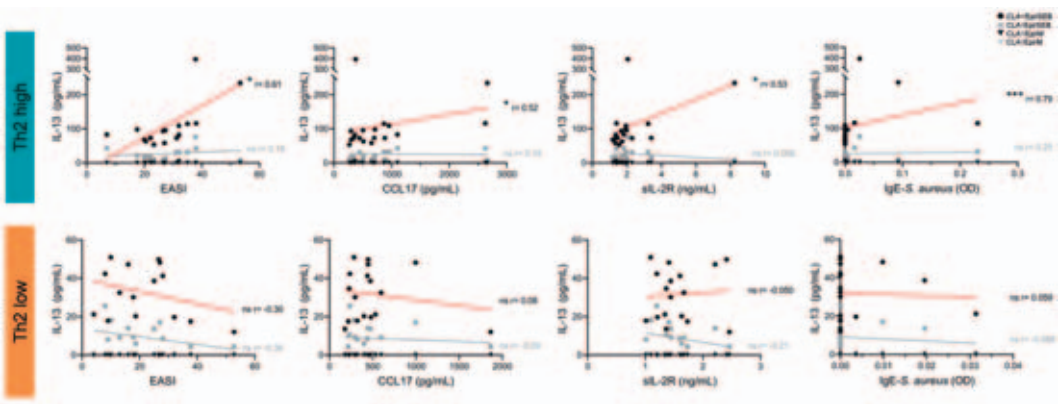
B

Cytokine (pg/mL)	CLA+/Epi/SEB Th2 high Median (25-75% percentiles)	CLA+/Epi/SEB Th2 low Median (25-75% percentiles)	p Value
IL-13	82.40 (71.70-113.5)	32.57 (18.81-44.87)	****
IL-4	49.46 (32.89-96.85)	24.66 (14.43-42.21)	**
IL-5	0.00 (0.00-4.92)	0.00 (0.00-0.00)	ns
IL-17A	86.19 (49.62-180.0)	143.8 (72.87-241.2)	ns
IL-22	115.1 (69.79-174.3)	166.4 (49.75-300.7)	ns
IFN-γ	454.0 (332.2-797.3)	509.3 (223.5-1069)	ns

C

Clinical data	Th2 high Median (25-75% percentiles)	Th2 low Median (25-75% percentiles)	p Value
Age	42.00 (26.50-51.50)	36.00 (26.50-47.00)	.39
Years since diagnosis	15.50 (8.75-27.50)	30.00 (19.50-35.00)	.036
SCORAD	58.49 (50.00-67.35)	62.50 (57.85-69.85)	.31
EASI	27.00 (21.25-33.50)	18.60 (10.00-28.00)	.11
IGA	3.00 (3.00-4.00)	3.00 (2.25-4.00)	.29
Pruritus (VAS)	7.00 (6.00-8.00)	7.00 (5.00-9.00)	.89
Eosinophilia (10³/uL)	0.59 (0.49-0.79)	0.38 (0.21-0.72)	.23
Total IgE (kU/L)	4300 (1218-7292)	1212 (368.5-5365)	.16

The IL-13 response enables the stratification of a clinically homogeneous population into Th2 high and Th2 low groups. (A) IL-13 levels (pg/mL) in AD (*n* = 35) and control subjects (C, *n* = 8). Dotted line indicates the median of SEB-induced CLA+ T-cell IL-13 response in AD. **(B)** IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ (pg/mL) produced by SEB-induced CLA+ T-cell cocultures was compared between Th2 high (*n* = 14-18) and Th2 low (*n* = 15-17) groups. Data presented as the median (25-75% percentiles) and compared with the Mann-Whitney test. **(C)** Clinical characteristics were compared between Th2 high (*n* = 12-17) and Th2 low (*n* = 13-17) groups. Data presented as the median (25-75% percentiles) and compared with the Mann-Whitney test. EASI, eczema area and severity index; IGA, investigator's global assessment; M, untreated; SCORAD, scoring atopic dermatitis; VAS, visual analogue scale. ns: p>.05; **: p<.01; ****: p<.0001



In the Th2 high group, unlike the Th2 low, the SEB-induced CLA+ T-cell IL-13 response directly correlates with eczema area and severity index (EASI), CCL17, sIL-2R and *S.aureus*-specific IgE plasma levels. IL-13 (pg/mL) from 24-hour cocultures was correlated with EASI (*n* = 17 for Th2 high and *n* = 15 for Th2 low), and plasma levels of CCL17 (*n* = 18 for Th2 high and *n* = 17 for Th2 low), sIL-2R (*n* = 18 for Th2 high and *n* = 17 for Th2 low), and *S. aureus*-specific IgE (*n* = 17 for Th2 high and *n* = 17 for Th2 low). Correlations performed with R Spearman test and represented with linear regression. M, untreated; ns: p>.05; *: p<.05; ***: p<.001

Allergen sensitization stratifies IL-31 production by CLA+ memory T cells in atopic dermatitis[4]

HDM induces IL-31 in CLA+ memory T cells cultured with autologous lesional epidermal atopic dermatitis cells, which correlates with pruritus, and plasma CCL27 and periostin. (A) IL-31 (pg/mL) produced by CLA+/- memory T-cell cocultures in basal conditions (M) or stimulated with HDM in AD-(*n* = 58) and C-(*n* = 11) derived samples. Statistics are represented as median with 95% confidence interval. Wilcoxon test and Mann-Whitney test were used for comparisons within the same group or between two groups, respectively. **(B)** Correlations of CLA+/Epi/HDM IL-31 response in group 1 with pruritus (*n* = 17), and plasma levels of CCL27 (*n* = 17), and periostin (*n* = 13). Correlations tested with R Spearman and represented with linear regression. C, control subjects; VAS, visual analogue scale. *: p<.05; **: p<.01; ****: p<.0001

A

Clinical data	Group 1 Median (25-75% percentiles)	Group 2 Median (25-75% percentiles)	p Value
Age	28.00 (18.50-44.50)	36.00 (27.00-45.75)	.063
Years since diagnosis	15.50 (11.00-25.75)	24.00 (13.00-33.50)	.28
SCORAD	59.63 (30.00-61.32)	59.27 (42.10-68.00)	.44
EASI	22.65 (19.38-27.93)	25.00 (15.25-32.00)	.92
IGA	4.00 (3.00-4.00)	3.00 (3.00-4.00)	.35
Pruritus (VAS)	7.00 (6.50-8.00)	8.00 (6.25-9.00)	.31
Eosinophilia (10³/uL)	0.73 (0.21-1.06)	0.46 (0.24-0.61)	.16
sp IgE response (OD)	18747 (12287-20456)	5338 (393.0-16830)	<.0001
Total IgE (kU/L)	2000 (606.9-5000)	568.0 (110.5-1792)	.0027

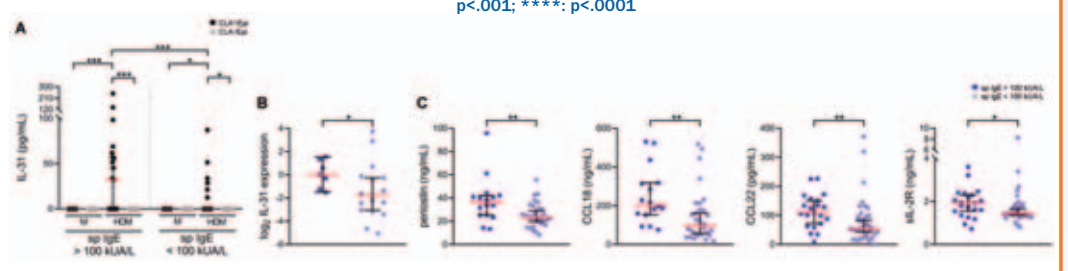
B

Cytokine (pg/mL)	CLA+/Epi/HDM Group 1 Median (25-75% percentiles)	CLA+/Epi/HDM Group 2 Median (25-75% percentiles)	p Value
IL-13	242.7 (123.7-471.8)	5.22 (0.00-41.24)	****
IL-4	57.34 (32.55-103.9)	0.00 (0.00-5.53)	****
IL-5	67.72 (12.04-108.9)	0.00 (0.00-8.71)	****
IL-17A	24.83 (6.98-49.20)	0.00 (0.00-7.75)	***
IL-22	57.18 (11.16-149.0)	0.00 (0.00-0.00)	****
IFN-γ	0.00 (0.00-22.97)	0.00 (0.00-7.32)	ns

C

	Term name	Term ID	adj. p
Group 1	response to external stimulus	GO:0009605	1.69 e-6
	response to cytokine	GO:0034097	4.20 e-6
	inflammatory response	GO:0006954	6.16 e-5
	immune system process	GO:0002376	8.35 e-5
	defense response	GO:0006952	1.62 e-4
	cell population proliferation	GO:0008283	2.02 e-4
	cellular response to chemokine	GO:1990869	2.02 e-4
	response to interleukin-1	GO:0070555	9.73 e-4
Group 2	anatomical structure morphogenesis	GO:0009653	0.022
	developmental process	GO:0032502	0.022
	anatomical structure development	GO:0048856	0.022
	regulation of multicellular organismal process	GO:0051239	0.022
	negative regulation of locomotion	GO:0040013	0.028
	regulation of developmental process	GO:0050793	0.033
	cyclic-nucleotide-mediated signalling	GO:0019935	0.038
	multicellular organism development	GO:0007275	0.040

Patients with IL-31 response by HDM-activated CLA+ T cells show higher HDM-specific and total IgE and more inflammatory profile than patients with no IL-31 response. (A) Clinical characteristics were compared between group 1 (*n* = 15-17) and group 2 (*n* = 31-41) of patients. Data presented as the median (25-75% percentiles) and compared with the Mann-Whitney test. Bold values indicate significant data. **(B)** IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ (pg/mL) produced by HDM-induced CLA+ T-cell cocultures were compared between groups 1 (*n* = 13-17) and 2 (*n* = 35-41) of patients. Data presented as the median (25-75% percentiles) and compared with the Mann-Whitney test. **(C)** Functional enrichment of all differential expressed gens (gene-array performed in *n* = 3 group 1 and *n* = 13 group 2 cutaneous lesions, considering differential expressed genes those with a fold change of 1.5 or greater and a p value of less than .05) up-regulated in group 1 and group 2 of patients with GO biological process. For group 1, summary of enriched biological processes; for group 2, representation of all enriched biological processes of significant terms (Benjamini-Hochberg FDR < .05). EASI, eczema area and severity index; IGA, investigator's global assessment; GO, gene ontology; OD, optical density; SCORAD, scoring atopic dermatitis; sp IgE, HDM-specific IgE; VAS, visual analogue scale. ns: p>.05; ***: p<.001; ****: p<.0001



Patients with HDM-specific IgE > 100 kUA/L show increased IL-31 response by HDM-induced CLA+ T cells, IL-31 mRNA expression in lesional skin and plasma levels of periostin, CCL18, CCL22 and sIL-2R compared to those with HDM-specific IgE < 100 kUA/L. (A) IL-31 (pg/mL) from atopic dermatitis culture supernatants separated according to sp IgE levels (> 100 kUA/L *n* = 21, < 100 kUA/L *n* = 37). Statistics are represented as median with 95% confidence interval, and Wilcoxon test and Mann-Whitney test were used for comparisons within the same group or between two groups, respectively. **(B)** IL-31 mRNA expression in cutaneous lesions (high *n* = 7, low *n* = 17) and **(C)** plasma levels of periostin (ng/mL), CCL18 (ng/mL), CCL22 (pg/mL) and sIL-2R (ng/mL) were compared between high (*n* = 16-21) and low (*n* = 30-37) sp IgE groups. Statistics are represented as median with 95% confidence interval, Mann-Whitney test was used for comparisons. M, untreated; sp IgE, HDM-specific IgE. *: p<.05; **: p<.01; ***: p<.001

Conclusions

This translational approach based on the coculture of skin-homing CLA+ memory T cells with autologous lesional epidermal cells from adult non-treated moderate-to-severe atopic dermatitis patients allows identifying Th2 high and Th2 low responders from a clinically homogeneous population based on the SEB-CLA+-IL-13 axis that differently correlate with EASI and CCL17, the best biomarker for atopic dermatitis. Additionally, it allows stratifying patients into IL-31 producers and non-producers by HDM-induced CLA+ T cells in relation with allergen sensitization status by functionally analysing the CLA+ T-cell immune response, and correlating IL-31 response with patient's pruritus and CCL27, a biomarker of clinical response to anti-IL-31RA treatment. The current findings may help to explore the complex heterogeneity of AD pathophysiology from a more functional point of view and guide patient's stratification for directed therapies.

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Contact: lidia.sans@ub.edu

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