UNIVERSITAT DE BARCELONA

The role of CLA+T lymphocytes in the development of Atopic Dermatitis

Lídia Sans de San Nicolàs

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Doctoral Thesis

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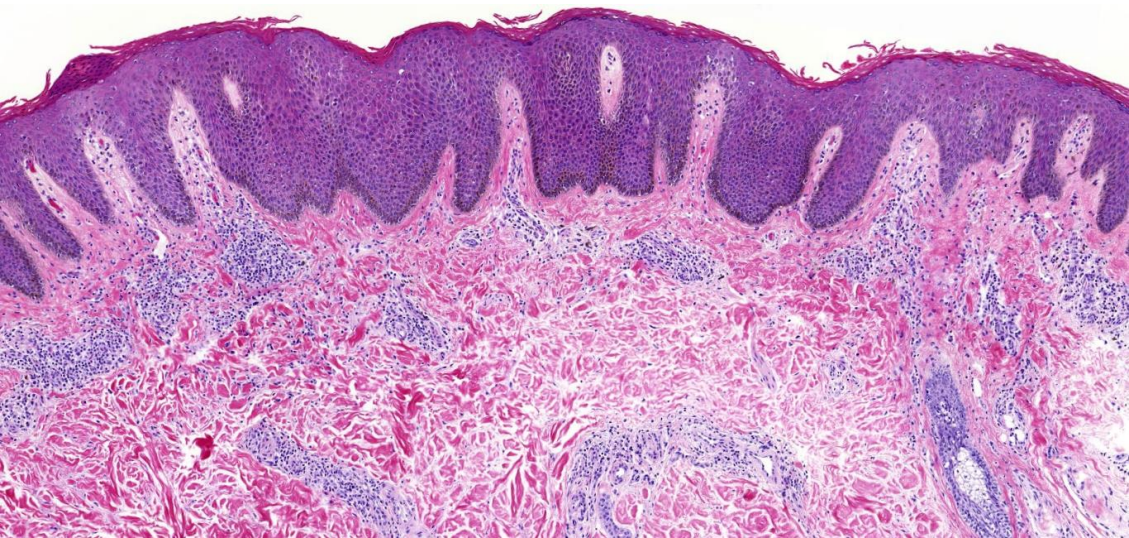
Barcelona, 2023



UNIVERSITAT DE BARCELONA

Department of Cellular Biology, Physiology and Immunology

PhD Program in Biomedicine

The role of CLA$^+$ T lymphocytes in the development of Atopic Dermatitis

Thesis submitted by

Lídia Sans de San Nicolàs

to qualify for the degree of Doctorate by the Universitat de Barcelona

Directed by Dr. Luis F. Santamaria Babí

Lídia Sans de San Nicolàs
PhD Candidate

Dr. Luis F. Santamaria Babí
Director and tutor

Barcelona, 2023

"La ciencia siempre vale la pena,
porque sus descubrimientos,
tarde o temprano,
siempre se aplican."
Severo Ochoa

A l'avi i la iaia,
per formar part d'aquesta història.

ABSTRACT

Atopic dermatitis is the most common chronic immune-mediated inflammatory skin disease affecting up to 20% of children and 10% of adults. The complex pathophysiology of the disease comprises genetic susceptibility, epidermal barrier dysfunction, cutaneous dysbiosis with abundance of *S. aureus*, an abnormal cutaneous immune system activation with a core Th2 response and pruritus. Cutaneous Lymphocyte-associated Antigen $(CLA)^+$ T cells represent the subset of memory T cells that belong to the cutaneous immune system. CLA^+ T cells recirculate between blood and skin through the thoracic duct, specifically respond to skin-related antigens, and represent over 90% of T cells infiltrating the skin. Therefore, CLA^+ T cells constitute peripheral cellular biomarkers and, because they can be found in general circulation, they are a source of translational information on the immunological mechanisms taking place in the skin during disease. We have studied atopic dermatitis in the context of the cutaneous immune response through the effector function of CLA^+ T cells. For this, we have stablished a novel *ex vivo* model of adult non-treated moderate-to-severe atopic dermatitis based on circulating CLA^+ T cells cocultured with a suspension of autologous epidermal cells obtained from lesional biopsies in the same patients. Then, we have studied the T-cell effector function in response to relevant disease triggers such as *S. aureus* microorganism and house dust mite (HDM) allergen, as well as the association between cytokine response to the stimuli and patient's clinical data. First, the study of IL-13 response to *S. aureus* enterotoxin B (SEB) by CLA^+ T cells defined two groups of patients, Th2 high and Th2 low, within a clinically homogeneous population. In the Th2 high group, in contrast to the Th2 low group, the IL-13 response positively correlated with severity, in terms of EASI score, and levels of CCL17, sIL-2R and *S. aureus*-specific IgE in plasma. Additionally, in this group the IL-13 response directly correlated with CCL26 and indirectly correlated with LCN2 mRNA expression in cutaneous lesions. Conversely, in the Th2 low group, the CLA^+ T-cell response to SEB skewed towards Th17, Th22 and Th1. Next, the role of the neuroimmune cytokine IL-31 was examined in our model by studying the CLA^+ T-cell response to HDM, and a bimodal (present or absent) IL-31 response in relation with the HDM-specific IgE levels in plasma was observed. Patients producing IL-31 by HDM-activated CLA^+ T cells showed increased HDM-specific and total IgE levels and reported an increased inflammatory profile compared to patients with no IL-31 response. Interestingly, the IL-31 response directly correlated with patient's pruritus intensity and plasma levels of CCL27 and periostin. Of note, patients with no IL-31 response reported raised presence of HDM-specific and total IgE levels compared to control subjects, suggesting that the degree of IgE sensitization to HDM in this group was not enough for inducing IL-31 response. In summary, this novel *ex vivo* model of adult non-treated moderate-to-severe atopic dermatitis has allowed to functionally identify Th2 high and Th2 low responders from a clinically homogeneous population based on the SEB-CLA^+-IL-13 axis, as well as stratifying patients into IL-31 producers and non-producers in relation with the degree of IgE sensitization to HDM by analyzing the CLA^+ T-cell response to HDM and its association with clinical features. Altogether, this translational work expands the understanding of the heterogeneous inflammatory response of the disease and may contribute to improving the effectiveness of targeted therapies.

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AMPs: Antimicrobial peptides

APCs: Antigen presenting cells

CCL: C-C chemokine ligand

CCR: C-C chemokine receptor

CGRP: Calcitonin gene-related peptide

CLA: Cutaneous Lymphocyte-associated Antigen

CTACK: Cutaneous T cell-attracting chemokine

DRG: Dorsal root ganglia

EASI: Eczema Area and Severity Index

EMA: European Medicines Agency

Epi: Epidermal cells suspension from lesional biopsies

FcεRI: high-affinity IgE receptor

FDA: Food and Drug Administration

FLG: Filaggrin

γc: Gamma chain

HDM: House dust mite

HLA: Human leucocyte antigen

ICAM-1: Intercellular adhesion molecule 1

ICOS: Inducible T cell costimulator

IFN: Interferon

Ig: Immunoglobulin

IL: Interleukin

ILC: Innate lymphoid cells

INV: Involucrin

JAK: Janus kinase

JAKi: JAK inhibitor

KC: Keratinocyte

LFA-1: Lymphocyte-function-associated antigen 1

LOR: Loricrin

mAb: Monoclonal antibody

MHC-II: Major histocompatibility complex class II

NMFs: Natural moisturizing factors

OSMR: Oncostatin M receptor

PAR2: Protease-activated receptor 2

PSGL-1: Platelet-selectin glycoprotein ligand 1

S. aureus*: Staphylococcus aureus*

SALT: Skin-associated lymphoid tissue

SCORAD: SCORing Atopic Dermatitis

SEB: *S. aureus* enterotoxin B

STAT: Signal transducer and activation of transcription molecule

TARC: Thymus- and activation-regulated chemokine

Tc: T cytotoxic cell

T_{CM}: Central memory T cells

TCR: T-cell receptor

T_{EM}: Effector memory T cells

TEWL: Transepidermal water loss

Th: T helper cell

TJ: Tight junction

TSLP: Thymic stromal lymphopoietin

VAS: Visual Analogue Scale

VCAM-1: Vascular cell adhesion protein 1

VLA-4: Very later antigen

INTRODUCTION

1. Atopic dermatitis

Atopic dermatitis is a chronic inflammatory skin disorder characterized by highly pruritic and recurrent eczematous lesions over a dry, thickened skin with a fluctuating course. It is the most common inflammatory skin disease, affecting up to 20% of children and 10% of adults in developed countries, and the non-fatal skin condition with the highest disease burden (Langan et al. 2020).

Clinically, atopic dermatitis is extremely heterogeneous as it presents a wide spectrum of features (or phenotypes), such as age of onset, severity, and the presence of atopic comorbidities. The disease may initiate at any age, but it usually develops in early childhood and persists into adulthood. Most of the patients have a mild disease, and 30% of patients develop a moderate-to-severe form. There is no gender predilection, and prevalence varies among ethnic groups and geographic locations, being most frequent in Sweden, United Kingdom, Iceland, Finland, and Denmark, while the lowest prevalence is found in Kazakhstan, China, Tajikistan, Armenia, and Uzbekistan (Global Burden of Disease project 2017 data)(Laughter et al. 2021). Atopic dermatitis is associated with increased risk of numerous comorbidities. It frequently co-occurs with other atopic diseases, including food allergy, asthma and allergic rhinitis (with or without conjunctivitis) in the so-called “atopic march”, and most patients have a personal or family history of these atopic diseases. Patients often experience other non-atopic comorbid conditions, such as inflammatory bowel disease, and psychosocial comorbidities, such as sleep disruption, anxiety, and depression, with a substantial impact on a patient’s quality of life. Therefore, atopic dermatitis can be defined as a systemic inflammatory disease with multiple comorbidities extending beyond the well-recognized atopic associations.

Histologically, acute atopic dermatitis lesions are characterized by mild epidermal thickening (acanthosis), intercellular oedema (spongiosis), and eosinophilic and lymphocytic dermal infiltrates mostly $CD4^+$ Cutaneous Lymphocyte-associated

Antigen $(CLA)^+$ memory T lymphocytes (Acevedo et al. 2020; Leung et al. 1983)). At chronic stages, lichenified skin presents prominent acanthosis with elongation of the rete ridges, thickening of the stratum corneum (hyperkeratosis), incomplete maturation of keratinocytes (KCs) with retention of the nucleus in the stratum corneum (parakeratosis), minimal spongiosis, a dense perivascular dermal infiltration of mononuclear cells dominated by mast cells, and fibrosis (Figure 1) (Weidinger et al. 2018). Of note, non-lesional (unaffected) skin from atopic dermatitis patients already shows increased epidermal thickness, spongiosis and dermal infiltration of lymphocytes compared to normal skin from healthy subjects (Suárez-Fariñas et al. 2011).

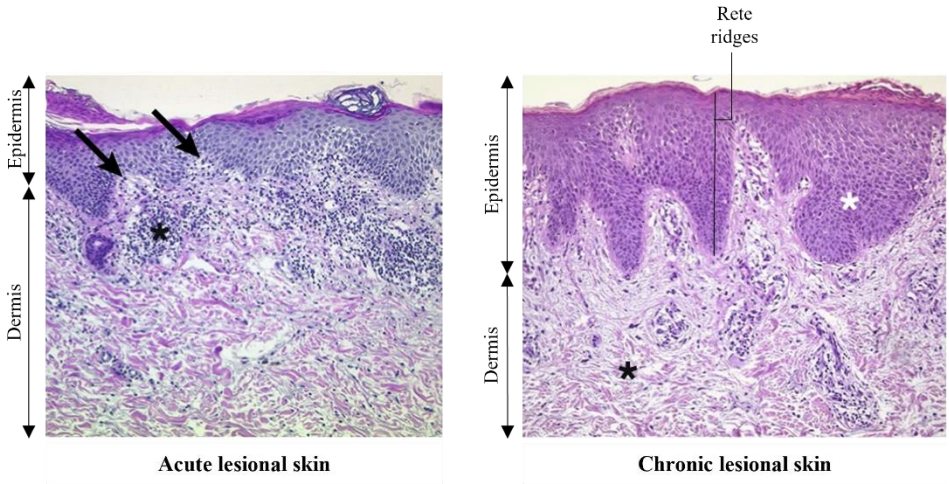

Figure 1. Histopathological features of lesional atopic dermatitis skin. Compared to acute cutaneous lesions, chronic epidermal layer shows marked acanthosis (white asterisk) with elongated rete ridges, prominent hyperkeratosis and parakeratosis, and minimal spongiosis (arrows). Within the dermal layer, lymphocytes and eosinophils infiltrate acute lesions (left panel, black asterisk), and chronic lesions show a denser infiltrate with mononuclear cells and increased mast cells along with fibrosis (right panel, black asterisk). Adapted from Weidinger et al. 2018.

The understanding of the complex atopic dermatitis pathophysiology has progressed substantially, in part, due to the development of new targeted therapies in the last decade. There are many mechanistic drivers that can promote and interact each other, including genetic predisposition, epidermal barrier dysfunction, cutaneous dysbiosis, abnormal T-cell driven cutaneous

inflammation, and neuroimmune interactions, as it will be reviewed in the following sections.

1.1. Genetic susceptibility

Association studies, basically genome-wide and targeted high-throughput approaches, have identified up to 34 probable atopic dermatitis susceptibility loci, most of which include genes involved in adaptive and innate immunity, and epidermal differentiation (Weidinger et al. 2018). They account for less than 20% of heritability, and molecular mechanisms have not been identified in most of the loci. Loss-of-function mutations in the *FLG* gene, encoding for the epidermal barrier protein filaggrin, are the most significantly associated genetic variant for atopic dermatitis, and it confers a 3-5-fold increased risk to develop disease (Irvine et al. 2011). This mutation leads to a reduction in filaggrin expression with generalized skin dryness as downstream effect of disturbed skin barrier formation and increased transepidermal water loss (TEWL). Nonetheless, less than 50% of individuals carrying *FLG* mutations develop atopic dermatitis disease. Beyond *FLG* mutations, other genetic and epigenetic alterations are pointed out to contribute to disease (Marin et al. 2020).

In addition to the possible contribution of genetic and epigenetic factors to atopic dermatitis susceptibility, numerous studies have revealed that the association between altered epidermal barrier, cutaneous microbiome, and immune and neuronal systems, are required to initiate and/or exacerbate atopic dermatitis lesions.

1.2. Epidermal barrier dysfunction

As the body's largest organ conferring initial barrier against external agents, skin integrity is essential to maintain its physical, chemical, and immunological protective functions. A compromised epidermal barrier is observed in both non-lesional and lesional atopic dermatitis skin in the so-called "core" signature

(Möbus et al. 2021), characterized by increased pH, TEWL and permeability, decreased water retention and altered lipid composition.

The epidermal barrier is fundamentally composed of KCs, whose migration from the inner basal layer to the outer stratum corneum is directly proportional to their differentiation state. In the stratum corneum, terminal differentiated KCs (corneocytes) are embedded in a lipid matrix, following the "brick and mortar" model. Keratin filaments crosslink to other structural proteins (FLG, loricrin (LOR) and involucrin (INV)) and lipids to form the cornified envelope, conferring structural stability and flexibility to KCs. The expression of keratins is dysregulated in atopic dermatitis; for instance, keratin 6 and keratin 16, with a role in repairing injured epidermis, are overexpressed and associated with aberrant KCs proliferation. The expression of FLG, LOR and INV is also downregulated in atopic dermatitis KCs. Among them, FLG is the major structural protein of the stratum corneum and its degradation by caspase-14 produces natural moisturizing factors (NMFs) that promote epidermal maturation and desquamation, skin hydration and maintenance of the acidic pH of the skin. Downregulated FLG expression is found in lesional and non-lesional skin, with subsequent alterations in barrier permeability, microbial defence, hydration, and pH. The lipidic matrix surrounding corneocytes is enriched in hydrophobic free fatty acids, cholesterol and ceramides forming packed layers to stimulate the expression of antimicrobial peptides (AMPs) and maintain the pH to inhibit the growth of pathogenic microorganisms, and alterations in the lipid packaging results in shortened fatty acid chains and increased barrier permeability, TEWL and reduced skin hydration. Likewise, proteases and metalloproteinases also interfere with the epidermal composition by increasing the degradation of lipid-processing enzymes, reducing tight junction (TJ) protein expression, and inducing inflammatory cell aggregation, among others. Under the stratum corneum, TJs maintain KC adhesion in the stratum granulosum creating a second physical barrier to prevent TEWL and limit the penetration of allergens, microbes, and irritants. Some TJs

from the claudin family members such as claudins 1, 3, 4, 8, 23 are downregulated in the atopic dermatitis skin (Figure 2) (Beck et al. 2022; Czarnowicki et al. 2017b).

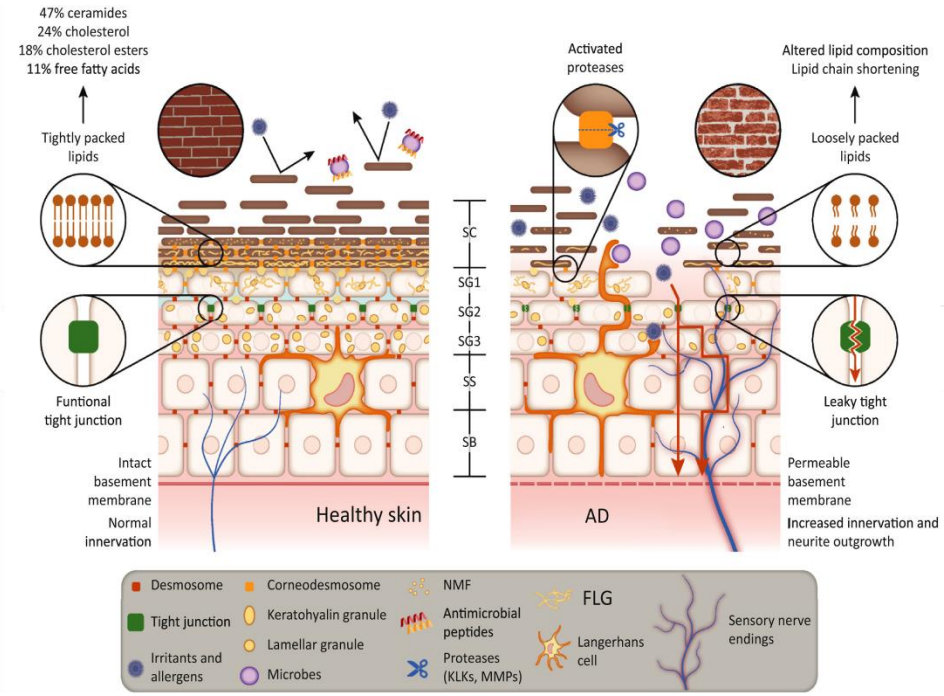

Figure 2. The key components of skin showing the differences between normal healthy skin and atopic dermatitis skin. In the "brick and mortar" model, the bricks represent corneocytes, and the mortar represents the extracellular lipids and other extracellular matrix components. Alteration of components including microbiome, corneocytes, antimicrobial peptides, structural proteins and lipids, NMF and tight junctions are found in atopic dermatitis skin. AD, atopic dermatitis; FLG, filaggrin; KLK, kallikrein; MMP, matrix metalloproteinase; NMF, natural moisturizing factor; SB, stratum basale; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum. Adapted from Beck et al. 2022.

In short, skin barrier damage in atopic dermatitis is promoted by an abnormal differentiation of KCs, alterations in the synthesis and maintenance of structural proteins and lipids, and TJ loss of integrity. The T helper (Th) type 2 inflammatory response broadly affects the skin barrier function, and a disrupted barrier function simultaneously induces a Th2 immune response. Thus, a disturbed epidermal barrier function, with an increased pH and reduced antimicrobial capability, makes easer the penetration of allergens, irritants and toxins from the environment and

the skin colonization by *Staphylococcus aureus* (*S. aureus*), which ultimately activate the cutaneous immune system.

1.3. Cutaneous dysbiosis

The cutaneous microbiome is the collection of microorganisms, such as bacteria, fungi and viruses, and the surrounding environment that naturally occupy the skin, and relates symbiotically with the cutaneous barrier to maintain skin homeostasis. Bacteria are the most abundant microbial microorganisms within the cutaneous microbiome, whose composition is site-specific and suffers temporal changes with age (Lee and Kim 2022). The skin of atopic dermatitis patients is characterized by an imbalance of the microbial species that constitute the microbiome (dysbiosis), that encompasses reduced microbial diversity and increased abundance of the genus *Staphylococcus* and specially *S. aureus*. In fact, up to 90% atopic dermatitis patients show *S. aureus* colonization in lesional and non-lesional skin, whilst no more than 10% of healthy individuals report *S. aureus* colonization (Ong and Leung 2010; Tauber et al. 2016). *S. aureus* colonization results from a combination of factors that are not mutually excluded including elevated pH, abnormal lipid profiles, deformed corneocytes, deficiency of AMPs and NMFs, overactivation of T helper immune response and microbial dysbiosis (Kim et al. 2019). Microbial dysbiosis includes increased colonization by commensal *S. epidermidis* and *S. hominis*, but at lesser extent than *S. aureus*, which under physiological conditions produce AMPs, as well as induce KCs to produce AMPs, to limit the growth of *S. aureus*.

Reduced microbial diversity as well as abundance of *S. aureus* are associated with disease flares (Kong et al. 2012). *S. aureus* colonization is also linked with disease exacerbation and influences the disease phenotype and endotype (Simpson et al. 2018). In this sense, patients colonized with *S. aureus* have more severe disease, increased eosinophil counts, serum total Immunoglobulin (Ig) E, C-C chemokine ligand 17 (CCL17), periostin and lactate dehydrogenase, greater allergen sensitization and skin barrier dysfunction (increased TEWL), compared to non-

colonized patients. *S. aureus* secretes a broad range of virulence factors that affect KCs and immune cells, further contributing to microbial dysbiosis, skin barrier disruption and dysregulated T-cell-mediated inflammation (Figure 3). Among them, *S. aureus* superantigens induce B-cell differentiation with production of IgE antibodies, and they also induce T-cell activation by binding to major histocompatibility complex class II (MHC-II) molecules on the surface of antigen presenting cells (APCs) and the T-cell receptor (TCR) on T cells, allowing both cell types to interact without antigenic presentation (Kim et al. 2019). The superantigens' effect on KCs leads to epidermal barrier disruption and induced expression of epithelial chemokines to promote leucocyte infiltration. *S. aureus* enterotoxin B (SEB) is the most prevalent *S. aureus* superantigen in atopic dermatitis lesions (Totté et al. 2016) and sensitization to SEB is associated with disease severity (Breuer et al. 2000). Interestingly, application of SEB on intact atopic skin leads to induction of dermatitis through accumulation and activation of T cells expressing SEB-sensitive TCR Vβ chains (Skov et al. 2000).

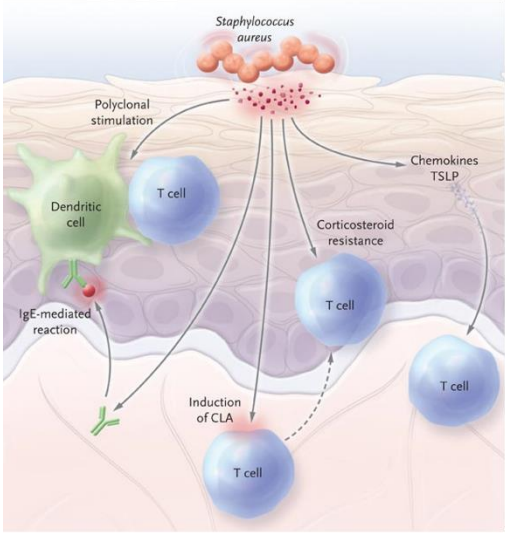

Figure 3. Multiple pathways of *S. aureus*–driven sensitization and inflammation. By virtue of several mechanisms, *S. aureus* and its products provide signals that favor sensitization and inflammation. *S. aureus*–derived ceramidase increases the permeability of the stratum corneum, and the superantigenic capacity of *S. aureus* enterotoxins activates T cells in an allergen-independent manner. *S. aureus* induces the expression of the skin-homing receptor cutaneous lymphocyte-associated antigen (CLA) on T cells. Keratinocyte-derived chemokines, thymic stromal lymphopoietin (TSLP), and interleukin-31 secretion are induced and augmented by *S. aureus* enterotoxins. They also contribute to corticosteroid resistance in T cells and alter the activity of regulatory T cells. *S. aureus*–specific IgE generated by the immune system can bind to FcεRI receptors on dendritic cells and initiate an IgE-mediated reaction to this microbe. From Bieber 2008.

Although infections by various microorganisms could trigger or exacerbate disease, including fungi (*e.g.*, *Malassezia spp.*) (Langan et al. 2020), extended knowledge agrees with the crosstalk between a dysfunctional epidermal barrier,

infections mediated by *S. aureus* and a predominant immune T-cell dysregulation for initiation and worsen of atopic dermatitis lesions. Overall, *S. aureus* colonization is part of a vicious cycle of an altered epidermal barrier that facilitates the attachment of *S. aureus*, followed by the production of virulence factors that activate the cutaneous immune system, leading in turn to sustained epidermal barrier dysfunction and *S. aureus* colonization.

1.4. Abnormal immune response

Cutaneous inflammation has a pivotal role in the pathogenesis of atopic dermatitis, with T cells being the most skin-infiltrating lymphocytes (Czarnowicki et al. 2017a). Lesions are mainly presented with dysregulated expression of Th2-associated genes but altered activation of other helper T-cell pathways like Th22, Th17 and Th1 has also been identified (Gittler et al. 2012). The complex immune network underlying cutaneous inflammation also involves innate (basophils, dendritic cells, eosinophils, innate lymphoid cells (ILC), mast cells) and other adaptive (B cells) immune cells.

1.4.1. Th2-driven cutaneous inflammation

Atopic dermatitis has traditionally been considered a type 2 immunity-driven disease, with implications of the innate and antigen-specific adaptive immune systems. Type 2 inflammation is characterized by overexpression of cytokines interleukin (IL)-4, IL-5, IL-13, and IL-31, with overlapping roles in some cases. In addition to the classic Th2 lymphocytes, innate immune cells (such as ILC2, mast cells, basophils, or eosinophils) can produce these cytokines. In fact, non-lesional skin already presents a pro-Th2 non-specific inflammation triggered by alarmins, such as IL-33 and thymic stromal lymphopoietin (TSLP), that activate ILC2 residing in the skin. The local response of ILC2 with production of type 2 cytokines subsequently promotes the adaptive immune response by activation of Th2 cells and eosinophils (Figure 4) (Langan et al. 2020). Even though there is an increasing interest in understanding the biologic role ILC2 in atopic dermatitis,

the relevance of these cells in the disease flare is elusive since blockade of alarmins with targeted therapies does not affect the disease course. Furthermore, $CD4^+$ T cells are the predominant lymphocyte population within atopic dermatitis skin and the main drivers of the pathogenesis, as demonstrated by clinical trials and mechanistic studies (Trier and Kim 2023).

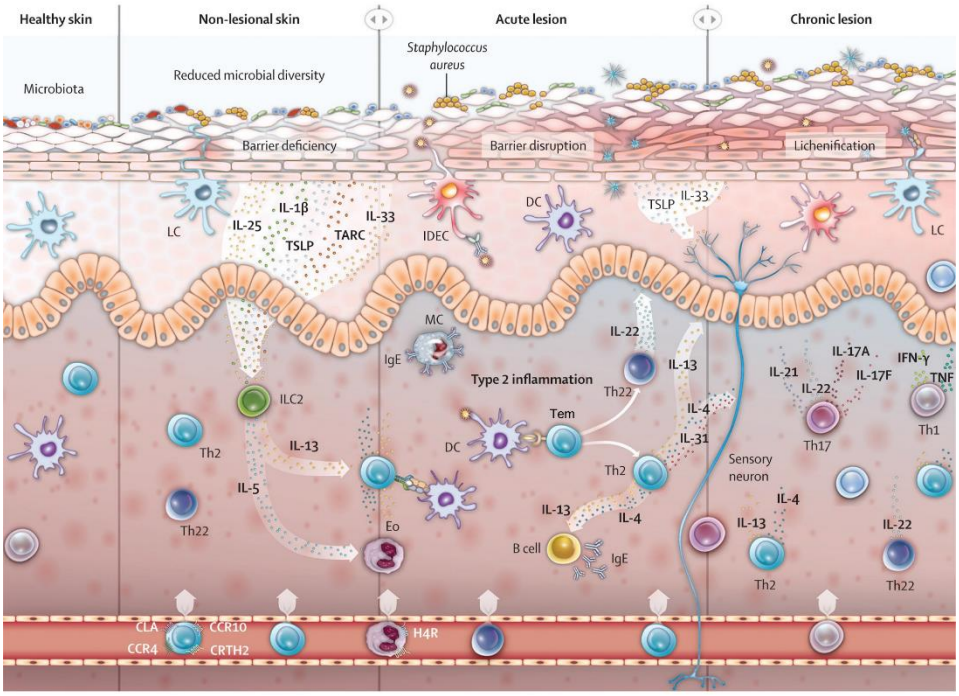

Figure 4. Stage-based pathogenesis and main mechanisms of atopic dermatitis. Non-lesional skin has an epidermal barrier dysfunction with a reduced diversity of the surface microbiome. In lesional skin, Langerhans cells, inflammatory epidermal dendritic cells bearing specific IgE bound to the high affinity receptor for IgE, and dermal dendritic cells take up allergens and antigens. In acute lesions, Th2 and Th22 cytokines (IL-13, IL-4, IL-31 and IL-22) are secreted. The type-2 cytokines IL-4, IL-13, and IL-31 directly activate sensory nerves, which promotes pruritus. With increasing chronicity, there is a progressive increase of keratinocyte-derived and Th2/Th22-cell-derived cytokines, and recruitment of Th17 and Th1 cells. CCR, CC chemokine receptor; CLA, cutaneous lymphocyte-associated antigen; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cell; DC, dendritic cell; Eo, eosinophil; H4R, histamin 4 receptor; IDEC, inflammatory dendritic epidermal cell; ILC, innate lymphoid cell; LC, Langerhans cell; MC, mast cell; Tem, effector memory T cell; Th, T helper cell. Adapted from Langan et al. 2020.

Atopic dermatitis skin shows increased presence of IL-13, whilst for IL-4 limited protein levels and near undetectable expression are found, which is reflected in dominance of IL-13 pathways in the lesion (Koppes et al. 2016; Tsoi et al. 2019). They are essential cytokines that act on both immune and structural cells. They

are involved in the regulation of class switching and IgE production by B cells; in inflammation, by inducing KCs to secrete chemokines CCL17 and CCL22 that, together with other molecular interactions, orchestrate the recruitment of C-C chemokine receptor type 4 $(CCR4)^{+}$ T lymphocytes with skin tropism from the peripheral blood to the skin; in the epidermal barrier disruption and dysbiosis, by inducing downregulation of epidermal structural proteins and AMPs; and pruritus, by direct binding to their receptors on skin sensory neurons, among others (Beck et al. 2022). Both cytokines bind to a common receptor subunit, the IL-4Rα, which is part of the so-called type I and type II receptors. IL-4 binds to both type I receptor, which is complemented with the common gamma chain (γc) subunit, and type II receptor, whose other subunit is IL-13Rα1. In contrast, IL-13 binds to type II receptor, and to the decoy receptor IL-13Rα2 (Figure 5)(Bieber 2020). Hematopoietic cells, such as lymphocytes, express large amounts of γc subunit and low amounts of IL-13Rα1, while non-hematopoietic structural cells, such as KCs and fibroblasts, preferentially express IL-13Rα1 and IL-13Rα2. The relative abundancy of both cytokines, as well as the preferential location of their receptors, have provided a deeper insight of their contribution in the disease pathogenesis. In this regard, IL-4 is more relevant for displaying a Th2 central activity (*e.g.,* promotion of differentiation of T cells into a Th2 phenotype and generation of humoral immunity), and IL-13 has a prominent role in mediating inflammation, epidermal barrier dysfunction and dysbiosis in the periphery, that is in the skin, where it is more abundant that IL-4. These features changed the historically paradigm that atopic dermatitis was primarily driven by IL-4 to the concept that IL-13 is the key cytokine with a wide impact on disease pathogenesis (Bieber 2020).

Other less studied Th2 cytokines are IL-31 and IL-5. IL-31 was first described in 2004 by Dillon et al. demonstrating its involvement in the development of pruritus and dermatitis-like skin lesions in mice (Dillon et al. 2004). IL-31 signals through the binding to a heterodimeric receptor composed of the IL-31RA chain and the

oncostatin M receptor (OSMR) β chain. Since then, several studies have revealed a central role of IL-31 in inflammation, epidermal barrier dysfunction, tissue remodelling, and pruritus (Nemmer et al. 2021). For its part, cytokine IL-5 is recognized as key mediator for eosinophil maturation, differentiation, survival, and recruitment in most organs, including the skin, where they act as important effector cells in allergic reactions (Möbus et al. 2022).

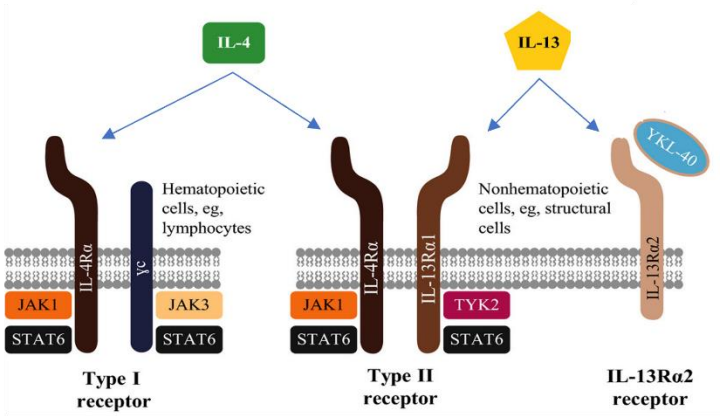

Figure 5. IL-4 and IL-13 receptors structure. Upon binding of its ligand, IL-4Rα associates with the common γ chain (type I receptor) to exert signaling through JAK1 and JAK3. IL-13 also binds to IL-4Rα but uses IL-13Rα1 (type II receptor) for signaling via JAK1 and JAK2. IL-13 also binds to IL-13Rα2, which associates with YKL40. The signaling transducing machinery linked to this complex has not been completely elucidated. Adapted from Bieber 2020.

In the classical model of atopic dermatitis pathogenesis, Th2 cytokines were described to participate in the acute stages and Th1 cytokines had a role in chronic stages. Nowadays, this model has evolved to a more complex scenario including a Th2 and Th22 inflammatory profile in acute stages that extends to chronic stages with the addition of Th17 and Th1 immune responses (Figure 4).

1.4.2. Additional T helper pathways: Th22, Th17 and Th1

Th22 cells are the main producers of IL-22, which is upregulated in atopic dermatitis skin and drives epidermal hyperplasia and barrier defects. The expression of its receptor, IL-22R, is limited to epithelial cells, where, in normal conditions, it induces defence mechanisms against pathogens to preserve barrier

integrity. Varying degrees Th17 cells, characterized by production of IL-17A and low levels of IL-22, and Th1 cells, characterized by interferon (IFN)-γ production, are confined to chronic stages of disease. Recent studies using RNA sequencing of biopsy specimens from moderate-to-severe patients have defined a "dynamic" signature of atopic dermatitis, based on the progressive upregulation of Th2, Th22, Th17 and Th1 immune responses from non-lesional skin towards lesional skin (Möbus et al. 2021). Th17 and Th1 profiles are also associated with ethnic groups; for instance, Asian patients show a prominent activation of Th17 pathway, whilst African American patients are characterized for an absent Th1 and Th17 inflammatory profile (Czarnowicki et al. 2019).

1.4.3. Inflammatory endotypes

Endotype is defined as the molecular mechanisms underlying the disease's visible features/phenotype (Czarnowicki et al. 2019). Considering the immune complexity of atopic dermatitis, identification of inflammatory endotypes might be a big step towards the personalized medicine instead of the "one-size-fits-all" therapeutic approach. For this, principal component analyses on serum proteomics have attempted to stratify atopic dermatitis patients according to their cytokine profile. Thijs et al. described four clusters of adult moderate-to-severe patients by analysing 147 serum mediators, and each cluster was characterized by a specific biomarker profile and clinical characteristics. Based on the patterns of mediator expression, 48% of patients belonged to two of the clusters and showed high Th2 cytokine levels, and 52% of patients belonged to the other two clusters and shared low Th2 cytokine levels (Thijs et al. 2017). These findings drove to the definition of "Th2 high" and "Th2 low" endotypes, that were further confirmed in an independent cohort of adult severe patients by using a panel of 143 serum mediators (Bakker et al. 2021). In this study, cluster analysis identified three of the clusters to be comparable to the previous identified clusters ("Th1/Th2/Th17 dominant", "Th2/Th22/PARC dominant", and "Th2/eosinophil inferior"). Interestingly, one of the clusters was distinguished from the other clusters as being

"skin-homing chemokines/IL-1R1 dominant" cluster with the highest levels of CCL17, CCL22 and CCL27, which are related with the skin-specific homing of $CD4^+$ T cells.

The stratification of patients into inflammatory endotypes based on the serum cytokine profile has confirmed the substantial immune heterogeneity of the disease, with contribution of Th22, Th17 and Th1 immune axis in addition to the Th2 inflammatory pathways.

1.5. Pruritus

Pruritus (or itch) is a cardinal symptom of atopic dermatitis and has a significant negative impact on patients' quality of life. In chronic skin diseases like atopic dermatitis, chronic itch sensations can lead to persistent scratching responses that contribute to epidermal barrier disruption and activation of the immune system, that ultimately stimulate sensory nervous system to promote more itch sensation. A vicious itch-scratch cycle is stablished accordingly, as the result of a crosstalk between epithelial barrier, immune system, and peripheral nervous system (Mack and Kim 2018). Epithelial and immune cells act as sentinels in the skin and communicate with the sensory nervous system through the release of itch-causing factors, known as pruritogens. Exogenous environmental trigger factors (*e.g.*, allergens, irritants) can also activate the sensory nervous system in the skin. Then, two types of itch signalling pathways can be activated, the histaminergic and non-histaminergic, which transduce the pruritic signal via the spinal cord and contralateral spinothalamic tract to the central nervous system (Figure 6) (Steinhoff et al. 2022).

The most studied pruritogen is histamine, which is predominantly released by mast cells and basophils, and induces the histaminergic itch signalling pathway by stimulating histamine H1 and H4 receptors on sensory neurons. Nevertheless, the poor clinical efficacy of antihistamines for pruritus relief in atopic dermatitis patients (Bieber 2022; Werfel et al. 2019) has drawn attention towards

understanding pathways that mediate non-histaminergic itch. The latter is driven to a great extent by the adaptive Th2 immune response, by stimulating specific sensitive neurons through a Janus kinase (JAK)-dependent mechanism (Oetjen et al. 2017). IL-31 was the first cytokine recognized to mediate itch (Dillon et al. 2004) through the direct binding to its receptor IL-31RA, which is abundantly expressed on dorsal root ganglia (DRG) neurons, that is the site were cell bodies of cutaneous sensory nerves are located. IL-31 also binds OSMRβ, that is widely expressed in several sites, including DRG neurons (Sonkoly et al. 2006). IL-31 involvement in itch goes beyond the pruritic signal, since it induces nerve fiber elongation and branching with the consequent increased density of neuronal networks in the skin (Feld et al. 2016). The Th2 cytokines IL-4 and IL-13 also induce neuronal activation by direct binding to their receptors IL-4Rα and IL-13Rα1 expressed on sensory neurons innervating the skin. Interestingly, IL-31, IL-4 and IL-13 are upregulated in pruritic atopic dermatitis skin compared to psoriatic skin, that is another inflammatory skin disease (He et al. 2021; Sonkoly et al. 2006).

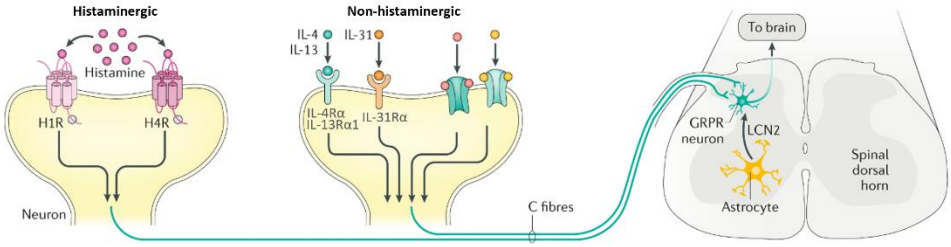

Figure 6. Histaminergic and non-histaminergic itch signalling pathways in atopic dermatitis. Sensory neurons (C-fibres) expressing histamine H1 receptor (H1R) and H4R respond to histamine release. In the non-histaminergic pathway, Th2 immunity cytokines IL-4, IL-13 and IL-31 activate sensory neurons expressing their own specific receptors. C fibres relay the signal to the dorsal horn of spinal cord, where reactive astrocytes produce lipocalin 2 (LCN2). LCN2 sensitizes a pruritic processing neuronal network involving neurons expressing gastrin-releasing peptide receptor (GRPR). These events induce a long-term reactive state of astrocytes in the dorsal horn of the spinal segments, which transduce the stimulus to the brain via the contralateral spinothalamic tract, resulting in chronic pruritus. Adapted from Weidinger et al. 2018.

The stimulation of cutaneous sensory neurons and neuronal dysfunction induce the release of neuropeptides to the skin, which act directly on immune cells thereby inducing neurogenic inflammation with production of Th2 cytokines that

contribute to epidermal barrier dysfunction and enhanced neuronal function. Altogether a neuroimmune communication by means of Th2 cytokines and skin sensory neurons regulates pruritus in atopic dermatitis, and these neuroimmune circuits are significantly involved in the cycle of Th2 immune dysregulation, skin barrier dysfunction and pruritus (Steinhoff et al. 2022).

1.6. Interplay between Th2 inflammation and epidermal barrier dysfunction, cutaneous dysbiosis and pruritus

Atopic dermatitis presents a predominantly Th2-skewed immune dysregulation that interacts with the epidermal barrier dysfunction, cutaneous dysbiosis and pruritus to drive disease pathogenesis. Controlling skin hydration, antimicrobial activity and limiting the penetration of allergens, irritants and toxins are among the principal roles of the skin barrier, but an altered epidermal barrier function is affected by Th2 inflammatory molecules like IL-13, IL-4 and IL-31 that modulate the expression of structural protein (*e.g.,* FLG, LOR, INV, keratins) and lipid components (reduced expression of elongases ELOVL1, ELOVL3 and ELOVL6 that compromises the lipid packaging). Other mechanisms that contribute to epidermal barrier dysfunction are mutations in genes encoding epidermal structural components, cutaneous dysbiosis and physical disruptions from scratching (Langan et al. 2020). Th2 cytokines IL-4 and IL-13 also promote *S. aureus* colonization by suppressing the production of relevant AMPs by KCs, such as human beta defensin-2 and -3, and cathelicidin (Beck et al. 2022), and inducing the production of fibronectin to facilitate its adherence to the stratum corneum (Cho et al. 2001a; Cho et al. 2001b). In addition, the non-histaminergic itch form is a behavioral extension of Th2 inflammation, that is driven by IL-4, IL-13 and IL-31 binding to their receptors on the skin sensory neurons. In turn, skin barrier weakness, dysbiosis with abundance of *S. aureus*, and pruritus, induce Th2 immune responses, therefore stablishing a dynamic interaction between Th2 immunity and epidermal barrier dysfunction, altered cutaneous microbiome and itch perception.

2. Translational research in atopic dermatitis

Atopic dermatitis is only observed in humans and atopic dermatitis-like animal models cannot mimic the full spectrum of clinical, epidermal barrier-related, histological, neurophysiological, and immunological features that represent the disease. In this regard, novel approaches in experimental studies using human samples that better represent patients' uniqueness are more convenient for translational purposes (Eyerich et al. 2019; Florian et al. 2020).

The T-cell-mediated nature of the disease is reflected by an altered pattern of cytokines and cells, both in the peripheral blood and in the lesions, that may be treated as potential therapeutic targets. Translational research, with the aim of translating basic scientific knowledge into applicable results that directly benefit human health, is based on the "therapeutic generation cycle" (Figure 7). The starting point of this model are bench (laboratory research) studies of the disease pathogenesis by skin and blood profiling to identify cells and molecules related with the pathogenic mechanisms of the disease, leading to the formulation pathogenic hypothesis. Then, candidate cells and molecules are addressed in clinical trials with targeted therapies and based on the reported clinical efficacy, the pathogenic hypothesis and therapeutic target will be validated or refused. Multiple cycles of this process allow the generation of rational therapies directed against clinically relevant mechanisms of the disease and, as a result, better defining the key drivers of disease pathogenesis.

Owing to the complex pathogenesis of atopic dermatitis with contributions of genetic, environmental, skin microbiome, immunologic, neurophysiologic, and epidermal barrier factors, many topical and systemic treatments have been tested for atopic dermatitis management. Moderate-to-severe patients usually require of systemic therapy including conventional systemic agents (corticosteroids, cyclosporine A, etc.) that broadly target inflammation, and the newest biological

treatments targeting specific cellular products or molecular pathways as a result from translational research.

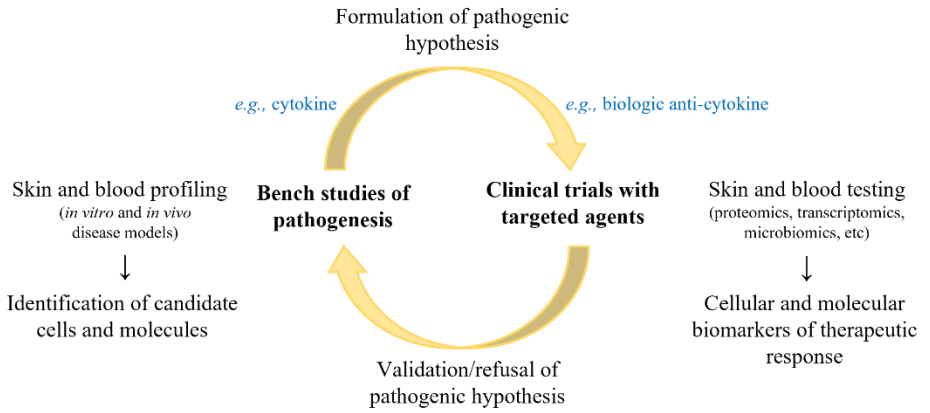

Figure 7. Scheme of the therapeutic generation cycle in translational research. Consecutive cycles allow identifying the best therapeutic agents for the treatment of disease and a better understanding of the key players of disease pathogenesis. Adapted from a conference presentation by Dr. E Guttman-Yassky in 2022.

The use of humanized monoclonal antibodies (mAbs), that selectively target a specific molecule, has provided a great step to the precision medicine. For atopic dermatitis disease, it was not until 2017 that dupilumab targeting IL-4Rα, which is the shared receptor of IL-4 and IL-13, was the first biologic being approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as a first-line treatment for moderate-to-severe disease. The significant improvement in signs and symptoms of the disease, including pruritus, promoted its use in patients from all ages. Dupilumab significantly improves the molecular signature of atopic dermatitis at epidermal barrier (reduced expression of genes related with epidermal hyperplasia, upregulation of genes with structural, lipid metabolism and barrier-related functions), microbiome (increased microbial diversity and lowered abundance of *S. aureus*), and inflammatory (blockade of IL-4Rα on both B and Th2 cells, decreased percentage of proliferating Th2 and Th22 cells, inhibition of eosinophil- and Th2-associated chemokines) levels (Bakker et al. 2021; Callewaert et al. 2020; Hamilton et al. 2014; Simpson et al. 2023). Given the dominant effect of IL-13 over IL-4, mAbs targeting IL-13 have been

developed with clinical efficacy, such as tralokinumab, approved by both FDA and EMA in 2021, that prevents binding of IL-13 to its receptors IL-13Rα1 and IL-13Rα2 leading to skin clearance, increased microbial diversity, reduced *S. aureus* abundance and pruritus, and improved quality of life (Beck et al. 2023; Wollenberg et al. 2021). Besides it, lebrikizumab, that impairs the formation the receptor complex between IL-13Rα1 and IL-4Rα thereby inhibiting IL-13 signal transduction through IL-13Rα1 (but not IL-13Rα2), is currently under phase III studies (Bieber 2020). Blocking the pruritic IL-31 signalling pathway is currently being tested with nemolizumab, that targets the subunit IL-31RA, and has demonstrated notable improvement in chronic itch in phases II and III clinical trials, as well as improvement of pro-inflammatory markers in the skin (Sidbury et al. 2022). Other type 2 immunity mediators have been tested, such as therapies targeting IL-5 (mepolizumab) and IgE (omalizumab), but studies were discontinued due to lack of efficacy (Trier and Kim 2023). Given the heterogeneous immune response, other molecules besides Th2 immunity have been tested. The mAb fezakinumab targeting IL-22 is in phase II clinical trial and has reported clinical efficacy as well as improvement in epidermal markers for inflammation and epidermal proliferation in patients with elevated basal IL-22 expression in cutaneous lesions (Brunner et al. 2019). Therapeutic strategies against antigen presentation by targeting the T-cell costimulatory molecule OX40 and its ligand (OX40L) on APCs, which promote T-cell clonal expansion and survival of Th2 cells, are under phase II and III clinical trials and are also providing good clinical results together with reductions in hyperplasia measures and Th1/Th2/Th17/Th22-related genes in lesional skin (Guttman-Yassky et al. 2023; Guttman-Yassky et al. 2019; Weidinger et al. 2023). By contrast, systemic immunomodulatory therapies targeting the innate immune response (IL-1α, IL-17C, IL-33 and TSLP) and the IL-17 signalling pathway did not demonstrate significant clinical improvements (David et al. 2023). Altogether, the evolution of atopic dermatitis pathogenic concepts, mainly through the translational research and based on selective therapeutic strategies, has clinically validated Th2

cytokines IL-13, IL-4, IL-31, and Th22 cytokine IL-22 to be central to the pathogenesis of atopic dermatitis, whereas the innate immune response may no longer be of significant relevance in the disease pathogenesis (Table I).

Table I. Systemic immunomodulatory therapies for moderate-to-severe atopic dermatitis. Adapted from Trier et al. 2023.

	Target	Mechanism	Clinical evidence	Status
Monoclonal antibody	IL-4Rα	Th2 cytokines IL-4 and IL-13	✓	Approved
	IL-13	Th2 cytokine	✓	Approved
	IL-31RA	Th2 cytokine IL-31	✓	Phase III
	IL-22	Th22 cytokine	✓	Phase II
	OX40	T-cell costimulatory molecule	✓	Phase II-III
	OX40L	T-cell costimulatory molecule	✓	Phase II-III
	IgE	Th2 related - basophils and mast cells	✗	Discontinued
	IL-5	Th2 cytokine - eosinophil biology	✗	Discontinued
	IL-17A	Th17 cytokine	✗	Discontinued
	IL-23p19	Th17 axis	✗	Discontinued
	IL-1α	Alarmin (innate immune system)	✗	Discontinued
	IL-17C	Alarmin (innate immune system)	✗	Discontinued
	IL-33	ILC2 alarmin (innate immune system)	✗	Discontinued
	TSLP	ILC2 alarmin (innate immune system)	✗	Discontinued
Small molecule	CCR4	Skin-homing $CCR4^{+}$ T-cell chemotaxis	✓	Phase II
	JAK1	JAKi	✓	Approved
	JAK1/2	JAKi	✓	Approved

In addition to mAbs, oral JAK inhibitors (JAKi) are the most recently developed treatment for atopic dermatitis, which are an evolution of directed therapies that take advantage of the knowledge generated with biological treatments. JAK together with signal transducer and activation of transcription (STAT) molecules, are involved in the signal transduction of several cytokine receptors, including those implicated in atopic dermatitis pathogenesis (Figure 8)(Chovatiya and Paller 2021). Therefore, the interest in JAKi small molecules derives from their ability to simultaneously block multiple cytokine pathways. Among the multiple JAKi that are currently being tested, the inhibition of JAK1 is of especial interest due to the broad atopic dermatitis relevant cytokines that signal through this molecule.

First-generation JAKi targeting JAK1 and JAK2 baricitinib was approved by the EMA in 2020, but the broad effect of JAKi could lead to increased risk of other diseases. For this reason, second-generation JAKi with increased selectivity and improved safety profile were developed, and second-generation JAK1-selective inhibitors upadacitinib and abrocitinib were approved in the past two years by the FDA and the EMA (Trier and Kim 2023). In addition to JAKi, specific targeting of CCR4, which is a hallmark of memory Th2 cells, with the small-molecule CCR4-antagonist RPT193 reported improvement of clinical signs and symptoms in phase II studies (Bissonnette et al. 2021) (Table I).

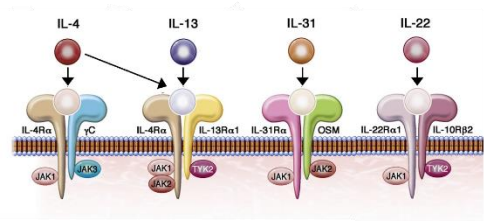

Figure 8. Relevant cytokines in atopic dermatitis bind to JAK-STAT–dependent receptors to propagate inflammation and itch. IL-4 binds to a heterodimer consisting of the IL-4Rα and common γ chain subunits and is dependent on JAK1/3. IL-13 binds to a heterodimer consisting of IL-4Rα and IL-13Rα1 (JAK1/2, TYK2), while IL-31 and IL-22 bind to IL-31Rα/OSM (JAK1/2) and IL-22Rα1/IL-10Rβ2 (JAK1, TYK2) heterodimers, respectively. Adapted from Chovatiya et al. 2021.

3. Human skin immune system

3.1. Skin-associated lymphoid tissue

The human skin is the largest and more exposed interface with the environment. Besides constituting a physical barrier, it has an active role in host defence as an immunologic organ where innate and adaptive immune systems interwine. In 1983, J. W. Streilein proposed the existence of the skin-associated lymphoid tissue (SALT) as a functional unit of cutaneous immune surveillance consisting of: 1) Langerhans cells, which are APCs within the epidermis with the capacity to accept, process and present antigens, 2) subpopulations of circulating T lymphocytes with affinity for the skin, 3) KCs, which are cutaneous resident cells that secrete immune-regulatory molecules and determine the T-cell affinity and immune recognition, and 4) skin-draining peripheral lymph nodes that accept the skin-derived immunogenic signals and connect this multicellular system (Streilein 1983).

3.2. CLA$^+$ memory T cells

CLA$^+$ T cells represent the subset of memory T lymphocytes that belong to the adaptive immune system of the skin. In fact, CLA is expressed on the surface of over 90% infiltrating T cells in the cutaneous sites but less than 20% of T cells in non-cutaneous sites. It is also expressed on around 15% of all human T cells in peripheral blood (Picker et al. 1990). The CLA molecule is a carbohydrate induced by the fucosyltransferase VII-dependent post-translational modification of the platelet (P)-selectin glycoprotein ligand 1 (PSGL-1) (Fuhlbrigge et al. 1997), and it is induced on T cells during the transition from naive (CD45RA$^+$) to memory (CD45R0$^+$) in the skin-draining lymph nodes (Picker et al. 1993).

3.3. Skin tropism and homing of memory T cells

Extravasation is the process by which lymphocytes in the blood cross the endothelium and localize to specific tissues. The guidance of lymphocytes to distinct peripheral tissues is not random but occurs through specific molecular interactions (adhesion molecules and chemokine receptors on T lymphocytes, and their respective ligands displayed or expressed on the surface of vascular endothelial cells in the target tissue) that mediate the cell trafficking.

After leaving the thymus, naive T cells are in continuous circulation through the secondary lymphoid organs such peripheral lymph nodes and the spleen. In tissue draining lymph nodes, naive T cells recognize antigens presented by APCs and undergo clonal expansion and differentiate into antigen-experienced T cells with a memory phenotype, which can be divided into central memory T cells (T_{CM}) that localize in lymphoid tissues, and effector memory T cells (T_{EM}) that patrol non-lymphoid peripheral sites. During priming, local microenvironment and cellular interactions with tissue-derived APCs promote T lymphocytes to express a specific set of tissue-homing receptors to orchestrate the interaction with organ-specific endothelial cells and migration to distinct target tissues where they will exert effector responses. The T lymphocytes with skin-homing capability express

the CLA molecule and those with gut-tropism express the α4β7 integrin on their surface. How migration of memory T cells to other tissues is orchestrated is still to be deciphered. Then, memory T cells enter the blood circulation through the lymphatic system and travel to the antigen-rich tissue where immune response needs to be activated (Figure 9) (Agace 2006; Robert and Kupper 1999).

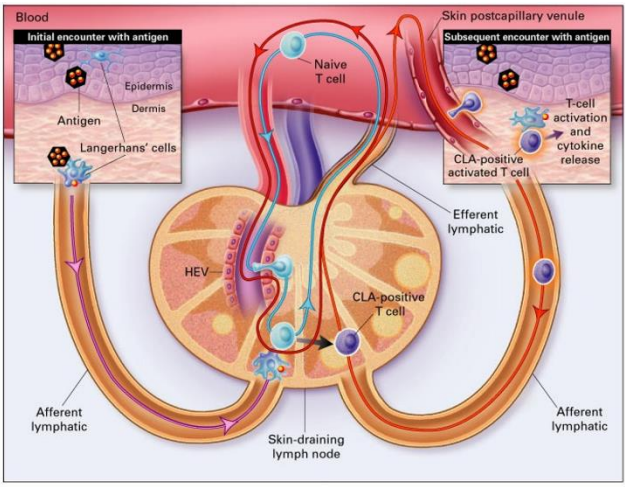

Figure 9. Movement of naive T cells and CLA-positive, skin-homing memory T cells. Naive T cells are continuously recirculating between blood and lymphoid organs. They can extravasate through the HEV into the lymph node, where they accumulate in areas that are already rich in T cells, and they can exit the node through efferent lymphatics and return to the blood. This pattern of movement is represented by the blue loop. Antigen-presenting cells that reside in the skin, such as Langerhans' cells and dermal dendritic cells, internalize foreign antigens in the skin and migrate to the lymph nodes through afferent lymphatics. When a naive T cell encounters the antigen for which it is specific on an antigen-presenting cell in a skin-draining lymph node, it is activated and becomes a memory T cell (black arrow), with the expression of CLA and chemokine receptors. Endowed with these skin-homing molecules, antigen-specific, CLA-positive T cells possess the molecular keys that allow them to migrate to skin, the site where the antigen was first encountered. Some of these CLA-positive memory T cells retain the capacity to exit HEV and enter the lymph node. The red loops represent the two possible patterns of movement of memory T cells. CLA, cutaneous lymphocyte-associated antigen; HEV, high endothelial venules. From Robert et al. 1999.

CLA functions as an adhesion molecule and it is the ligand for both endothelial-cell (E)-selectin and P-selectin expressed on the endothelium of post-capillary venules. Also, expression of the CLA epitope on the sialomucin CD43 T cells supports the selective E-selectin, but not P-selectin, binding (Fuhlbrigge et al. 2006). The multistep process that allows memory T cell migration to skin also involves interactions between lymphocyte-function-associated antigen 1 (LFA-1) and very later antigen 4 (VLA-4) integrins expressed on T cells with intercellular

adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1) respectively, located on the cutaneous endothelium (Santamaria Babí et al. 1995a). In addition to this, CCR4 and CCR10, which are preferentially expressed on CLA^+ $CD4^+$ T cells (Campbell et al. 1999; Hudak et al. 2002), bind to the KC-derived chemokines CCL17 or thymus- and activation-regulated chemokine (TARC) and CCL27 or cutaneous T cell-attracting chemokine (CTACK) respectively, during this process (Chong et al. 2004; Homey et al. 2002) (Figure 10). The selective migration of CLA^+ effector memory T cells is key for the maintenance of a local effective immune response in the skin.

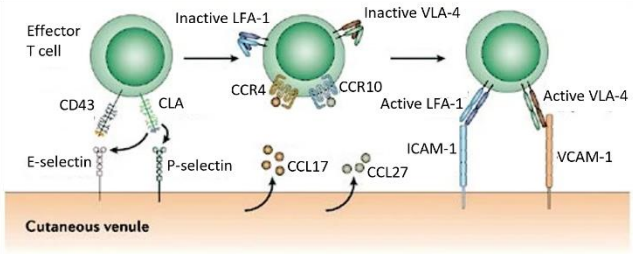

Figure 10. Multistep process for selective migration of CLA-positive memory T cells into the skin. First, the tethering of effector memory T cells on endothelium is mediated by CLA binding to P-selectin and/or E-selectin on the surface of the cutaneous post capillary venules. Then, T cells roll on the endothelial surface much more slowly and bind to chemokines (CCL17 and CCL27) through specific receptors on T cells (CCR4 and CCR10, respectively), and integrins LFA-1 and VLA-4 on T cells are activated so that they can bind to ICAM-1 and VCAM-1 expressed on the cutaneous postcapillary venules, respectively. Finally, CLA-positive memory T cells extravase trough the endothelial layer into the cutaneous tissue. Adapted from Agace 2006.

3.4. De-homing and recirculation of CLA^+ memory T cells

As transendothelial migration of CLA^+ memory T cells towards the cutaneous tissues needs of the interaction between all mentioned molecules on T cells and endothelial cells, the potential therapeutic of trafficking blockade through LFA-1/ICAM-1 interaction was explored. Efalizumab, a mAb to the α-chain of LFA-1, led to clinical improvement and decreased cutaneous T cell infiltration (Gottlieb et al. 2002; Hassan et al. 2007) in parallel to accumulation of CLA^+ memory T cells in blood in patients with atopic dermatitis and psoriasis (Harper et al. 2008; Vugmeyster et al. 2004). This lymphocytosis may be consequence of 1) the blockade of transendothelial migration of CLA^+ T cells towards the skin, and 2)

the accumulation of skin-infiltrating CLA$^+$ memory T cells that left the cutaneous tissue to the bloodstream trough the lymphatic drainage (de-homing) (Ferran et al. 2013). Supporting the de-homing, CLA$^+$ memory T cells were found in the afferent skin-derived lymph (Hunger et al. 1999; Yawalkar et al. 2000). When blockade was released, it led to disease exacerbation that may be consequence of a quick entrance of accumulated CLA$^+$ T cells from the blood to the skin (Johnson-Huang et al. 2012; Takiguchi et al. 2007). These observations provided translational evidence for CLA$^+$ memory T-cell recirculation between skin and blood through the thoracic duct during cutaneous inflammation (Figure 11) (Sans-de San Nicolàs et al. 2023) (included in APPENDIX II). Additionally, skin-tissue "resident" CLA$^+$ T cells share transcriptomic, phenotypic, and functional signatures with circulating CLA$^+$ T cells and are clonally related (Klicznik et al. 2019; Strobl et al. 2021). Thus, circulating CLA$^+$ T cells are a useful as a tool to study the disease-associated T-cell changes occurring in cutaneous lesions.

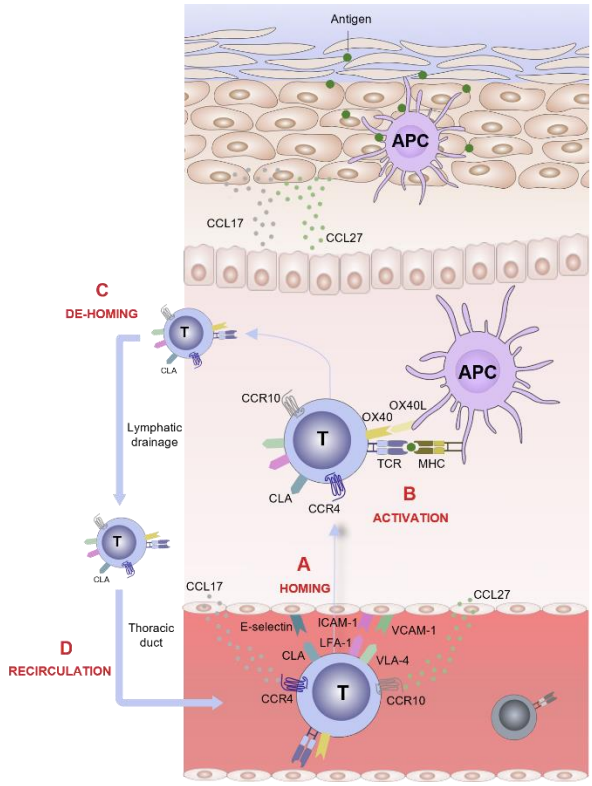

Figure 11. Homing, de-homing and recirculation capability of CLA$^+$ memory T cells. Skin-homing CLA$^+$ T cells migrate into the skin (A) through a multistep process based on molecular interactions of CLA, LFA-1 and VLA-4 expressed on T cells, with E-selectin, ICAM-1 and VCAM-1 respectively, present on cutaneous endothelial cells. Additional interactions between CCR4 and CCR10 expressed on CLA$^+$ T cells, and their ligands CCL17 and CCL27 respectively, produced by keratinocytes, are required during this process. Local activation of CLA$^+$ T cells (B) implies their involvement in the cutaneous immune response and, by virtue of their de-homing (C) and skin-blood recirculation capacities (D), they reflect in the periphery the cutaneous abnormalities present in the lesions. APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; MHC, major histocompatibility complex; TCR, T-cell receptor. Adapted from Sans-de San Nicolàs et al. 2023.

3.5. CLA^+ T lymphocytes represent the cutaneous adaptive immune system

Due to the selective migration and recirculation capacity of CLA^+ T cells, as well as the preferential skin-related antigen-specific responses with a broad production of disease-derived mediators, they constitute a subset of memory T cells closer to SALT characteristics (Table II)(Streilein 1983) and, accordingly, they may be contemplated representative of the adaptive immune system of the skin. For this reason, circulating CLA^+ T cells have been proposed as cellular peripheral biomarkers of the inflammatory responses occurring in the skin (Ferran et al. 2013). A biomarker is "a defined characteristic that is measured as an indicator of normal biologic processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions" according to the FDA.

Table II. Similarities of skin-associated lymphoid tissues (SALT) and CLA^+ T cells properties. From Streilein 1983.

SALT properties	CLA^+ T cells properties
Only a subset of T cells displays skin affinity.	Selective skin homing.
Skin-related lymphocytes produce immunoregulatory molecules.	Memory phenotype with broad capacity for cytokine production.
Immune recognition of antigens in the skin.	Preferentially respond to antigens related to skin.

Several features of CLA^+, but not systemic CLA^-, T cells support them as a source of translational information in a diversity of human T-cell-mediated skin diseases with different pathological mechanisms. First, CLA^+ T cells specifically respond to relevant antigens in skin diseases, as exemplified in Table III (Sans-de San Nicolàs et al. 2023) (included in APPENDIX II). Additionally, changes in phenotype and numbers of CLA^+ T cells during clinical manifestation and in response to treatments in distinct T-cell-mediated skin diseases have been reported, as shown in Table IV (Ferran et al. 2013; De Jesús-Gil et al. 2021; Sans-de San Nicolàs et al. 2023) (included in APPENDIX II). Last, CLA^+ T cells can be effortlessly isolated from peripheral blood, which is more accessible than tissue

biopsies are, and lower volumes are needed to obtain skin-infiltrating T cells compared to biopsy specimens, making them minimally invasive biomarkers.

Table III. Antigen-specific CLA$^+$ T-cell response in human cutaneous diseases. Adapted from Sans-de San Nicolàs et al. 2023.

Disease	Antigen	Reference
Contact dermatitis	Nickel	Santamaria-Babi LF, et al. J Exp Med 1995
	Cobalt	Blom LH, et al. Contact Dermatitis 2021
	Chromium	
Drug-induced allergic reactions	Amoxicillin and benzylpenicillin	Blanca M, et al. Blood Cells Mol Dis 2003
	Piperacillin	Sullivan A, et al. J Allergy Clin Immunol 2018
Herpes simplex	Herpes simplex virus type 2	Koelle DM, et al. J Clin Invest 2002
	Herpes simplex virus type 1	Hensel MT, et al. J Virol 2017
Leprosy	*Mycobacterium leprae*	Sieling PA, et al. Immunology 2007
Psoriasis	*Streptococcus pyogenes*	Diluvio L, et al. J Immunol 2006
	Candida albicans	De Jesús-Gil C, et al. Int J Mol Sci 2021
Skin dengue infection	Dengue virus	Rivino L, et al. Sci Transl Med 2015
Vitiligo	Melan-A	Ogg GS, et al. J Exp Med 1998

Table IV. Involvement of CLA$^+$ T cells in the pathogenesis of human T-cell-mediated cutaneous diseases. Adapted from Ferran et al. 2013., de Jesús-Gil et al. 2021., and Sans-de San Nicolàs et al. 2023.

Disease	Pathogenesis involvement	Reference
Acute graft versus-host disease	Increase of CLA$^+$ regulatory T cells at engraftment is associated with prevention of skin acute GVHD.	Engelhardt BG, et al. Bone Marrow Transplant 2011
	Upregulated expression of CLA on CD38^{bright} CD8$^+$ T cells.	Khandelwal P, et al. Biol Blood Marrow Transplant 2020
Alopecia areata	Percentage of CLA$^+$ T cells decreases in parallel with good clinical course.	Yano S, et al. Acta Derm Venereol 2002
	Increased CLA$^+$ Th2/Tc2 and Th22 and decreased CLA$^+$ regulatory T cells compared to healthy controls.	Czarnowicki T, et al. Allergy 2018
Drug-induced allergic reactions	CLA$^+$ T cells express the HLA-DR activation marker in parallel to the cutaneous symptoms in β-lactams hypersensitivity.	Blanca M, et al. Allergy 2000
	Expansion of CLA$^+$ T cells producing IL-13 in patients with drug rash with eosinophilia and systemic symptoms (DRESS).	Teraki Y, et al. Dermatology 2017
Leprosy	Increased CLA$^+$ CD4$^+$ and CD8$^+$ T cells in patients with acute type 1 reaction compared to non-reactional borderline lepromatous patients and healthy controls.	Dos Santos LN, et al. PLoS One 2016
Lichen planus	CLA$^+$ T cells accumulate in the epithelium of skin and oral lichen planus.	Walton LJ, et al. J Oral Pathol Med 1997
Non-lymphoid skin cancer	Decreased expression of endothelial cell adhesion molecules reduces CLA$^+$ T-cell recruitment in metastasic melanoma[1] and squamous[2] and basal[3] cell carcinomas, favouring tumor progression	[1]Weishaupt C, et al. Clin Cancer Res 2007 [2]Clark RA, et al. J Exp Med 2008 [3]Verhaegh M, et al. Eur J Dermatol 1998
	CLA$^+$ CD8$^+$ effector memory T cells are associated with favourable clinical outcome after CTLA4 blockade in metastastic melanoma.	Jacquelot N, et al. J Clin Invest 2016
Papuloery-throderma	Higher proportion of CD4$^+$ and CD8$^+$ T cells producing IL-4, IL-13, IL-31, and IL-22 within the CLA$^+$ than the CLA$^-$ T-cell subset.	Takamura S, et al. J Dermatol 2021

Table IV. Continued.

Disease	Pathogenesis involvement	Reference
Primary cutaneous T-cell lymphoma	T cells co-express CLA, CCR4 and CLA in Sézary syndrome, thus infiltrating skin and lymph nodes.	Campbell JJ, et al. Blood 2010
	T cells co-express CLA and CCR4, but not CCR7, therefore tumour cells are found in the skin only.	
Psoriasis	Circulating CLA^+ $CD8^+$ T cells correlate with severity (PASI).	Sigmundsdóttir H, et al. Clin Exp Immunol 2001
	Streptococcus pyogenes tonsillar infection produces CLA^+ T cells that migrate to psoriatic lesions.	Diluvio L, et al. J Immunol 2006
	Circulating CLA^+ $CD3^+$ and CLA^+ $CD4^+$ T cells are increased and express the activation marker HLA-DR[1], and inversely correlate with PASI and body surface area (BSA)[2], in acute disease.	[1]Pont-Giralt M, et al. J Invest Dermatol 2006 [2]Ferran M, et al. Eur J Dermatol 2008
	Decreased circulating CLA^+ Vγ9Vδ2 T cells inversely correlate with PASI and increase with successful treatment.	Laggner U, et al. J Immunol 2011
	Psoriatic lesions produce chemokines that can attract CLA^+ T cells to the skin such as CCL20, CCL27, CXCL8, CXCL9, CXCL10, CXCL11[1] and CXCL16[2]	[1]Mabuchi T, et al. J Dermatol Sci 2012 [2]Günther C, et al. J Invest Dermatol 2012
	S. pyogenes extract induces preferential CLA^+ T cell activation with secretion of Th1, Th17[1] and Th9[2] cytokines and induce keratinocyte activation hyperplasia[1]	[1]Ferran M, et al. J Invest Dermatol 2013 [2]Ruiz-Romeu E, et al. J Invest Dermatol 2018
	Decrease of circulating CLA^+ T cells with clinical efficacy of TNF-α inhibitors.	Cordiali-Fei P, et al. Mediators Inflamm 2014
	CLA^+ $CD8^+$ T cells accumulate in psoriatic skin, while CLA^+ $CD4^+$ T cells recirculate and patrol the skin compartment.	Diani M, et al. Clin Immunol 2017
	Candida albicans induce primarily Th17, but also Th9, response confined to CLA^+ T cells.	De Jesús-Gil C, et al. Int J Mol Sci 2021
Rosacea	Demodex infestations and papulopustular rosacea influence CLA^+ $CD4^+$ T-cell subsets.	Gazi U, et al. Parasite Immunol 2019
Scleroderma	CLA^+ $CD8^+$ T cells producing IL-13 accumulate in lesions and produce cytotoxic granules	Fuschiotti P, et al. Arthritis Rheum 2013
Vitiligo	Increased frequencies of CLA^+ T cells producing IFN-γ and IL-9.	Czarnowicki T, et al. J Allergy Clin Immunol 2019
	Elevated expression of CXCL9 in keratinocytes attracts CLA^+ $CD8^+$ T cells to the skin.	Ahn Y, et al. J Invest Dermatol 2020

Effector and central memory, both $CD4^+$ and $CD8^+$, T cells express the CLA molecule (Czarnowicki et al. 2020). Apart from them, other T-cell subsets, such as Vγ9Vδ2 T cells (Laggner et al. 2011), $NKG2D^+$ $CD8^+$ T cells (Jacquemin et al. 2020), regulatory T cells (Clark and Kupper 2007), and mucosa-associated invariant T cells (Teunissen et al. 2014), and non-T-cell subsets like effector memory B cells (Yoshino et al. 1999) and ILC2 (Salimi et al. 2013), express the cell-surface antigen CLA. Nonetheless, the knowledge on the involvement of

these cell types in the pathogenesis of human T-cell-mediated skin diseases is still limited.

In summary, circulating CLA^+ memory T cells reflect the immunological mechanisms taking place in skin during cutaneous inflammation. Thus, the analysis of this subset of memory T lymphocytes, through their isolation from peripheral blood by the CLA cell-surface marker on T cells, provides opportunity for better deciphering the immunopathogenesis of T-cell-mediated skin diseases as atopic dermatitis.

4. CLA^+ T lymphocytes and atopic dermatitis

Lesions of atopic dermatitis are characterized by abundant infiltration of CLA^+ $CD4^+$ T cells (Acevedo et al. 2020; Bilsborough et al. 2006; Leung et al. 1983), and they preferentially respond to a variety of antigens over CLA^- T cells (Table V) (Czarnowicki et al. 2017c). Besides this, they are associated with different features of the disease, including clinical aspects, treatment response and biomarkers (Table VI)(Sans-de San Nicolàs et al. 2023)(included in APPENDIX II).

Table V. Antigen-specific CLA^+ T cells in atopic dermatitis. Adapted from Czarnowicki et al. 2017.

Antigen	Reference
Dermatophagoides pteronyssinus	Santamaria-Babi LF, et al. J Exp Med 1995
Casein	Abernathy-Carver KJ, et al. J Clin Invest 1995
Staphylococcus aureus	Torres MJ, et al. Clin Exp Allergy 1998
CGRP neuropeptide	Antúnez C, et al. Br J Dermatol 2009
Autoallergen Hom s 2	Heratizadeh A, et al. Br J Dermatol 2011
Birch pollen	Campana R, et al. J Allergy Clin Immunol 2016

CLA^+ memory T cells from atopic dermatitis patients display features of *in vivo* activation, with increased expression of activation markers CD25, CD40L, inducible T cell costimulator (ICOS) and human leucocyte antigen (HLA)-DR, and spontaneous proliferation after isolation from peripheral blood (Akdis et al. 1997; Czarnowicki et al. 2015c; Santamaria Babí et al. 1995b). Circulating CLA^+,

both $CD4^+$ and $CD8^+$, T cells express the main type 2 cytokines IL-4, IL-5, IL-13 and IL-31, and other cytokines such as IL-17A, IL-21, IL-22, IL-9, IFN-γ, TNF-α and GM-CSF (Akdis et al. 1999; Akdis et al. 1997; Czarnowicki et al. 2021; Czarnowicki et al. 2020; Czarnowicki et al. 2015a; Czarnowicki et al. 2015b; Santamaria Babí et al. 1995b), and induce eosinophil survival and production of IgE by B cells (Akdis et al. 1999; Akdis et al. 1997). Additionally, the receptor for TSLP, which promotes the generation of memory T cells with preferential differentiation towards Th2, is expressed on CLA^+ $CCR4^+$ $CCR10^+$ effector memory Th2 cells (Tatsuno et al. 2015).

Table VI. Involvement of CLA^+ T cells in atopic dermatitis. Adapted from Sans-de San Nicolàs et al. 2023.

Role	Involvement of CLA^+ T cells	Reference
Pathogenesis	CLA^+ T cells express $CD25^2$, $CD40L^2$, HLA-$DR^{1,2,4}$ and $ICOS^4$, and induce IgE production by B cells and eosinophil survival[3].	[1]Santamaria Babi LF, et al. J Exp Med 1995 [2]Akdis M, et al. J Immunol 1997 [3]Akdis M, et al. J Immunol 1999 [4]Czarnowicki T, et al. J Allergy Clin Immunol 2015
	CLA^+ T cells spontaneously produce IL-4[1], IL-$13^{2,3}$ and IL-5[3].	[1]Santamaria Babi LF, et al. J Exp Med 1995 [2]Akdis M, et al. J Immunol 1997 [3]Akdis M, et al. J Immunol 1999
	CLA^+ T cells express *S. aureus*-related TCR variable Vβ segments[1,2,3].	[1]Torres MJ, et al. Clin Exp Allergy 1998 [2]Strickland I, et al. J Invest Dermatol 1999 [3]Davison S, et al. Clin Exp Immunol 2000
	CLA^+ T cells preferentially produce IL-31, a cytokine involved in pruritus.	Bilsborough J, et al. J Allergy Clin Immunol 2006
	CGRP neuropeptide selectively activates CLA^+ T cells to express IL-13 and HLA-DR.	Antúnez C, et al. Br J Dermatol 2009
	TSLP receptor is mostly expressed on CLA^+ effector memory Th2 cells.	Tatsuno K, et al. J Invest Dermatol 2015
	Severe adult disease shows expansion of CLA^+ Th2/Tc2 and Th22/Tc22, and decrease of CLA^+ Th1/Tc1, and CLA^+ T-cell IL-13 expression correlates with IgE levels and severity (SCORAD).	Czarnowicki T, et al. J Allergy Clin Immunol 2015; 136(1)
	Paediatric disease shows CLA^+ Th2/Th1 imbalance with no alteration of CLA^+ Th22 cells.	Czarnowicki T, et al. J Allergy Clin Immunol 2015; 136(4)
	Amplified infiltrates of CLA^+ T cells in lesional skin compared to healthy controls	Acevedo N, et al. Sci Rep 2020
	Reduced DNA methylation of IL-13 gene correlates with increased IL-13 mRNA expression in $CD4^+$ CLA^+ T cells.	Acevedo N, et al. Sci Rep 2020
	Circulating allergen-specific and CLA^+ T cells share the same TCRB CDR3 regions as T cells infiltrating the skin.	Roesner LM, et al. Allergy 2022

Table VI. Continued.

Role	Involvement of CLA^+ T cells	Reference
Mechanism of action of therapies	Decreased expression of IL-13, IL-4, IL-5 and IL-22 by circulating $CD4^+$ CLA^+ T cells, as well as reduced proliferation of this subset, under dupilumab treatment.	Bakker DS, et al. J Invest Dermatol 2021
	An antagonist of CCR4, which co-express with CLA in T cells, improves clinical symptoms of moderate-to-severe disease	Bissonnette R, et al. Exp Dermatol 2021
	Persistent IL-13^+ CLA^+ T-cell populations in patients under remission with dupilumab.	Jack C, et al. J Invest Dermatol 2023
	Increased frequencies of CLA^+ Th17 cells in patients responding to dupilumab.	Simpson EL, et al. J Allergy Clin Immunol 2023
	Targeting OX40, which is increased in CLA^+ $CD4^+$ T cells[1], as well as OX40L in antigen presenting cells, improves disease severity[2,3,4] in moderate-to-severe patients.	[1]Elsner JS, et al. Acta Derm Venereol 2020 [2]Guttman-Yassky E, et al. J Allergy Clin Immunol 2019 [3]Guttman-Yassky E, et al. Lancet 2023 [4]Weidinger S, et al. Br J Dermatol 2023
Biomarkers	CCL17 attracts CLA^+ memory Th2 cells to inflammed skin through the CCR4 receptor.	Campbell JJ, et al. Nature 1999
	CCL27 facilitates migration of $CCR10^+$ CLA^+ $CD4^+$ T cells to the skin.	Hudak S, et al. J Immunol 2002

Blood phenotyping studies have reported enhanced frequencies of CLA expression in atopic dermatitis, together with expanded CLA^+ Th2/T cytotoxic (Tc)2 and Th22/Tc22 and decreased CLA^+ Th1/Tc1 profiles in adult severe patients (Czarnowicki et al. 2015b). Studies involving paediatric and adult patients confirmed a dominant CLA^+ Th2 signature across all age groups, with reduced CLA^+ Th1 frequencies that raise with age (Czarnowicki et al. 2020). In addition to this, CLA^+ T_{CM} and T_{EM} atopic dermatitis populations show higher activation than psoriatic populations, and frequencies of IL-13^+ CLA^+ T cells and CLA^+ T_{CM} and T_{EM} correlate with disease severity and serum levels of total IgE (Czarnowicki et al. 2015b; Czarnowicki et al. 2015c). Interestingly, dysregulated epigenetic signatures affecting key inflammatory molecules have been described in an atopic dermatitis $CD4^+$ CLA^+ T-cell subset, including a reduced DNA methylation of the IL-13 gene promoter that correlates with increased IL-13 mRNA expression in these cells (Acevedo et al. 2020).

Atopic dermatitis flares and worsening of the disease are frequently associated with environmental triggers (Werfel et al. 2015). Exogenous proteases derived from allergens activate protease-activated receptor 2 (PAR2)-mediated

mechanisms to cause skin barrier disruption; in turn, a compromised epidermal barrier prompts the entrance of allergens in the skin and the subsequent activation and expansion of allergen-specific cutaneous T lymphocytes, mainly of the Th2 profile (Beck et al. 2022). Atopic dermatitis patients display a significant T cell proliferative response to allergens, such as house dust mite (HDM), confined to the skin-homing CLA^+ memory T-cell subset (Santamaria Babí et al. 1995b). Additionally, an emerging study showed that the TCR repertoire from circulating allergen-specific and CLA^+ T cells largely overlaps with that found in skin-infiltrating T cells, raising evidence for circulating CLA^+ T cells as relevant T cells that infiltrate the skin and respond to allergens locally (Roesner et al. 2022).

Another environmental factor is *S. aureus* which, as previously discussed, is closely related to disease flares and severity. CLA^+ T cells express significant TCR Vβ segments specific for *S. aureus* superantigens over CLA^- T cells in atopic dermatitis patients and compared to psoriatic patients and healthy controls (Davison et al. 2000; Strickland et al. 1999; Torres et al. 1998), which may explain the accumulative T cells in atopic dermatitis lesions upon application of SEB to intact skin (Skov et al. 2000). Superantigens can induce the expression of CLA on T cells in an IL-12-dependent manner (Leung et al. 1995), contributing to increasing the memory T-cell population with skin-homing capacity to maintain the continuous T-cell activation and, thus, perpetuate the lesions.

The fact that CLA^+ T cells are antigen-experienced T cells with a memory phenotype, explain their recognition of epitopes in allergens and *S. aureus*. Similarly, calcitonin gene-related peptide (CGRP) neuropeptide favours a Th2-type skewing condition by production of IL-13 and the expression of the activation marker HLA-DR restricted to CLA^+ memory T cells in atopic dermatitis patients, providing evidence of crosstalk between immune and nervous systems (Antúnez et al. 2009).

The research of biomarkers to predict and monitor treatment effects has revealed serum CCL17 as the most reliable biomarker in atopic dermatitis, both at baseline

and following therapy (Renert-Yuval et al. 2021). CCL17 is a chemoattractant of Th2 cells and is closely related to CLA^+ T cells, since its receptor CCR4 is mainly expressed on $CD4^+$ CLA^+ memory T cells (Campbell et al. 1999) and regulatory T cells (Hirahara et al. 2006), and atopic dermatitis CLA^+ memory Th2 cells home to human skin grafts in SCID mice in response to CCR4 (Biedermann et al. 2002). As for targeted therapies, oral CCR4-antagonist RPT193 shows clinical efficacy in moderate-to-severe patients (Bissonnette et al. 2021). On its behalf, the presence of CCL27 in stratum corneum, whose receptor CCR10 is preferentially expressed on $CD4^+$ CLA^+ memory T cells (Hudak et al. 2002), has been proposed as a biomarker of response to nemolizumab (anti-IL-31RA) treatment (Sidbury et al. 2022). Additionally, dupilumab (anti-IL-4Rα) treatment reduces the expression of IL-4, IL-5, IL-13 and IL-22 in $CD4^+$ CLA^+ T cells (Bakker et al. 2021), but increased frequencies of IL-13^+ CLA^+ T cells during clinical remission compared to healthy controls (Jack et al. 2023) as well as increased CLA^+ Th17 cells in responder patients have been reported (Simpson et al. 2023). Moreover, targeted therapies against OX40, which is highly expressed on $CD4^+$ CLA^+ memory T cells favouring the expansion of Th2 cells (Elsner et al. 2020) and CLA^+ regulatory T cells (Dahabreh et al. 2023), as well as targeting OX40L on APCs have shown clinical efficacy (Guttman-Yassky et al. 2023; Guttman-Yassky et al. 2019; Weidinger et al. 2023).

AIM AND OBJECTIVES

The general aim of this doctoral thesis is to study the effector function of circulating CLA^+ memory T lymphocytes in the pathogenesis of atopic dermatitis by developing a new *ex vivo* coculture model made of circulating CLA^+ or CLA^- memory T cells with autologous lesional epidermal cells from adult non-treated moderate-to-severe patients and its activation with clinically relevant triggers. On that purpose, the specific objectives that we set up are the following:

1. To characterize the CLA^+ T-cell-dependent response profiles induced by SEB and HDM and compare the profile of mediators in atopic dermatitis cocultures with control cocultures.
2. To study the contribution of epidermal cells in the CLA^+ T-cell response.
3. To neutralize relevant mediators for the disease to inquire into their effect on the production of other mediators and the gene expression on autologous epidermal cells.
4. To associate clinical data from the patients with the objectives 1, 2 and 3.

The aforementioned objectives were of great help for defining the *ex vivo* model, but the present work exceeded goals and the CLA^+ T-cell *in vitro* response, either triggered by SEB or HDM, was used for stratifying patients into groups and further characterize them by associating the CLA^+ T-cell response with clinical parameters such as Eczema Area and Severity Index (EASI), pruritus intensity and exposition to aeroallergens.

PUBLICATIONS

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

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LETTER

Allergy EAACI WILEY

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.

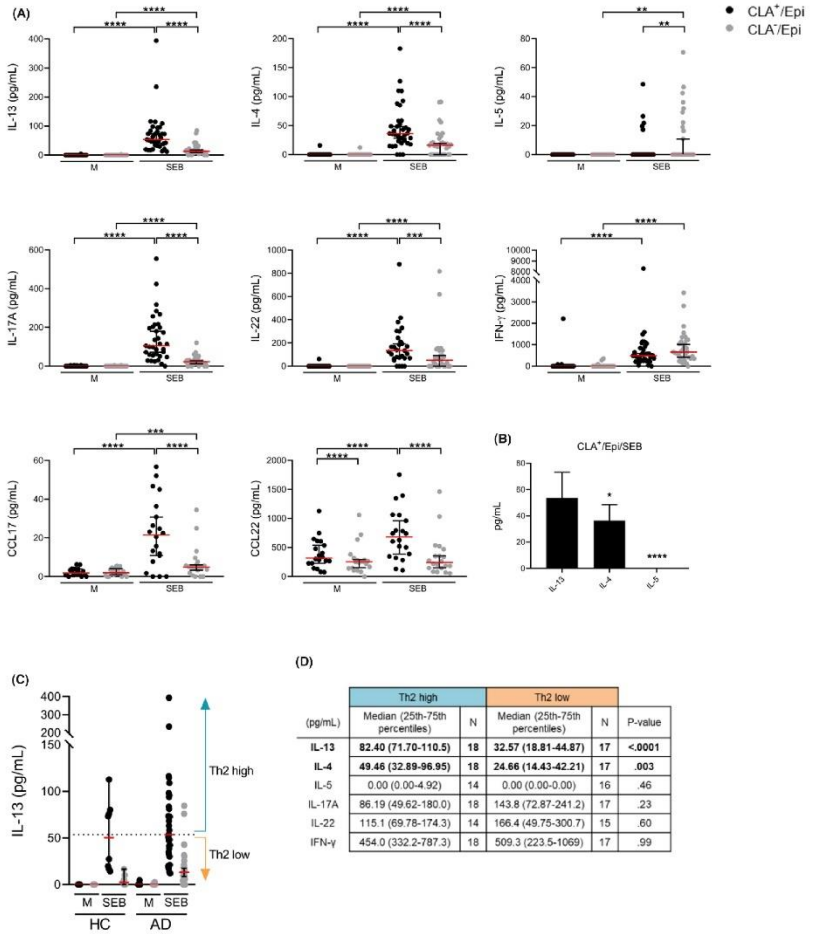
Our results showed that IL-13, together with IL-4, IL-17A, IL-22, CCL17, and CCL22, was preferentially produced by circulating memory CLA$^+$ T cells upon activation with SEB in the presence of autologous lesional epidermal cells (Figure 1A). Interestingly, SEB activation of the CLA$^+$/Epi cocultures 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.



(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. **: $p < .01$; ***: $p < .001$; ****: $p < .0001$

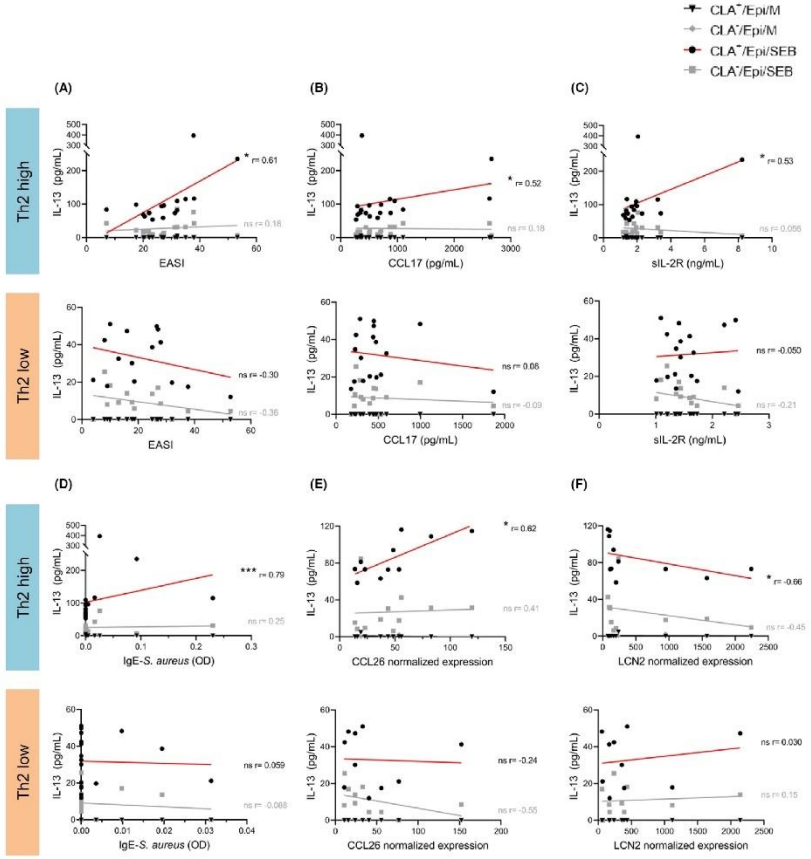


FIGURE 2 In the Th2 high group, SEB-triggered CLA⁺/Epi IL-13 response directly correlates with EASI, CCL17, sIL-2R, and anti-*S. aureus* IgE plasma levels and CCL26 mRNA expression in cutaneous lesions and inversely correlates with LCN2 mRNA expression in cutaneous lesions. IL-13 (pg/mL) from 24-hour 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.

Lídia Sans-De San Nicolàs[1]
Ignasi Figueras-Nart[2]
Montserrat Bonfill-Orti[2]
Carmen De Jesús-Gil[1]
Irene García-Jiménez[1]
Antonio Guilabert[3]
Laia Curto-Barredo[4]
Marta Bertolín-Colilla[4]
Marta Ferran[4]
Esther Serra-Baldrich[5]
Anna Zalewska-Janowska[6]
Yui-Hsi Wang[7,8]
Michael D. Howell[9]
Ramon M. Pujol[4]
Luis F. Santamaria-Babí[1]

[1]*Grup d'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*
[6]*Psychodermatology Department, Rheumatology and Clinical Immunology, Medical University of Lodz, Lodz, Poland*
[7]*Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA*
[8]*Type 2 Inflammation and Fibrosis Cluster, Immunology and Inflammation Research, Sanofi, Cambridge, Massachusetts, USA*
[9]*DermTech, Inc, La Jolla, California, USA*

Correspondence
Luis F. Santamaria-Babí, Translational Immunology, Parc Científic de Barcelona, Baldiri i Reixac, 10, 08028 Barcelona, Spain.
Email: luis.santamaria@ub.edu

ORCID

Lídia Sans-De San Nicolàs https://orcid.org/0000-0002-2828-2842
Luis F. Santamaria-Babí https://orcid.org/0000-0002-1674-6654

REFERENCES

1. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet*. 2020;396(10247):345-360.
2. DeJesús-Gil C, Sans-de San Nicolàs L, García-Jiménez I, et al. The translational relevance of human circulating memory cutaneous lymphocyte-associated antigen positive T cells in inflammatory skin disorders. *Front Immunol*. 2021;12:1-7.
3. Simpson EL, Villarreal M, Jepson B, et al. Patients with atopic dermatitis colonized with staphylococcus aureus have a distinct phenotype and endotype. *J Invest Dermatol*. 2018;138(10):2224-2233.
4. Santamaria-Babi LF. Atopic dermatitis pathogenesis: lessons from immunology. *Dermatol Pract Concept*. 2022;12(1):1-6.
5. Thijs JL, Strickland I, Bruijnzeel-Koomen CAFM, et al. Moving toward endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis. *J Allergy Clin Immunol*. 2017;140(3):730-737.

SUPPORTING INFORMATION

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

SUPPORTING INFORMATION

METHODS

Patients

The study included 35 consented adult patients with moderate-to-severe AD (based on SCORAD and EASI) and 8 consented controls under institutional review board-approved protocols at the Hospital de Bellvitge, Hospital General de Granollers, Hospital del Mar and Hospital de la Santa Creu i Sant Pau (Spain). Exclusion criteria included any topical or systemic (corticosteroid and antibiotic) anti-inflammatory treatment for the last 2 or 4 weeks prior to the study, respectively. AD patients' clinical data are summarized in Figure S1C.

Circulating memory CLA^+ and CLA^- T cells and epidermal cell isolation

Peripheral blood mononuclear cells were isolated by Ficoll (GE Healthcare, Princeton, NJ, USA) gradient and effector and central memory $CD45RA^-$ CLA^+ and CLA^- T (T_{EM} and T_{CM}) lymphocytes were purified using three consecutive immunomagnetic separations (Miltenyi Biotech, Bergisch Gladbach, Germany) as previously described.[1] First, $CD14^+$ and $CD19^+$ cells were depleted, then $CD45RA^+$ and $CD16^+$ lymphocytes were depleted and finally $CD45R0^+$ T cells were divided into CLA^+ and CLA^- T-cell subpopulations. Sample purity was > 95% and < 10% of CLA-positivity in the CLA^+ and CLA^- T-cell subpopulations respectively.[1] In order to obtain T_{EM} and T_{CM} cells we depleted $CD45RA^+$ cells, thus terminal differentiated memory T_{EMRA} cells could not be studied with this approach.

Punch skin biopsy samples, from active lesions for AD, were incubated in dispase solution (Corning, Corning, NY, USA) overnight at 4ºC, and the epidermal sheet was peeled off from the dermis. The epidermis was cut into pieces and incubated with trypsin (Biological Industries, Kibbutz Beit Haemek, Israel) for 15 minutes at 37ºC. The epidermal tissue was mechanically disaggregated by pipetting and the cell suspension was transferred to fresh culture media (1:1 volume) (RPMI, 10% FBS, 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA)). Finally, the epidermal cells suspension (Epi) was obtained by means of centrifugation. It contains all cell types at the same proportion present in the epidermis, being keratinocytes the major cell type.

Coculture of memory $CLA^{+/-}$ T cells with epidermal cells and stimulation

Ex vivo cocultures consisted of 5 x 10^4 circulating memory $CLA^{+/-}$ T cells plated together with 3 x 10^4 autologous lesional epidermal cells (CLA^+/Epi or CLA^-/Epi, respectively), in 96-well U-bottom plates (Falcon, Corning, Corning, NY, USA), in the culture media described above. Cocultures were left untreated (M) or stimulated with SEB (Sigma-Aldrich) at 100 ng/mL, PMA (25 ng/mL) and Ionomycin (2 µg/mL) (Sigma-Aldrich), or CD3/CD28 beads (1:1 ratio) (Gibco, Life Technologies, Carlsbad, CA, USA), final well concentration respectively. In cultures containing only T cells or epidermal cells, the mentioned amounts of each cell type were used, and activation with SEB was performed in the same way. Supernatants were collected after 24 hours and kept at -20ºC for mediators' quantification.

Cytokine and chemokine quantification

ProcartaPlex immunoassay (Invitrogen, Waltham, MA, USA) was used to measure IL-13, IL-4, IL-5, IL-17A, IL-22, IFN-γ, CCL17 and CCL22 concentration in collected culture supernatants with the MAGPIX instrument (Invitrogen) and analysed with ProcartaPlex Analyst software version 1.0 (Invitrogen). Values below the standard curve were treated as zero.

ELISA

Plasma from AD patients was analysed for CCL17 and sIL-2R (Invitrogen) by using precoated ELISA kits.

S. aureus extract (ATCC strain number 10832 (Wood-46)) was grown in liquid medium followed by a thermal shock for 2 hours at 60ºC; then, washes with PBS were performed, it was sonicated, and maintained in sterile conditions. *S. aureus* was diluted in coating buffer (50 nM NaHCO3 in mili-Q H_2O

pH 9.6) to a final concentration of 5 µg/mL and incubated in Nunc-Immuno MicroWell 96-well solid plates (Sigma-Aldrich) for 3 hours at 37ºC. Wells were washed 5 times with PBS, blocked with 5% skim milk powder in PBS overnight at 4ºC, and washed again 5 times in PBS-Tween 0.05%. Plasma samples were added to coated wells and incubated for 2 hours at 37ºC. Wells were washed 5 times with PBS-Tween and incubated with alkaline phosphatase conjugated goat anti-human IgE (Sigma-Aldrich) diluted 1:250 in PBS-1% skim milk for 90 minutes at 37ºC. Following another 5 washes with PBS-Tween, p-nitrophenyl phosphate substrate (Sigma-Aldrich) was incubated in the dark for 30 minutes at room temperature, and finally enzymatic reaction was stopped by adding 3 M NaOH solution. Plates were read within the next 30 minutes at 405 nm and 570 nm. The titer of reactive IgE was taken as the optical density (OD) at 405 nm after subtracting the background signal (570 nm) and negative control well signal, with only PBS-1% skim milk.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from AD lesional skin biopsies with TRIzol (Invitrogen). cDNA was synthesized with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, MA, USA) and preamplified with the TaqMan PreAmp Master Mix (2x) (Applied Biosystems). Taqman Gene Expression Master Mix and FAM-labelled probes (Applied Biosystems) (Figure S2A) were used for qRT-PCR in an ABI Prism 7900HT instrument (Applied Biosystems), and data was processed by SDS analysis software version 2.4.1 (Applied Biosystems). Gene expression was calculated by using the Δ - Δ cycle threshold (Ct) method (with the mean cycle threshold value for *RPLP0* and the gene of interest for each sample). The equation 1.8e (Ct*RPLP0* - Ctgene of interest) x 10^4 was used to obtain the normalized values.[2]

Statistical analysis

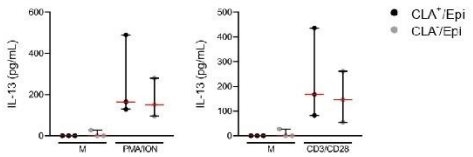
Data are presented as scatter dot plots and the median ± 95% confidence interval (CI). Differences between two groups were conducted with the Mann-Whitney test, and differences within the same group were done with the Wilcoxon test. When comparing data between the Th2 high and Th2 low groups, for continuous variables the sample median (25th-75th percentiles) was shown, whereas for categorical variables the raw numbers (percentages) were shown and Fisher's exact test was used. Values in bold indicate statistical significance. Spearman correlation coefficient was used for correlations and linear regression for representation. Statistics and data presentation were conducted with GraphPad Prism software version 8 (GraphPad Software Corporation, San Diego, CA, USA). P-value of less than .05 was considered significant.

SUPPLEMENTARY REFERENCES

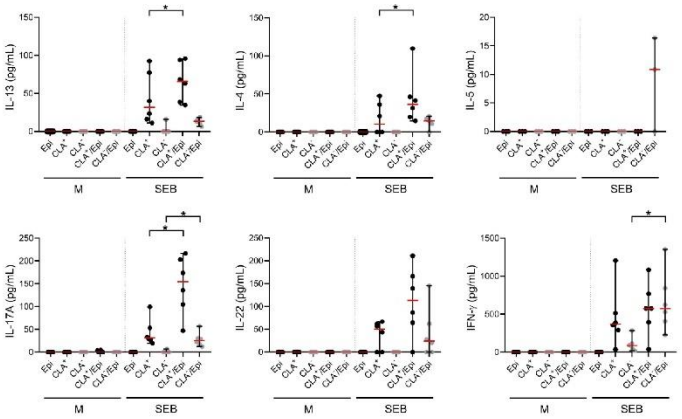
1. Ferran M, Galván AB, Rincón C, et al. Streptococcus induces circulating CLA+ memory T-cell-dependent epidermal cell activation in psoriasis. *J Invest Dermatol*. 2013;133(4):999-1007.

2. Chan JR, Blumenschein W, Murphy E, et al. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med*. 2006;203(12):2577-2587.

(A)



(B)



(C)

Clinical characteristics	Total AD patients		Th2 high		Th2 low		
	Median (25th-75th percentiles) or raw number (%)	N	Median (25th-75th percentiles) or raw number (%)	N	Median (25th-75th percentiles) or raw number (%)	N	P-value
Age	38.50 (27.75-48.50)	34	42.00 (28.50-51.50)	17	36.00 (26.50-47.00)	17	.39
Female sex (%)	17 (50.00)	34	6 (35.29)	17	11 (64.71)	17	.17
Years since diagnosis	24.00 (12.00-34.00)	33	15.50 (8.75-27.50)	16	30.00 (19.50-35.00)	17	.056
SCORAD	61.32 (54.50-68.17)	25	58.49 (50.00-67.30)	12	62.50 (57.89-69.85)	13	.31
EASI	25.75 (17.58-32.00)	32	27.00 (21.25-33.50)	17	18.60 (10.00-28.00)	15	.11
IGA	3.00 (3.00-4.00)	33	3.00 (3.00-4.00)	17	3.00 (2.25-4.00)	16	.29
Pruritus (VAS or NRS)	7.00 (6.00-8.25)	33	7.00 (6.00-8.00)	17	7.00 (5.00-9.00)	16	.89
Eosinophilia (x10^9/uL)	0.53 (0.25-0.75)	31	0.59 (0.45-0.75)	15	0.38 (0.21-0.72)	16	.23
Total IgE (kU/L)	2642 (701.0-6510)	26	4300 (1218-7292)	12	1212 (398.5-5365)	14	.18
Atopic comorbidities (%)	23 (69.70)	33	11 (64.71)	17	12 (75.00)	16	.71
Rhinitis (%)	14 (42.42)	33	5 (29.41)	17	9 (56.25)	16	.17
Asthma (%)	11 (33.33)	33	6 (35.29)	17	5 (31.25)	16	>.99
Conjunctivitis (%)	14 (42.42)	33	7 (41.18)	17	7 (43.75)	16	>.99
Food allergy (%)	6 (20.69)	29	1 (6.67)	15	5 (35.71)	14	.080

Figure S1. Unspecific stimulation of cocultures, epidermal cells suspension contribution to the SEB-triggered cocultures and clinical data of AD patients. (**A**) IL-13 quantification (pg/mL) in 24-hour cocultures unstimulated or stimulated with PMA/Ionomycin or CD3/CD28 beads (n=3). (**B**) IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ levels (pg/mL) were measured in culture supernatants from epidermal cells suspension, CLA$^+$ or CLA$^-$ T cells alone, or cocultures of CLA$^+$ and CLA$^-$ T cells after 24 hours unstimulated or stimulated with SEB (n=6 for IL-13/4/17A/22 and IFN-γ, n=3 for IL-5). (**C**) Clinical characteristics of the AD patients population (n=35), and stratified into the Th2 high (n=18) and Th2 low (n=17) groups. AD, atopic dermatitis; CLA, cutaneous lymphocyte-associated antigen T cells; EASI, eczema area and severity index; Epi, epidermal cells suspension; IGA, investigator's global assessment; M, untreated; NRS, numerical rating scale; SCORAD, scoring atopic dermatitis; SEB, staphylococcal enterotoxin B; VAS; visual analogue scale. *: $p < .05$.

(A)

		Th2 high		Th2 low		
Normalized expression	Taqman probe	Median (25th-75th percentiles)	N	Median (25th-75th percentiles)	N	P-value
IL-13	Hs00174379_m1	16.68 (10.64-27.36)	11	13.88 (5.28-27.50)	10	.31
IL-4	Hs00174122_m1	0.14 (0.09-0.31)	11	0.11 (0.07-0.17)	10	.40
IL-31	Hs01098710_m1	0.92 (0.12-2.54)	11	0.42 (0.27-1.36)	9	.55
IL-5	Hs01548712_g1	1.41 (0.81-2.49)	11	0.92 (0.49-2.04)	10	.38
CCL17	Hs00171074_m1	257.9 (133.5-643.4)	11	207.0 (64.14-482.4)	10	.35
CCL22	Hs01574247_m1	445.8 (231.4-615.1)	11	285.5 (91.15-533.8)	10	.22
CCL26	Hs00171146_m1	43.78 (19.05-55.80)	11	28.56 (14.61-60.76)	10	.60
TSLP	Hs00263639_m1	20.69 (13.86-35.74)	11	22.61 (19.21-34.80)	10	.65
IL-22	Hs01574154_m1	9.35 (6.64-26.01)	11	7.43 (2.78-17.54)	10	.28
IL-9	Hs00174125_m1	2.19 (0.17-16.85)	11	1.46 (0.55-2.49)	8	.51
IL-17A	Hs00174383_m1	0.40 (0.14-1.84)	11	0.54 (0.09-1.20)	9	.94
IL-17F	Hs00369400_m1	0.38 (0.10-0.98)	10	0.30 (0.04-1.11)	9	.58
IL-20	Hs00218888_m1	4.84 (3.25-16.70)	11	8.75 (4.01-17.85)	10	.58
IL-21	Hs00222327_m1	1.04 (0.49-2.88)	11	1.32 (0.38-2.05)	9	.60
CXCL8	Hs00174103_m1	58.82 (15.25-580.6)	11	28.21 (16.53-107.3)	10	.70
IFN-γ	Hs00174143_m1	1.39 (0.92-3.14)	11	1.16 (0.82-1.74)	10	.35
CXCL10	Hs00171042_m1	16.75 (6.89-72.58)	11	15.48 (7.59-32.94)	10	.47
TNF-α	Hs01113624_g1	49.93 (32.11-59.38)	11	41.57 (23.50-60.24)	10	.70
IL-1α	Hs00174092_m1	10.53 (6.59-36.18)	11	9.21 (5.73-16.24)	10	.31
LCN2	Hs01008571_m1	164.2 (108.8-949.8)	11	297.2 (139.3-607.6)	10	.65
LOR	Hs01894962_s1	2292 (1769-3027)	11	2148 (1068-3722)	10	.70
FLG	Hs00856927_g1	2475 (1372-3524)	11	1779 (1078-3429)	10	.76
RPLP0	Hs99999902_m1					

(B)

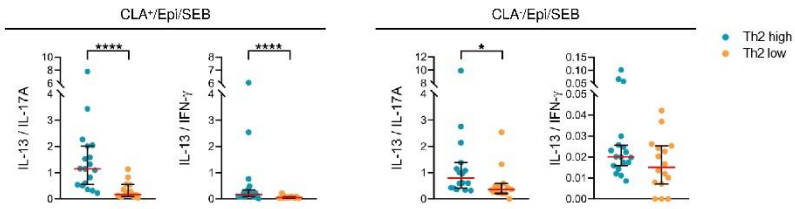


Figure S2. No differences in mRNA expression from AD lesional skin biopsies between the Th2 high and Th2 low groups, but higher SEB-induced CLA$^+$ Th2/Th17 and Th2/Th1 ratios in the Th2 high than the Th2 low group. (**A**) Taqman probes and normalized mRNA expression for each selected gene comparing the AD lesional biopsies of the Th2 high and Th2 low groups. (**B**) The ratio between the production (pg/mL) of IL-13 and IL-17A or IFN-γ by 24-hour CLA$^{+/-}$ T-cell cocultures stimulated with SEB was calculated for each sample (n=16 to 18 for Th2 high, n=13 to 16 for Th2 low). CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; M, untreated; SEB, staphylococcal enterotoxin B. *: $p < .05$; ****: $p < .0001$.

II. Allergen sensitization stratifies IL-31 production by memory T cells in atopic dermatitis patients

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EDITED BY
Jongdae Lee,
Guangzhou Medical University, China

REVIEWED BY
Hans David Brightbill,
Genentech Inc., United States
Patrick M. Brunner,
Icahn School of Medicine at Mount Sinai, United States

*CORRESPONDENCE
Luis F. Santamaria Babí
luis.santamaria@ub.edu

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Allergen sensitization stratifies IL-31 production by memory T cells in atopic dermatitis patients

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: The role of allergen sensitization in IL-31 production by T cells and specifically in the clinical context of atopic dermatitis (AD) has not been characterized.

Methods: The response to house dust mite (HDM) in purified memory T cells cocultured with epidermal cells from AD patients (n=58) and control subjects (n=11) was evaluated. AD-associated cytokines from culture supernatants, plasma proteins and mRNA expression from cutaneous lesions were assessed and related with the clinical features of the patients.

Results: HDM-induced IL-31 production by memory T cells defined two subsets of AD patients according to the presence or absence of IL-31 response. Patients in the IL-31 producing group showed a more inflammatory profile, and increased HDM-specific (sp) and total IgE levels compared to the IL-31 non-producing group. A correlation between IL-31 production and patient's pruritus intensity, plasma CCL27 and periostin was detected. When the same patients were analyzed based on sp IgE and total IgE levels, an increased IL-31 *in vitro* response, as well as type 2 markers in plasma and cutaneous lesions, was found in patients with sp IgE levels > 100 kUA/L and total IgE levels > 1000 kU/L. The IL-31 response by memory T cells was restricted to the cutaneous lymphocyte-associated antigen $(CLA)^+$ T-cell subset.

Conclusion: IgE sensitization to HDM allows stratifying IL-31 production by memory T cells in AD patients and relating it to particular clinical phenotypes of the disease.

KEYWORDS

atopic dermatitis, CCL27, CLA+ memory T cells, house dust mite, IgE, IL-31, periostin, pruritus

1 Introduction

IL-31 is a clinically relevant neuroimmune cytokine involved in epidermal barrier disruption, pruritus, inflammation and tissue remodeling in atopic dermatitis (AD) (1–3). It is enhanced in the sera from AD patients and correlates with disease severity (4).

The major source of IL-31 are $CD4^+$ T cells associated to a Th2 phenotype (5–8), but it is also produced by dendritic cells, mast cells, group 2 innate lymphoid cells, basophils, eosinophils or M2 macrophages (9–14). Its receptor, IL-31RA, is expressed by multiple cell types, including skin sensory neurons, keratinocytes and immune cells, activating the JAK/STAT, PI3K/AKT and MAPK/JNK pathways (9, 15, 16).

IL-31 and IL-31RA expression is increased in the AD skin and are part of the "core" signature characterized by the activation of the IL-31/IL-1 signaling pathway (17). The epidermal barrier function is affected by IL-31 modulation of keratinocyte differentiation directly or *via* other mediators including IL-20, IL-24, IL-33 and IL-1α (18–23). IL-31 binding to its receptor in sensory neurons stimulates itch signaling, neuronal branching and increases of cutaneous nerve fiber density (9, 24). A clear improvement of pruritus has been observed after IL-31RA blockade in moderate-to-severe AD patients (25–27). IL-31 initiates and maintains inflammation by promoting the release of proinflammatory cytokines and chemokines by myeloid cells, eosinophils, basophils and keratinocytes (10, 15, 18–20, 28–31).

Aeroallergens such as house dust mite (HDM) are associated to disease severity and total IgE levels in AD (32, 33). The relationship between IL-31 and allergens has been poorly characterized and mainly limited to canine models: HDM-sensitized dogs showed IL-31 expression in $CD3^+$ $CD4^+$ T cells in the skin lesions and production of IL-31 by Th2-polarized peripheral blood mononuclear cells (PBMC) stimulated with HDM (7, 34). Non-lesional skin from AD patients challenged with HDM induced IL-31 mRNA expression in three out of six HDM allergic patients (21). Increased frequencies of $IL\text{-}31^+$ $CD4^+$ and $CD8^+$ T cells in PBMC from HDM-sensitized patients have also been detected (35). Nevertheless, no studies have addressed the role of allergen in IL-31 production by memory T cells and its possible relationship with particular clinical features in AD patients.

In this study, HDM-induced IL-31 by cutaneous lymphocyte-associated antigen $(CLA)^+$ memory T cells correlated with patient's pruritus. Patients with elevated HDM-specific (sp) IgE (> 100 kUA/L) and total IgE levels (> 1000 kU/L) showed an increased IL-31 production by memory T cells compared to patients with lower sp and total IgE levels. These results show for the first time a relationship between allergen-specific T-cell-mediated IL-31 production, clinical status of the patients and the levels of allergen sensitization that can be of help to guide patient's identification in response to IL-31-directed therapies.

2 Materials and methods

2.1 Patients

Peripheral blood and two skin biopsies from active lesional areas were collected from 58 consented moderate-to-severe AD patients and 11 consented controls under institutional review board-approved protocols at the Hospital de Bellvitge, Hospital General de Granollers, Hospital del Mar and Hospital de la Santa Creu I Sant Pau (Spain). Exclusion criteria included topical or systemic anti-inflammatories for the last 2 or 4 weeks prior to the study, respectively.

Plasma samples were assessed for total IgE (kU/L) and HDM-specific IgE (response (OD) and kUA/L) by ImmunoCAP (Thermo Fisher Scientific, Waltham, MA, USA). Serum samples were used for lactate dehydrogenase (LDH) measurement (U/L) in a diagnostic laboratory. Patients' characteristics are summarized in Table S1.

2.2 Isolation of memory $CLA^{+/-}$ T cells and epidermal cells suspension

Central and effector memory $CD45RA^-$ T lymphocytes were purified from PBMC after Ficoll (GE Healthcare, Princeton, NJ, USA) gradient and three consecutive immunomagnetic separations (Miltenyi Biotech, Bergisch Gladbach, Germany) (36). First $CD14^+$ and $CD19^+$ cells were depleted, then $CD16^+$ and $CD45RA^+$ lymphocytes were depleted, and finally $CD45RA^+$ T cells were divided into CLA^+ and CLA^- T-cell subpopulations using the Miltenyi product ref 130-092-464. Sample purity was >95% of CLA-positivity for the CLA^+ T-cell subpopulation and <10% of CLA-positivity for the CLA^- T-cell subpopulation (36). Terminal differentiated memory T_{EMRA} cells were not studied with this approach because of $CD45RA^-$ cells depletion.

Punch skin biopsy samples were incubated in dispase solution (Corning, Corning, NY, USA) overnight at 4°C, and the epidermal

sheet was peeled off from the dermis. The epidermis was cut into pieces and incubated with trypsin (Biological Industries, Kibbutz Beit Haemek, Israel) for 15 minutes at 37°C. Then, the epidermal tissue was mechanically disaggregated by pipetting and the cell suspension was transferred to fresh culture media (1:1 volume) (RPMI supplemented with 10% FBS (Thermo Fisher Scientific) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA)). Finally, the epidermal cells suspension (Epi) was obtained by means of centrifugation. It contained all cell types at the same proportion present in the epidermis, being keratinocytes the major cell type.

2.3 Coculture of $CLA^{+/-}$ memory T cells with epidermal cells and stimulation

The coculture system was performed by seeding 3 x 10^5 autologous epidermal cells with 5 x 10^4 circulating CLA^+ or CLA^- memory T cells (CLA^+/Epi or CLA /Epi, respectively) in a 96-well U-bottom plate (Falcon, Corning, Corning, NY, USA), in the culture media described above. Whole ($CD4^+$ and $CD8^+$) CLA^+ memory T cells were used; however, we assume that results obtained are due to $CD4^+CLA^+$ memory T cells, since the majority of CLA^+ memory T cells are $CD4^+$ (80% $CD4^-$ and 20% $CD8^+$) and IL-31 is mainly produced by $CD4^-CLA^-$ T cells (6, 23). Cocultures were left untreated (M) or activated for 5 days with HDM extract kindly provided by LETI Pharma at 10 µg/mL or 24 hours with staphylococcal enterotoxin B (SEB) (Sigma-Aldrich) at 100 ng/mL final well concentration (37). In cultures containing only T cells or epidermal cells, the mentioned amounts of each cell type were used, and activation with HDM was performed in the same way. Collected supernatants were kept at -20°C.

For blocking assays, HLA-A/B/C (class I) (BioLegend, San Diego, CA, USA), HLA-DR (class II) (BioLegend), CD1a (Bio X Cell, Lebanon, NH, USA), IL-33 (R & D Systems, Minneapolis, Minnesota, USA) neutralizing antibodies, or respective mouse IgG2a isotype control (BioLegend), mouse IgG1 (BioLegend) and goat IgG isotype control (R & D Systems) were added to cocultures at 10 µg/mL (for HLA and CD1a blocking assays) or 1 µg/mL (for IL-33 blocking assay) final concentration.

2.4 Transwell culture

5 x 10^4 purified CLA^+ or CLA^- memory T cells were added to the top chamber of 0.4 µm pore size polycarbonate 96-well HTS Transwell culture insert (Corning) containing 3 x 10^4 autologous lesional epidermal cells stimulated with HDM at 10 µg/mL final well concentration in the bottom chamber. Supernatants were collected at day 5 of culture.

2.5 Cytokine and chemokine quantification

ProcartaPlex immunoassay (Invitrogen, Waltham, MA, USA) was used to measure IL-31, IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ concentrations in the culture supernatants with the MAGPIX instrument (Invitrogen) and analysed with ProcartaPlex Analyst software version 1.0 (Invitrogen). Values below the lower limit of quantification (LLOQ) were treated as zero. Plasma CCL22 levels were quantified with ProcartaPlex.

2.6 ELISA

Pre-coated ELISA kits were used for quantification of plasma levels of CCL27, CCL17, CCL18, sIL-2R (Invitrogen) and periostin (AdipoGen Life Sciences, San Diego, CA, USA).

2.7 RNA isolation, quantitative real-time PCR and gene-array analysis

Lesional skin biopsy specimens frozen in Tissue-Tek O.C.T. Compound (Sakura Finetek, Alphen aan den Rijn, The Netherlands) at -80°C were used for RNA isolation by using the TRIzol Reagent (Invitrogen).

For qRT-PCR, cDNA was obtained with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, MA, USA) and preamplified with the TaqMan PreAmp Master Mix (2x) (Applied Biosystems). Taqman Gene Expression Master Mix and FAM-labelled probes (Table S2) (Applied Biosystems) were used for qRT-PCR with an ABI Prism 7900HT instrument (Applied Biosystems). Data was processed by SDS analysis software version 2.4.1 (Applied Biosystems) and gene expression was calculated by using the Δ - Δ cycle threshold (Ct) method (with the mean cycle threshold value for RPLP0 and the gene of interest for each sample). The equation 1.8e (CtRPLP0 - Ctgene of interest) x 10^3 was used for normalizing the values (38).

For gene-array analysis, quality control of concentration and integrity of the isolated RNA was performed with the NanoDrop One (Thermo Fisher Scientific) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). PrimeView Human Gene Expression Arrays (Applied Biosystems) were processed at the Functional Genomics Facility of IRB Barcelona (Barcelona, Spain) and raw data (CEL files) were processed with Transcriptome Analysis Console version 4.0 (Applied Biosystems). Raw data were deposited in the Gene Expression Omnibus repository, accession number GSE226073. Genes with a fold change (FCH) of 1.5 or greater and a p value of less than .05 were considered differentially expressed genes (DEG). Pathway enrichment analysis was performed using g:Profiler web server (https://biit.cs.ut.ee/gprofiler/gost) with Gene Ontology (GO) biological process database and a Benjamini-Hochberg FDR of .05 or greater.

2.8 Statistical analyses

Data analysis and representation were performed with GraphPad Prism software version 8 (GraphPad Software Corporation, San Diego, CA, USA). Data are represented as the median ± 95% confidence interval (CI). Wilcoxon test was used to compare two

conditions within the same group and Mann-Whitney test was used to compare two groups. In tables, sample median (25th-75th percentiles) and Mann-Whitney test were used for continuous variables, raw numbers (percentages) and Fisher's exact test were used for categorical variables, and bold values indicate significant data. Correlations were examined using Spearman coefficient and represented with linear regression. Differences were considered significant at a P-value of less than .05 and showed as: (*) $p < .05$; (**) $p < .01$; (***) $p < .001$; (****) $p < .0001$.

3 Results

3.1 HDM induces IL-31 in memory T cells cultured with autologous lesional epidermal atopic dermatitis cells, which correlates with pruritus, and plasma CCL27 and periostin

AD-derived cocultures containing circulating memory CLA^+ T cells and autologous lesional epidermal cells (CLA^+/Epi) in the presence of HDM led to IL-31 production, whereas CLA^- T-cell AD cocultures (CLA^-/Epi) and control (C)-derived cocultures did not produced IL-31 upon stimulation (Figure 1A). Epidermal cells suspension promoted HDM-induced CLA^- T-cell-derived IL-31 response (Figure S1A) and it was produced in a time-dependent manner (Figure S1B).

AD patients were classified into two groups to further characterize clinical profiles based on the differential response to HDM: those producing IL-31 by CLA^+ T cells were arranged into group 1 (n=17), and those with no IL-31 production were arranged into group 2 (n=41) (Figure 1A).

Within group 1 of patients, HDM-induced IL-31 response directly correlated with pruritus ($r = 0.67$, $p = .0036$; Figure 1B) and plasma levels of CCL27 ($r = 0.62$, $p = .0090$; Figure 1C) and periostin ($r = 0.56$, $p = .050$; Figure 1D). Correlations within group 2 of patients could not be performed due to undetectable IL-31 production in the cocultures. As shown in Figure 1E, there was direct correlation between plasma CCL27 levels and SCORAD in group 1 ($r = 0.56$, $p = .032$) but not in group 2 ($r = 0.31$, $p = .091$). Additionally, HDM-induced CLA^+ T-cell IL-31 nearly correlated with SCORAD in group 1 of patients ($r = 0.49$, $p = .066$; not shown). There was no correlation between IL-31 *in vitro* response to HDM and plasma levels of HDM-specific (sp) IgE and total IgE, although there was a tendency for the former ($r = 0.46$, $p = .068$; Figure S2).

The IL-31 production by CLA^+ memory T cells was blocked by 59% by a neutralizing IILA class II, but not IILA class I, antibody (Figures 1F; S3A). Blocking of IL-33 and CD1a molecules did not affect IL-31 production (Figures S3B, C), but cell-cell contact between CLA^+ T cells and epidermal cells was necessary for IL-31 production as demonstrated by transwell cultures (Figure 1G).

IL-31 was simultaneously measured with other AD-derived mediators (IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ) and we found that IL-13, IL-4, IL-5, IL-17A and IL-22 production by HDM-induced CLA^+ T-cell AD cocultures was higher than that by C cocultures (Figure S4A). HDM-activated CLA^-/Epi cocultures from group 1 showed increased production of IL-13, IL-4, IL-5, IL-17A and IL-22 compared to group 2 (Figures 2A; S4B), revealing a more inflammatory phenotype in group 1 versus group 2. Also, in group 1 IL-31 production directly correlated with IL-13 ($r = 0.62$, $p = .0093$) and IL-4 ($r = 0.85$, $p < .0001$), but no correlation with IL-5, IL-17A, IL-22 and IFN-γ was observed (Figure 2B). Although group 2 of patients were defined for their null production of IL-31 by HDM-stimulated CLA^- T cells, there was an heterogeneous IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ response in the CLA^+ T-cell cocultures of this group (Figure S5). All correlations were tested with and without outlier patient data.

When stimulating cocultures with Staphylococcus aureus superantigen B (SEB), another clinically relevant stimulus of AD, IL-31 was specifically produced by CLA+ T cells but no significant differences were found between group 1 and group 2 of patients (Figure S6A). Additionally, therewas no correlation between HDM- and SEB-induced IL-31 production by memory T cells (Figure S6B).

3.2 Patients with memory T-cell IL-31 response to HDM show elevated plasma levels of sp and total IgE and a more inflammatory profile than patients with no IL-31 response

Patients from group 1 displayed increased sp IgE and total IgE levels compared with patients from group 2 (Table 1). No differences were found for severity, pruritus, eosinophilia, years since diagnosis and comorbidities. Although group 2 of patients had a reduced allergen sensitization degree when compared to group 1 of patients, they showed higher total IgE and sp IgE levels than controls (total IgE (kU/L): group 2 median = 568.00, C median = 21.10, $p < .0001$; sp IgE response (OD): group 2 median = 5338, C median = 27.00, $p < .0001$).

To further characterize both groups, we evaluated changes in lesional skin tissue from patients belonging to group 1 and group 2 using gene-array analysis. We identified two hundred six probe-sets (143 unique genes) up-regulated and one hundred probe-sets (80 gens) down-regulated in group 1 skin compared with group 2 skin (Figure S7A; Table S3). Among the upregulated genes there were IL-20, IL-24, CCL20, CXCL1, CCL2 and EGR1. Only for IL-20 was FDR of less than .05. Enrichment tests of DEG revealed that group 1 lesional skin was enriched for biological processes related to the immune system such as response to external stimulus, inflammatory response and cellular response to chemokine (Figure S7B; Table S4). On the contrary, group 2 lesional skin was enriched for developmental processes (Figure S7C; Table S5).

Interestingly, difference in IL-20 expression between group 1 and group 2 of patients by gene-array analysis was confirmed by qRT-PCR (Figure S7D), but higher number of patients should be analyzed to confirm this data due to significant overlap between both groups. Additionally, increase in IL-17A and IL-21 mRNA expression was detected in group 1 compared to group 2 (Figure S7D), and no differences were found for IL-31 mRNA expression in cutaneous lesions between both groups.

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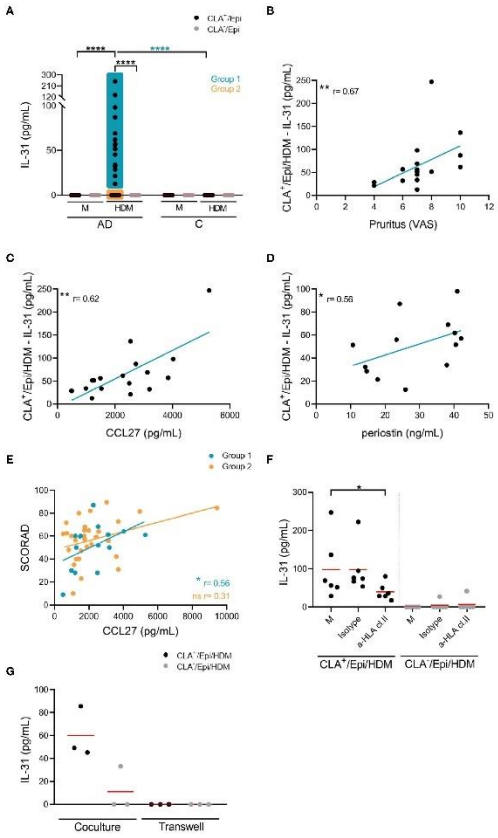

FIGURE 1
HDM induced IL-31 by memory T cells correlates with patient's pruritus and plasma CCL27 and periostin, partially depends on HLA class II molecules and the cell-cell contact with epidermal cells, and is restricted to CLA+ T-cells subset. **(A)** IL-31 (pg/mL) produced by CLA+/- T-cell cocultures in basal conditions (M) or stimulated with HDM in AD-(n=58) and C-(n=11) derived samples. Correlations of CLA^+/Epi/HDM IL-31 production in group 1 with **(B)** pruritus (n=17), and plasma **(C)** CCL27 (n=17) and **(D)** periostin (n=13) levels. **(E)** Correlation between plasma CCL27 levels and SCORAD in group 1 (n=15) and group 2 (n=31) of patients. **(F)** HDM activated $CLA^{+/-}$ T cell cocultures were treated with HLA class II neutralizing antibody or control IgG isotype at day 0, and IL-31 production (pg/mL) at day 5 was compared with respect to isotype values (n=6). Red bar indicates the mean. **(G)** $CLA^{+/-}$ T cells were added in the upper chamber and HDM-stimulated epidermal cells suspension in the lower chamber of the 96-well transwell plate and IL-31 (pg/mL) was measured at day 5. Data for three representative patients. AD, atopic dermatitis; C, control subjects; CLA, cutaneous lymphocyte associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated; SCORAD, scoring atopic dermatitis. ns: $p > .05$; *$p < .05$; **$p < .01$; ****$p < .0001$.

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A

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 (0.00-7.70)	***
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

B

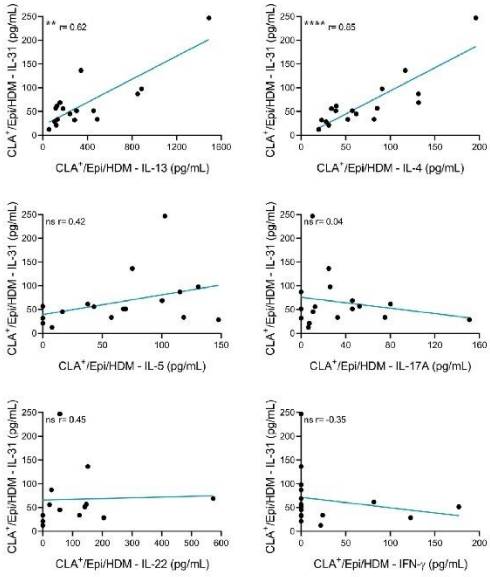

FIGURE 2
Patients producing IL-31 (group 1) by HDM-stimulated CLA+ T cells have a more inflammatory phenotype than patients with undetectable IL-31 (group 2). **(A)** IL-13, IL-4, IL-5, IL-17A, IL-22 and IFN-γ (pg/mL) produced by HDM-induced CLA+ T-cell cocultures was compared between groups 1 (n=13-17) and 2 (n=35-41) of patients. **(B)** In group 1, HDM-stimulated CLA+ T-cell-dependent IL-31 production was correlated with IL-13 (n=17), IL-4 (n=17), IL-5 (n=17), IL-17A (n=17), IL-22 (n=13) and IFN-γ (n=17). CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite. ns: p >.05; **p <.01; ***p <.001; ****p <.0001.

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TABLE 1 Differences in clinical characteristics between group 1 and group 2.

Clinical data	Group 1 Median (25-75% percentiles) or Total number (%)	N	Group 2 Median (25-75% percentiles) or Total number (%)	N	p Value
Age	28.00 (18.50-44.50)	17	36.00 (27.00-45.75)	40	.063
SCORAD	59.63 (30.00-61.32)	15	59.27 (42.10-68.00)	31	.44
EASI	22.65 (19.38 27.93)	16	25.00 (15.25 32.00)	38	.92
IGA	4.00 (3.00-4.00)	16	3.00 (3.00-4.00)	40	.35
Pruritus (VAS)	7.00 (6.50 8.00)	17	8.00 (6.25 9.00)	40	.31
Eosinophilia (10^3/uL)	0.73 (0.21-1.06)	16	0.46 (0.24-0.61)	34	.16
sp IgE response (OD)	**18747 (12287-20456)**	**17**	**5338 (393.0-16830)**	**41**	**<.0001**
Total IgE (kU/L)	**2000 (606.9-5000)**	**17**	**568.0 (110.5-1792)**	**41**	**.0027**
LDH (U/L)	209.0 (143.8-249.8)	6	193.0 (128.0-236.3)	26	.66
Years since AD diagnosis	15.50 (11.00-23.75)	16	24.00 (13.00-33.50)	38	.28
Comorbidities, n	13 (81.25)	16	27 (67.50)	40	.35
Rhinitis, n	8 (50.00)	16	23 (58.97)	39	.56
Asthma, n	6 (37.50)	16	16 (41.03)	39	>.99
Conjunctivitis, n	8 (50.00)	16	15 (38.46)	39	.55
Food allergy, n	2 (12.50)	16	11 (29.73)	37	.30

Categorical variables are presented as counts (percentages) and continuous variables are presented as medians (25th-75th percentiles). AD, atopic dermatitis; EASI, eczema area and severity index; IGA, investigator's global assessment; LDH, lactate dehydrogenase; OD, optical density; SCORAD, scoring atopic dermatitis; VAS, visual analogue scale. Bold values indicate significant data.

3.3 Patients with sp IgE > 100 kUA/L have increased IL-31 *in vitro* response to HDM confined to the CLA^+ memory T-cell subset compared to patients with sp IgE < 100 kUA/L

An alternative analysis in the same patients revealed that those with high levels of sp IgE (> 100 kUA/L) showed increased HDM-induced IL-31 response by memory T cells, only in the CLA^+ T-cell subset, than those with low levels of sp IgE (< 100 kUA/L) (Figure 3A). There was a trend towards positive correlation between IL-31 response by HDM-stimulated CLA T-cell cocultures and SCORAD in the high sp IgE group ($r = 0.28$, $p = .28$), whereas this trend was negative for the low sp IgE group ($r = -0.32$, $p = .094$; Figure 3B). Patients from the high sp IgE group showed increased eosinophilia and total IgE levels compared to patients from the low sp IgE group, as expected (Table 2). Additionally, in the high sp IgE there was a tendency towards more patients with conjunctivitis, a sign of HDM clinical symptom ($p < .1$).

In line with the previous observation, the IL-31 mRNA expression in cutaneous lesions was greater in the high than in the low sp IgE groups (Figure S8A). Furthermore, plasma levels of CCL18, CCL22, CCL27, periostin and sIL-2R were elevated in the high compared to the low sp IgE groups (Figures 3C; S8B). Although results were significant ($p < .05$) there was clear overlap between both groups and increased number of patients would be required.

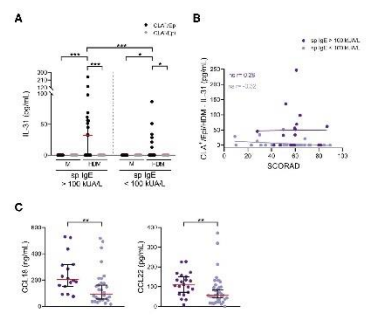

FIGURE 3
Enhanced IL-31 *in vitro* response and plasma levels of CCL18 and CCL22 in patients with sp IgE > 100 kUA/L. **(A)** IL-31 (pg/mL) from AD culture supernatants separated according to sp IgE levels (> 100 kUA/L n=21, < 100 kUA/L n=37). **(B)** Correlation between CLA^+/LPI/HDM IL-31 and SCORAD in high (n=17) and low (n=29) sp IgE groups. **(C)** Plasma levels of CCL18 (ng/mL) and CCL22 (pg/mL) were compared between high (n=16-21) and low (n=30-37) sp IgE groups. CLA, cutaneous lymphocyte associated antigen I cells; LPI, epidermal cells suspension; HDM, house dust mite; M, untreated; sp IgE, specific IgE to house dust mite; SCORAD, scoring atopic dermatitis. ns: $p > .05$; *$p < .05$; **$p < .01$; ***$p < .001$.

TABLE 2 Differences in clinical characteristics between patients with sp IgE >100 kUA/L and patients with sp IgE <100 kUA/L.

Clinical data	Sp IgE >100 kUA/L Median (25-75% percentiles) or Total number (%)	N	Sp IgE <100 kUA/L Median (25-75% percentiles) or Total number (%)	N	p Value
Age	36.00 (23.50-48.00)	21	34.50 (26.25-45.00)	36	.85
SCORAD	59.63 (51.00-62.76)	17	56.00 (37.50-66.38)	29	.52
EASI	27.30 (19.25 34.75)	20	22.10 (11.90 27.41)	34	.13
IGA	4.00 (3.00-4.00)	20	3.00 (3.00-4.00)	36	.21
Pruritus (VAS)	7.00 (6.50 8.00)	21	8.00 (6.25 9.00)	36	.40
Eosinophilia (10^3/uL)	**0.74 (0.47-1.08)**	**19**	**0.33 (0.19-0.56)**	**31**	**.0026**
sp IgE response (OD)	**19507 (17643-20456)**	**21**	**4031 (312.5-10513)**	**37**	**<.0001**
Total IgE (kU/L)	**3639 (1962-5000)**	**21**	**336.0 (107.0-1130)**	**37**	**<.0001**
LDH (U/L)	237.0 (124.5-329.3)	8	184.5 (122.0-219.3)	24	.13
Years since AD diagnosis	18.00 (13.50-32.75)	20	23.50 (9.50-30.00)	34	.99
Comorbidities, n	17 (80.95)	21	23 (65.71)	35	.36
Rhinitis, n	13 (61.90)	21	18 (52.94)	34	.58
Asthma, n	7 (33.33)	21	15 (44.12)	34	.57
Conjunctivitis, n	12 (57.14)	21	11 (32.35)	34	.094
Food allergy, n	2 (9.52)	21	11 (34.38)	32	.053

Categorical variables are presented as counts (percentages) and continuous variables are presented as medians (25th-75th percentiles). AD, atopic dermatitis; EASI, eczema area and severity index; IGA, investigator's global assessment; LDH, lactate dehydrogenase; OD, optical density; SCORAD, scoring atopic dermatitis; VAS, visual analogue scale. Bold values indicate significant data.

The IL-31 response by memory T cells activated with SEB showed no significant differences between high sp IgE and low sp IgE groups (Figure S8C). Additionally, no correlation between the HDM- and SEB-induced IL-31 production was observed in the high sp IgE and the low sp IgE groups (Figure S8D).

4 Discussion

In moderate-to-severe AD the role of allergen in T-cell mediated IL-31 production and its possible relationship with the clinical status of the patients is presently uncharacterized. Our results suggest that the degree of allergen sensitization allows stratifying patients for IL-31 production by T cells with different clinical features.

In lesional AD skin $CD4^-$ T cells are the most abundant infiltrating lymphocytes and they are mainly CLA^+ T cells (39, 40). Until now, T-cell-derived IL-31 production has been mainly analyzed by qRT-PCR and intracellular flow cytometry of polyclonal-activated PBMC in a low number of patients and without any association with the clinic (8, 21, 23, 35, 41). In our study, HDM-induced IL-31 production by memory T cells defined two groups of AD patients: the IL-31 producing group (group 1) and the IL-31 non-producing group (group 2). In group 1, compared with group 2, HDM-induced IL-31 in CLA^+ T cells positively correlated with patient´s pruritus intensity, and plasma levels of CCL27 and periostin. In addition, plasma levels of HDM-specific IgE and total IgE were increased in group1.

IL-31 is one of the main drivers of pruritus (2). For the first time, a direct correlation between memory T-cell-derived IL-31 response to HDM *in vitro* and patient's pruritus was demonstrated, which underlines the clinical relevance of this mechanism in AD. This translational observation stablishes a relationship between allergen and patient's pruritus through a mechanism restricted to a subset of circulating skin-homing memory T cells. The lack of difference in pruritus intensity between group 1 and group 2 of patients may be due to the broad range of pruritogens in AD, such as IL-13 or IL-4 (1), which could promote the itch signaling pathway in patients that do not produce IL-31 upon HDM stimulation. Since IL-31RA blockade improves symptoms in AD (25, 27, 42), further studies should explore the influence of allergen sensitization and the clinical response to anti-IL-31RA therapies in pruritus.

In group 1 of patients, IL-31 produced by CLA^+ T cells positively correlates with CCL27, being CLA^+ T cells and CCL27 mechanistically closely related, since CLA^+ T cells express the CCR10 receptor that specifically binds CCL27 (43, 44). The relationship between IL-31 producers and plasma CCL27 suggests that lesional keratinocytes produce the CLA-attracting chemokine CCL27 to facilitate further recruitment of CLA^+ T cells to the inflamed skin where, upon activation with HDM, would produce IL-31. CCL27 in the stratum corneum has been proposed as a

biomarker of clinical response to nemolizumab, an anti-IL-31RA, treatment in AD (42), supporting the significant correlation between CCL27 and SCORAD in group 1 of patients. Besides this, not all patients respond to nemolizumab treatment, suggesting that allergen sensitization may be of help to identify anti-IL-31RA responder patients.

Periostin is an extracellular matrix protein closely related to Th2 immune response with an emerging role in pruritus and barrier dysfunction (45–48). In canine models, epicutaneous application of HDM to sensitized dogs induced upregulation of Th2 signature, including IL-31 and periostin (49). Nevertheless, the association between periostin plasma levels and IL-31, for HDM-triggered memory T cells, has not previously been reported.

Group 1 of patients showed increased levels of IL-13, IL-4, IL-5, IL-17A and IL-22, all of them commonly found in AD lesional skin (17), in response to HDM by the CLA^- T-cell cocultures, suggesting a strong inflammatory response in this group. Moreover, the direct correlation of IL-31 with IL-13 and IL-4 underlines the allergen-specific and Th2-phenotype of IL-31, and it is also supported by the IL-4-dependent IL-31 production reported in polyclonal stimulated $CD4^+$ T-cell clones (5). The skin transcriptome confirmed the inflammatory signature in group 1 of patients, enriched in functions such as "response to external stimulus", "inflammatory response", "cellular response to chemokines" and "response to IL-1". Among the up-regulated genes in this group, IL-20 is associated with IL-31-induced barrier disruption (18), and has also been related with pruritus in murine AD models (50). On the contrary, group 2 of patients lacked a transcriptomic dysregulation of inflammatory genes.

Group 2 of patients responded in most of the endpoints evaluated in this study (*e.g.* severity, pruritus), which is given by the nature that they are AD patients, so they share some clinical characteristics with group 1 of patients. The most significant clinical difference between both groups was the degree of IgE sensitization to HDM, along with total IgE levels, which were related with the differential IL-31 response by skin-derived memory T cells upon stimulation with allergen. Thereby, two groups that apparently would be the same population may show distinct molecular mechanisms driving disease immunopathogenesis.

AD is associated with elevated IgE, sensitization to aeroallergens and eosinophilia (51). Total IgE levels correlate with patients' severity and are increased in patients sensitized to aeroallergens (33, 52). IgE levels are not a diagnostic requirement, but they are useful for determining prognosis, long-term outcome prediction or choosing therapy (53, 54). When stratifying patients according to their sp IgE levels, the increased IL-31 *in vitro* response in patients with high levels of sp IgE (over 100 kUA/L) supported the HDM contribution to disease activity in AD (32). Noteworthy, the increased IL-31 mRNA expression in lesional skin from high sp IgE patients may explain why topical exposure to HDM induced IL-31 only in a subset of HDM sensitized patients (21). The high sp IgE group also showed enhanced plasma levels of periostin, CCL27, CCL18, CCL22 and sIL-2R, thus associating the allergen-exposure and IL-31 response with Th2-immune response and general inflammation (55). To evaluate signs of HDM clinical symptoms and to compare the reactivity of CLA^- memory T cells to HDM with other allergens may be considered to complement these current findings.

A recent research described an increased IL-31RA expression on memory B cells from AD patients with total IgE levels > 1000 kU/L compared to controls, pointing to an association between IL-31-mediated mechanisms and atopic IgE-producing phenotype (56). For this reason, we classified patients according to their total IgE levels into high (> 1000 kU/L) and low (< 1000 kU/L) groups and found an increased HDM-induced memory T-cell IL-31, IL-13 and IL-4 response in the former group (Figure S9A), along with enhanced CCL26 mRNA expression in cutaneous lesions (Figure S9B) and plasma levels of CCL27, periostin, CCL17, CCL18, CCL22 and sIL-2R (Figure S9C). These data suggest that in allergen sensitized patients, IL-31 may play a role not only as T-cell-derived mediator by CLA^- T cells but also influencing IL-31RA-expressing B cells. This hypothesis is currently being investigated.

The knowledge generated from the present study is translationally relevant, since the allergen-induced IL-31 production by memory T cells was confined to the CLA^- T-cell subset, which reflects the skin-related mechanisms taking place in AD lesions and other inflammatory cutaneous diseases (57–61). Interestingly it has recently been reported that an early-term effect of dupilumab treatment on IL-13, IL-4, IL-5 and IL-22 expression is preferentially found in circulating $CD4^+$ CLA^- T cells from AD patients (62, 63). Besides this, circulating allergen-specific and CLA^+ T cells share the same TCRB CDR3 regions as lesional T cells from AD skin (64), supporting the relevance of our results for IL-31 produced by peripheral skin-homing T cells.

Circulating CLA^- T cells have been described to be the main memory T-cell subset producing IL-31 (23). When we complemented the analysis of T-cell mediated IL-31 response in the same patients by using SEB (65), IL-31 levels produced by CLA^+ memory T cells were lower than those observed after HDM stimulation, without significant differences between groups 1 and 2 of patients or sp IgE levels. Additionally, a correlation between HDM- and SEB-induced IL-31 response was not detected, suggesting different roles of these stimuli in T-cell-mediated IL-31 production in AD. The molecular mechanisms by which SEB and HDM activate T cells are different. SEB directly binds to HLA class II molecules on the surface of antigen presenting cells and stimulates T cells expressing particular TCR. SEB can also induce skin inflammation by the following mechanisms: the direct binding to antigen presenting cells in the skin, direct binding to MHC class II keratinocytes, and induction of IgE-mediated immune responses (66). On the other hand, HDM requires uptake, processing and presentation by antigen presenting cells, a process that can be favored by the presence of allergen-specific IgE on the surface of antigen presenting cells (67). Therefore, the IgE-mediated uptake and antigen presentation may be favored in group 1 of patients, since they present higher levels of HDM-specific IgE.

Other inflammatory cells have been shown to produce IL-31: group 2 innate lymphoid cells (ILC2), basophils, eosinophils, dendritic cells, macrophages and mast cells (9–14) A single study has shown IL-31 expression by ILC2 (12), but the clinical relevance of alarmins, which are needed for ILC2 activation, in AD is not clear since all targeted therapies against them failed in clinical trials (68). Regarding basophils, IL-31 has been shown in chronic spontaneous urticaria and healthy donors (10), and no study using AD-derived

basophils has shown IL-31 production. In eosinophils, IL-3 induces IL-31 production, but clinical relevance of eosinophils in AD has not been confirmed by anti-IL-5 therapy (14, 68). Dendritic cells express a hundred times less IL-31 than Th2 cells and whether dendritic cells produce physiologically relevant IL-31 quantities is underexplored (9). Recently, a new network comprising IL-31$^+$ M2 macrophages, basophils, periostin and TSLP has been described (13); nonetheless, the clinical relevance of these results are unknown due to lack of efficacy of TSLP blockade for AD treatment (68, 69). Mast cells from psoriatic skin and healthy donors express IL-31, but this expression has not been shown for AD (11). Altogether, although IL-31 can be produced by different cell sources besides CLA$^+$ memory T cells, no association with clinical pruritus has been reported, therefore their translational relevance is still under investigation. Additional studies on the involvement of IL-31 production by these non-CLA$^+$ memory T cells on epithelial cells in coculture assays may be of help to further understand more mechanisms of IL-31 in AD.

This study has some limitations. We tried to detect IL-31 by intracellular flow cytometry but it was a complex issue due to the lack of good tools (5), and plasma or serum IL-31 levels were difficult to detect. Additionally, sample size for gene-array analysis as well as qRT-PCR was limited and more detailed investigations on the interactions between lesional epidermal cells and CLA$^+$ memory T cells are needed.

Although the role of allergens in AD is still controversial and at the moment the stratification of response to anti-IL-31RA depending on IgE status has not been performed, it can be hypothesized that assessing specific IgE status to HDM in candidates for anti-IL-31RA therapy may eventually be helpful for identifying patients more prone to good clinical response to anti-IL-31RA and with a more favorable response in comparison to dupilumab or JAK inhibitors.

In summary, our findings bring new data on the mechanisms of allergen sensitization and IL-31 production by memory T cells in moderate-to-severe AD patients. The biphasic IL-31 response to HDM demonstrated with this translational *ex vivo* model may explain the partial IL-31 expression after HDM topical exposure in HDM-sensitized patients (21) and the heterogeneous response to anti-IL-31-directed therapies.

Data availability statement

The original contributions presented in the study are publicly available in the Gene Expression Omnibus repository, accession number GSE226073.

Ethics statement

The study involved human participants and was reviewed and approved by the Ethics Committee in Hospital de Bellvitge, Hospital del Granollers, Hospital del Mar and Hospital de la Santa Creu I Sant Pau (Spain). Participants provided written informed consent to participate in this study.

Author contributions

LS-dSN, IF-N, RP and LS-B contributed to the design of the study. IF-N, MB-O, AG, LC-B, MB-C, MF and ES-B included participants. LS-dSN, IF-N and IG-J participated in the investigation. LS-dSN analyzed the data and prepared figures. LS-dSN and LS-B wrote the manuscript. MF, RP and LS-B contributed to the funding acquisition. RP and LS-B supervised the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

AG has received grants from AbbVie, Janssen, Lilly, Novartis and Sanofi, and lecture fees from AbbVie, Almirall, Leo Pharma and Sanofi. ES-B is a consultant for AbbVie, Almirall, Galderma, Genentec, Leo Pharma, Lilly, Novartis, Pfizer, Pierre Fabre and Sanofi. LS-B is a consultant for AbbVie, Janssen, Leo Pharma, Novartis and Sanofi.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1124018/full#supplementary-material

References

1. Steinhoff M, Ahmad F, Pandey A, Datsi A, AlHammadi A, Al-Khawaga S, et al. Neuroimmune communication regulating pruritus in atopic dermatitis. *J Allergy Clin Immunol* (2022) 149(6):1875–98. doi: 10.1016/j.jaci.2022.03.010

2. Datsi A, Steinhoff M, Ahmad F, Alam M, Buddenkotte J. Interleukin-31: The "itchy" cytokine in inflammation and therapy. *Allergy Eur J Allergy Clin Immunol* (2021) 76(10):2982–97. doi: 10.1111/all.14791

3. Nemmer JM, Kuchner M, Datsi A, Olah P, Julia V, Raap U, et al. Interleukin-31 signaling bridges the gap between immune cells, the nervous system and epithelial tissues. *Front Med* (2021) 8:639097. doi: 10.3389/fmed.2021.639097

4. Raap U, Wichmann K, Bruder M, Ständer S, Wedi B, Kapp A, et al. Correlation of IL-31 serum levels with severity of atopic dermatitis. *J Allergy Clin Immunol* (2008) 122 (2):421–3. doi: 10.1016/j.jaci.2008.05.047

5. Stott B, Lavender P, Lehmann S, Pennino D, Durham S, Schmidt-Weber CB. Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation. *J Allergy Clin Immunol* (2013) 132(2):446–54. doi: 10.1016/j.jaci.2013.03.050

6. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol* (2004) 5(7):752–60. doi: 10.1038/ni1084

7. McCandless EE, Rugg CA, Fici GJ, Messamore JE, Aleo MM, Gonzales AJ. Allergen-induced production of IL-31 by canine Th2 cells and identification of immune, skin, and neuronal target cells. *Vet Immunol Immunopathol* (2014) 157(1–2):42–8. doi: 10.1016/j.vetimm.2013.10.017

8. Datsi A, Raba K, Kellermann S, van Lierop A, Olah P, Sorg R, et al. 029 the IL-31 producing circulating T cells subset represents a unique population of CLA+ CRTH2+ CCR4+ effector memory T cells. *J Invest Dermatol* (2019) 139(9):S219. doi: 10.1016/j.jid.2019.07.032

9. Cevikbas F, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol* (2014) 133(2):448–60. doi: 10.1016/j.jaci.2013.10.048

10. Raap U, Gehring M, Kleiner S, Rüdrich U, Eiz-Vesper B, Haas H, et al. Human basophils are a source of - and are differentially activated by - IL-31. *Clin Exp Allergy* (2017) 47(4):499–508. doi: 10.1111/cea.12875

11. Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K, et al. Antimicrobial peptides human β-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J Immunol* (2010) 184(7):3526–34. doi: 10.4049/jimmunol.0900712

12. Alkon N, Bauer WM, Krausgruber T, Goh I. Single cell analysis reveals innate lymphoid cell lineage infidelity in atopic dermatitis. *J Allergy Clin Immunol* (2022) 149 (2):624–39. doi: 10.1016/j.jaci.2021.07.025

13. Hashimoto T, Yokozeki H, Kurasuyama H, Satoh T. IL-31-generating network in atopic dermatitis comprising macrophages, basophils, thymic stromal lymphopoietin, and periostin. *J Allergy Clin Immunol Am Acad Allergy Asthma Immunol* (2022) 18:S0091–6749(22)01567–6. doi: 10.1016/j.jaci.2022.11.009

14. Kunsleben N, Rüdrich U, Gehring M, Novak N, Kapp A, Raap U. IL-31 induces chemotaxis, calcium mobilization, release of reactive oxygen species, and CCL26 in eosinophils, which are capable to release IL-31. *J Invest Dermatol* (2015) 135(7):1908–11. doi: 10.1038/jid.2015.106

15. Kasraie S, Niebuhr M, Baumert K, Werfel T. Functional effects of interleukin 31 in human primary keratinocytes. *Allergy Eur J Allergy Clin Immunol* (2011) 66(7):845–52. doi: 10.1111/j.1398-9995.2011.02545.x

16. Nakashima C, Otsuka A, Kabashima K. Interleukin-31 and interleukin-31 receptor: New therapeutic targets for atopic dermatitis. *Exp Dermatol* (2018) 27 (1):327–31. doi: 10.1111/exd.13533

17. Möbus L, Rodriguez E, Harder I, Stölzl D, Boraczynski N, Gerdes S, et al. Atopic dermatitis displays stable and dynamic skin transcriptome signatures. *J Allergy Clin Immunol* (2021) 147(1):213–23. doi: 10.1016/j.jaci.2020.06.012

18. Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Lüscher-Firzlaff J, et al. IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. *J Allergy Clin Immunol* (2012) 129(2):426–33. doi: 10.1016/j.jaci.2011.10.042

19. Hänel KH, Pfaff CM, Cornelissen C, Amann PM, Marquardt Y, Czaja K, et al. Control of the physical and antimicrobial skin barrier by an IL-31–IL-1 signaling network. *J Immunol* (2016) 196(8):3233–44. doi: 10.4049/jimmunol.1402943

20. Dai X, Shiraishi K, Muto J, Utsunomiya R, Mori H, Murakami M, et al. Nuclear IL-33 plays an important role in IL-31–Mediated downregulation of FLG, keratin 1, and keratin 10 by regulating signal transducer and activator of transcription 3 activation in human keratinocytes. *J Invest Dermatol* (2022) 142(1):136–44. doi: 10.1016/j.jid.2021.05.033

21. Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* (2006) 117(2):411–7. doi: 10.1016/j.jaci.2005.10.033

22. Singh B, Jegga AG, Shanmukhappa KS, Edukulla R, Khurana GH, Medvedovic M, et al. IL-31-driven skin remodeling involves epidermal cell proliferation and thickening that lead to impaired skin-barrier function. *PloS One* (2016) 11(8): e0161877. doi: 10.1371/journal.pone.0161877

23. Bilsborough J, Leung DYM, Maurer M, Howell M, Boguniewcz M, Yao L, et al. IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. *J Allergy Clin Immunol* (2006) 117(2):418–25. doi: 10.1016/j.jaci.2005.10.046

24. Feld M, Garcia R, Buddenkotte J, Katayama S, Lewis K, Muirhead G, et al. The pruritus- and TH2-associated cytokine IL-31 promotes growth of sensory nerves. *J Allergy Clin Immunol* (2016) 138(2):500–8. doi: 10.1016/j.jaci.2016.02.020

25. Silverberg JI, Pinter A, Alavi A, Lynde C, Bouaziz JD, Wollenberg A, et al. Nemolizumab is associated with a rapid improvement in atopic dermatitis signs and symptoms: subpopulation (EASI ≥ 16) analysis of randomized phase 2B study. *J Eur Acad Dermatol Venereol* (2021) 35(7):1562–8. doi: 10.1111/jdv.17218

26. Serra-Baldrich E, Santamaria-Babi LF, Silvestre JF. Nemolizumab: un innovador tratamiento biológico para el control de la interleuquina 31 (IL-31) clave en la dermatitis atópica y el prurigo nodular. *Actas Dermosifiliogr* (2022) 113(7):674–84. doi: 10.1016/j.ad.2021.12.014

27. Silverberg JI, Pinter A, Pulka G, Poulin Y, Bouaziz JD, Wollenberg A, et al. Phase 2B randomized study of nemolizumab in adults with moderate to severe atopic dermatitis and severe pruritus. *J Allergy Clin Immunol* (2020) 145(1):173–82. doi: 10.1016/j.jaci.2019.08.013

28. Horcjs-Hoeck J, Schwarz H, Lamprecht S, Maier E, Hainzl S, Schmittner M, et al. Dendritic cells activated by IFN-γ/STAT1 expresa IL-31 receptor and release proinflammatory mediators upon IL-31 treatment. *J Immunol* (2012) 188(11):5319–26. doi: 10.4049/jimmunol.1101044

29. Kasraie S, Niebuhr M, Werfel T. Interleukin (IL)-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. *Allergy Eur J Allergy Clin Immunol* (2010) 65(6):712–21. doi: 10.1111/j.1398-9995.2009.02255.x

30. Cheung PFY, Wong CK, Ho AWY, Hu S, Chen DP, Lam CWK. Activation of human eosinophils and epidermal keratinocytes by Th2 cytokine IL-31: Implication for the immunopathogenesis of atopic dermatitis. *Int Immunol* (2010) 22(6):453–67. doi: 10.1093/intimm/dxq027

31. Wong CK, Leung KML, Qin HN, Chow JYS, Choi AOK, Lam CWK. Activation of eosinophils interacting with dermal fibroblasts by pruritogenic cytokine IL-31 and alarmin IL-33: Implications in atopic dermatitis. *PloS One* (2012) 7(1):e29815. doi: 10.1371/journal.pone.0029813

32. Mittermann I, Wilkberg G, Johansson C, Lupinek C, Lundeberg L, Crameri R, et al. IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis. *PloS One* (2016) 11(5):e0156077. doi: 10.1371/journal.pone.0156077

33. Svensson A, Bartosik J, Christiansen J, Roscher I, Nilsson I, Schmidtchen A, et al. Sensitization to skin associated microorganisms in adult patients with atopic dermatitis is of importance for disease severity. *Acta Derm Venereol* (2013) 93(3):340–5. doi: 10.2340/00015555-1465

34. Tamamoto-Mochizuki C, Olivry T. IL-31 and IL-31 receptor expression in acute experimental canine atopic dermatitis skin lesions. *Vet Dermatol* (2021) 32(6):631–e169. doi: 10.1111/vde.13034

35. Szegedi K, van Lier A, Res PC, Chielie S, Bos JD, Kezic S, et al. House dust mite allergens der f and der p induce IL-31 production by blood-derived T cells from atopic dermatitis patients. *Exp Dermatol* (2018) 27(4):393–5. doi: 10.1111/exd.13438

36. Ferran M, Galván AB, Rincón C, Romeu ER, Sacrista M, Barboza E, et al. Streptococcus induces circulating CLA+ memory T-cell-dependent epidermal cell activation in psoriasis. *J Invest Dermatol* (2013) 133(4):999–1007. doi: 10.1038/jid.2012.418

37. Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte associated antigen, *via* stimulation of interleukin 12 production. *J Exp Med* (1995) 181(2):747–53. doi: 10.1084/jem.181.2.747

38. Chan JR, Blumenschein W, Murphy E, Diveu C, Wiekowski M, Abbondanzo S, et al. IL-23 stimulates epidermal hyperplasia *via* TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med* (2006) 203 (12):2577–87. doi: 10.1084/jem.20060244

39. Leung DYM, Bhan AK, Schneeberger EE, Geha RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* (1983) 71(1 PART 1):47–56. doi: 10.1016/0091-6749(83)90546-8

40. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* (1990) 136(5):1053–68.

41. Szegedi K, Kremer AE, Kezic S, Teunissen MBM, Bos JD, Luiten RM, et al. Increased frequencies of IL-31-producing T cells are found in chronic atopic dermatitis skin. *Exp Dermatol* (2012) 21(6):431–6. doi: 10.1111/j.1600-0625.2012.01487.x

42. Sidbury R, Alpizar S, Laquer V, Dhawan S, Abramovits W, Loprete L, et al. Pharmacokinetics, safety, efficacy, and biomarker profiles during nemolizumab

treatment of atopic dermatitis in adolescents. *Dermatol Ther* (2022) 12(3):631–42. doi: 10.1007/s13555-021-00678-7

43. Homey B, Wang W, Soto H, Buchanan ME, Wiesenborn A, Catron D, et al. Cutting edge: The orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCRL0) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J Immunol* (2000) 164(7):3465–70. doi: 10.4049/jimmunol.164.7.3465

44. Homey B, Alenius H, Müller A, Soto H, Bowman EP, Yuan W, et al. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* (2002) 8 (2):157–65. doi: 10.1038/nm0202-157

45. Masuoka M, Shiraishi H, Ochiai S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. *Journal Clin Investig* (2012) 122(7):2590–600. doi: 10.1172/JCI58978

46. Guttman-Yassky E, Bissonnette R, Ungar B, Suárez-Fariñas M, Ardeleanu M, Esaki H, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. *J Allergy Clin Immunol* (2019) 143 (1):155–72. doi: 10.1016/j.jaci.2018.08.022

47. Hashimoto T, Mishra SK, Olivry T, Yosipovitch G. Periostin, an emerging player in itch sensation. *J Invest Dermatol* (2021) 141(10):2338–43. doi: 10.1016/j.jid.2021.03.009

48. Mitamura Y, Nunomura S, Nanri Y, Ogawa M, Yoshihara T, Masuoka M, et al. The IL-13/periostin/IL-24 pathway causes epidermal barrier dysfunction in allergic skin inflammation. *Allergy Eur J Allergy Clin Immunol* (2018) 73(9):1881–91. doi: 10.1111/all.13437

49. Olivry T, Mayhew D, Paps JS, Linder KE, Peredo C, Rajpal D, et al. Early activation of Th2/Th22 inflammatory and pruritogenic pathways in acute canine atopic dermatitis skin lesions. *J Invest Dermatol* (2016) 136(10):1961–9. doi: 10.1016/j.jid.2016.05.117

50. Zhiping L, Xiao S, Chen W, Re Z, Yang H, Steinhoff M, et al. IL-20 promotes cutaneous inflammation and peripheral itch sensation in atopic dermatitis. *FASEB J* (2022) 36(6):e22334. doi: 10.1096/fj.202101800R

51. Leung DYM. Role of IgE in atopic dermatitis. *Curr Opin Immunol* (1993) 5 (6):956–62. doi: 10.1016/0952-7915(93)90112-6

52. Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? *Pediatr Allergy Immunol* (2004) 15(1):86–8. doi: 10.1046/j.0905-6157.2003.00106.x

53. Chow S, Seow CS, Dizon MV, Godse K, Poong H, Chan V, et al. A clinician's reference guide for the management of atopic dermatitis in asians. *Asia Pac Allergy* (2018) 8(4):e41. doi: 10.5415/apallergy.2018.8.e41

54. Kliski V, Karlsson O, Remitz A, Reitamo S. High serum total IgE predicts poor long-term outcome in atopic dermatitis. *Acta Derm Venereol* (2015) 95(8):943–7. doi: 10.2340/00015555-2126

55. Leonard A, Wang J, Yu L, Liu H, Estrada Y, Greenlees L, et al. Atopic dermatitis endotypes based on allergen sensitization, reactivity to staphylococcus aureus antigens, and underlying systemic inflammation. *J Allergy Clin Immunol Pract* (2020) 8(1):236–247.e3. doi: 10.1016/j.jaip.2019.08.013

56. Unger N, Gomez-Casado C, van Hecop A, Pongratz G, Bleck D, Homey B. 027 memory b cells of atopic individuals preferentially express IL-31RA: A putative role of IL-31 in b cell biology. *J Invest Dermatol* (2022) 142(12):S185. doi: 10.1016/j.jid.2022.09.036

57. Ferran M, Santamaria-Babi LF. Pathological mechanisms of skin homing T cells in atopic dermatitis. *World Allergy Organ J* (2010) 3(3):44–7. doi: 10.1097/WOX.0b013e3181d675f8

58. Czarnowicki T, Santamaria-Babi LF, Guttman-Yassky E. Circulating CLA+ T cells in atopic dermatitis and their possible role as peripheral biomarkers. *J Allergy Clin Immunol* (2017) 72(3):366–72. doi: 10.1111/all.13080

59. DeJesús-Gil C, Sans-de San Nicolás L, García-Jiménez I, Ferran M, Celada A, Chiriac A, et al. The translational relevance of human circulating memory cutaneous lymphocyte-associated antigen positive T cells in inflammatory skin disorders. *Front Immunol* (2021) 12:652613. doi: 10.3389/fimmu.2021.652613

60. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet* (2020) 396 (10247):345–60. doi: 10.1016/S0140-6736(20)31286-1

61. Santamaria Babi LF. Atopic dermatitis Pathogenesis :Lessons from immunology. *Dermatol Pr Concept* (2022) 12(1):e2022152. doi: 10.5826/dpc.1201a152

62. Bakker DS, van der Wal MM, Heeb LEM, Giovannone B, Asamoah M, Delemarre EM, et al. Early and long-term effects of dupilumab treatment on circulating T-cell functions in patients with moderate-to-Severe atopic dermatitis. *J Invest Dermatol* (2021) 141(8):1943–53. doi: 10.1016/j.jid.2021.01.022

63. Roesner LM, Bridgewood C, McGonagle D, Wittmann M. Dupilumab: An opportunity to unravel *In vivo* actions of IL-4 and IL-13 in humans. *J Invest Dermatol* (2021) 141(8):1879–81. doi: 10.1016/j.jid.2021.02.007

64. Roesner LM, Farag AK, Pospich R, Traidl S, Werfel T. T Cell receptor sequencing specifies psoriasis as a systemic and atopic dermatitis as a skin-focused, allergen driven disease. *Allergy* (2022) 77(9):2737–47. doi: 10.1111/all.15272

65. Sans De San Nicolás L, Figueras Nart I, Bonfill Ortí M, De Jesús Gil C, García Jiménez I, Guilabert A, et al. SEB-induced IL-13 production in CLA+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis. *Allergy* (2022) 77 (11):3448–51. doi: 10.1111/all.15424

66. Skov L, Olsen JV, Giorno R, Schlievert PM, Baadsgaard O, Leung DYM. Application of staphylococcal enterotoxin b on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol* (2000) 105(4):820–6. doi: 10.1067/mai.2000.105524

67. Novak N, Valenta R, Bohle B, Laffer S, Haberstok J, Kraft S, et al. FcεRI engagement of langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. *J Allergy Clin Immunol* (2004) 113(5):949–57. doi: 10.1016/j.jaci.2004.02.005

68. Trier AM, Kim BS. Insights into atopic dermatitis pathogenesis lead to newly approved systemic therapies. *Br J Dermatol* (2022) ljac016. doi: 10.1093/bjd/ljac016

69. Simpson EL, Parnes JR, She D, Crouch S, Rees W, Mo M, et al. Tezepelumab, an anti-thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: A randomized phase 2a clinical trial. *J Am Acad Dermatol* (2019) 80(4):1013–21. doi: 10.1016/j.jaad.2018.11.059

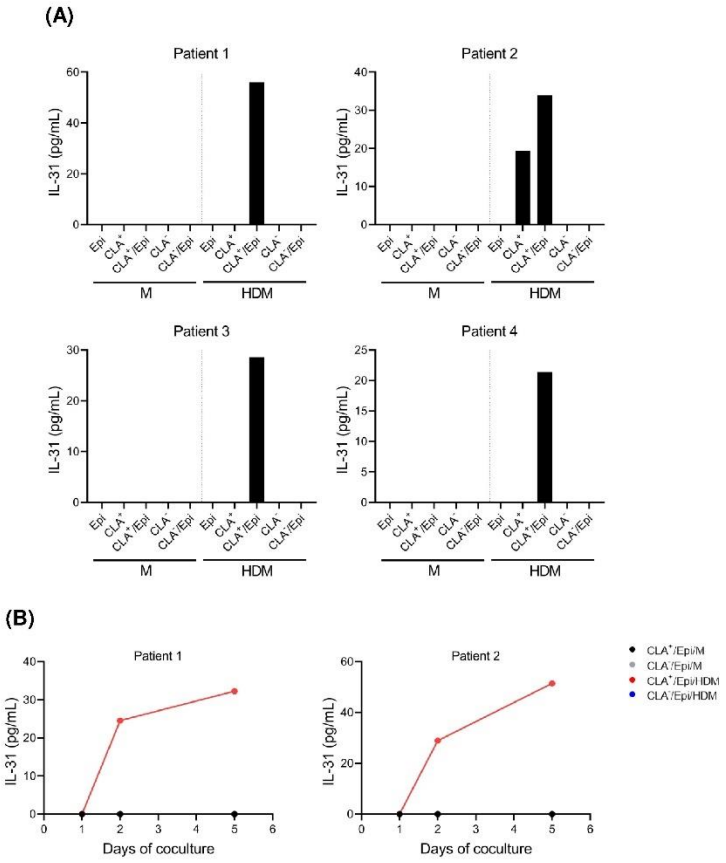


Figure S1. Epidermal cells suspension promotes CLA⁺ T-cell IL-31 response to HDM, which is produced in a time-dependent manner. (A) IL-31 cytokine levels (pg/mL) were measured in culture supernatants from epidermal cells suspension, CLA^+ or CLA^- T cells alone, or cocultures of CLA^+ and CLA^- T cells after 5 days in basal conditions (M) or stimulated with HDM. Data for four representative patients. **(B)** IL-31 cytokine levels (pg/mL) were measured in cocultures of CLA^+ and CLA^- T cells after one, two or five days in basal conditions (M) or stimulated with HDM. Data for two representative patients. CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated.

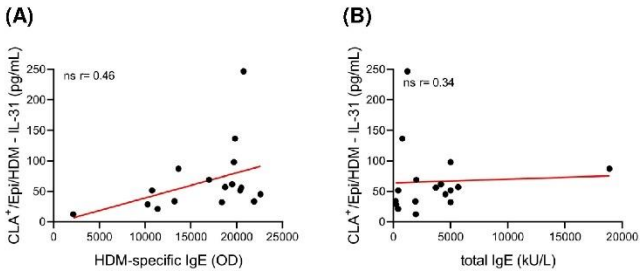
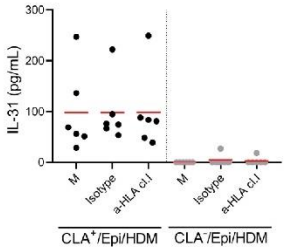


Figure S2. In group 1 of patients, there is no correlation between IL-31 *in vitro* response and plasma levels of HDM-specific and total IgE. Correlation of CLA^+/Epi/HDM IL-31 production with **(A)** HDM-specific IgE (OD) and **(B)** total IgE (kU/L) plasma levels in group 1 of patients (n=17). CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; OD, optical density. ns: $p > .05$.

(A)



(B)

	IL-31 (pg/mL)		
	Patient 1	Patient 2	Patient 3
CLA+/Epi/M	0.00	0.00	0.00
CLA+/Epi/HDM	247.03	45.26	33.68
CLA+/Epi/HDM isotype	185.32	28.60	44.60
CLA+/Epi/HDM a-IL-33	259.68	31.19	25.80

(C)

	IL-31 (pg/mL)		
	Patient 1	Patient 2	Patient 3
CLA+/Epi/HDM	128.62	136.46	33.68
CLA+/Epi/HDM isotype	106.58	88.62	45.64
CLA+/Epi/HDM a-CD1a	121.91	91.64	23.26

Figure S3. HDM-induced CLA+ T-cell IL-31 production is not affected by HLA class I molecule, IL-33 and CD1a neutralization. **(A)** HDM-activated $CLA^{+/-}$ T-cell cocultures were treated with HLA class I neutralizing antibody or control IgG isotype at day 0, and IL-31 production (pg/mL) at day 5 was compared with respect to isotype values (n=6). Red bar indicates the mean. $CLA^{+/-}$ T-cell cocultures were activated with HDM and treated with **(B)** IL-33 and **(C)** CD1a neutralizing antibodies or control IgG isotype at day 0 and IL-31 (pg/mL) was measured at day 5. Data for three representative patients. CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated.

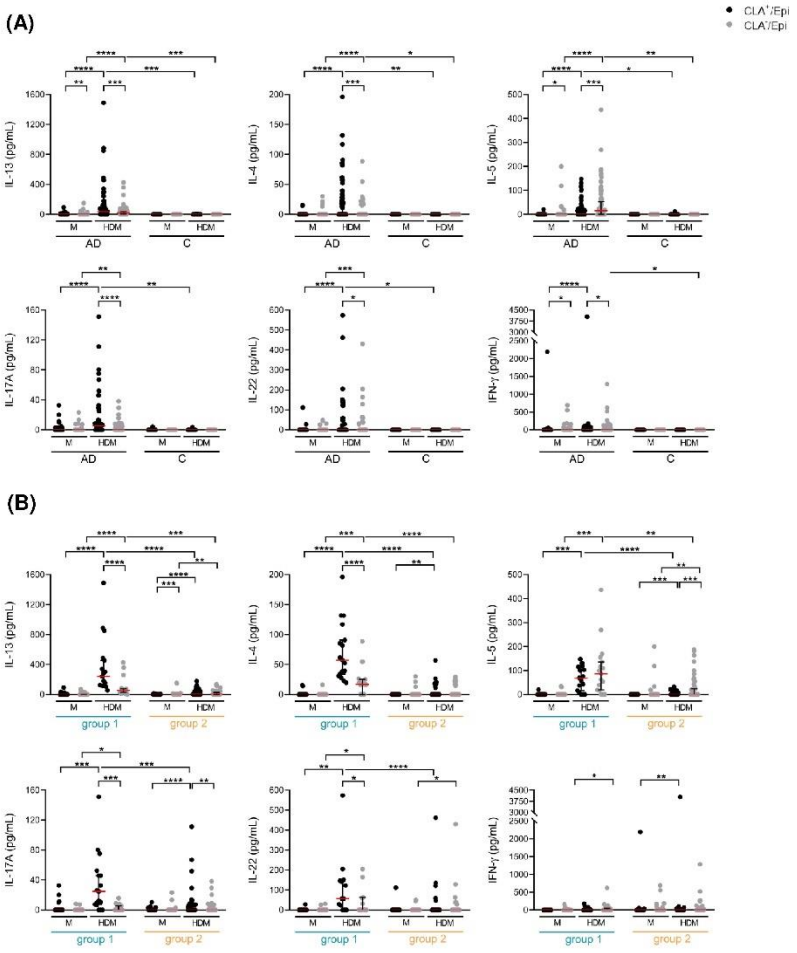


Figure S4. HDM induces IL-13, IL-4, IL-5, IL-17A and IL-22 production in AD-derived memory CLA⁺ T cells compared to controls, and it is increased in group 1 compared to group 2 of AD patients. (A) Quantification (pg/mL) of AD-derived mediators by $CLA^{+/-}$ T-cell cocultures in basal conditions (M) or stimulated with HDM in AD (IL-13/4/5/17A and IFN-γ n=58, IL-22 n=48) and control subjects (n=11). **(B)** Quantification (pg/mL) of mediators in $CLA^{+/-}$ T-cell cocultures in basal conditions (M) or stimulated with HDM was compared between group 1 (n=13-17) and group 2 (n=35-41) of patients. AD, atopic dermatitis; C, control subjects; CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated. *: $p < .05$; **: $p < .01$; ***: $p < .001$; ****: $p < .0001$.

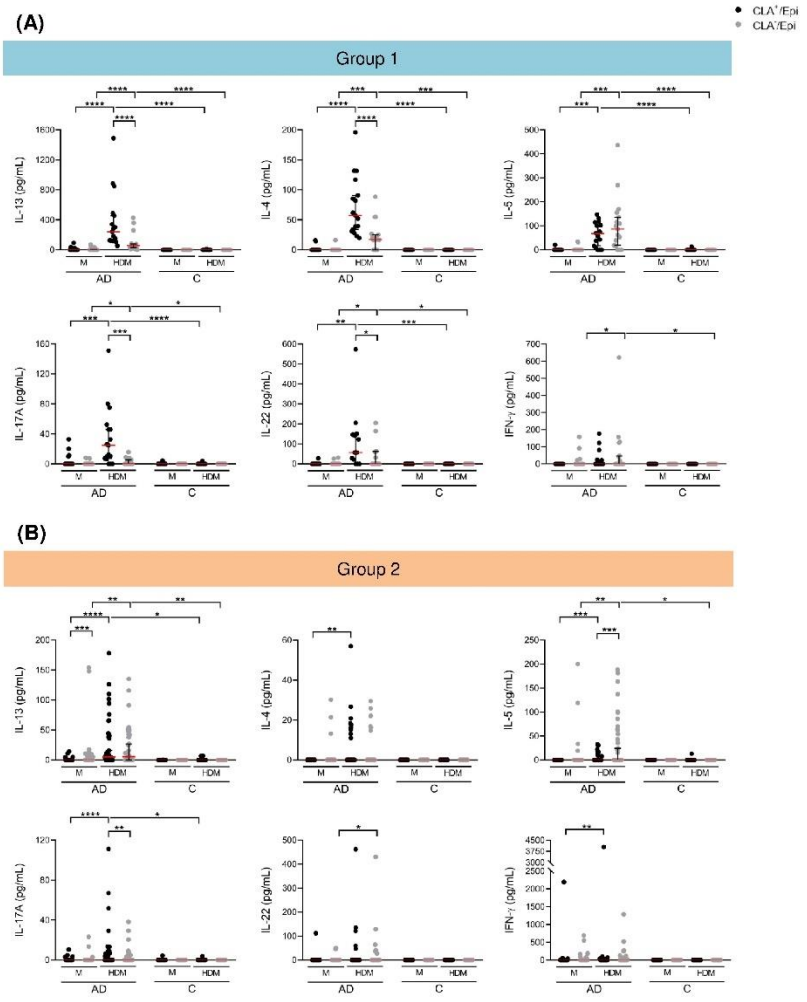


Figure S5. Production of AD-mediators in group 1 and group 2 AD cocultures compared to control subjects cocultures. Production (pg/mL) of AD-derived mediators by $CLA^{+/-}$ T-cell cocultures in basal conditions (M) or stimulated with HDM was compared between (**A**) group 1 (n=13-17) and control subjects (n=11), and (**B**) group 2 (n=35-41) and control subjects (n=11). AD, atopic dermatitis; C, control subjects; CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated. *: $p < .05$; **: $p < .01$; ***: $p < .001$; ****: $p < .0001$.

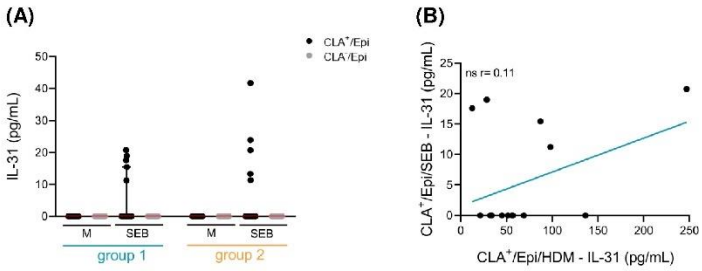
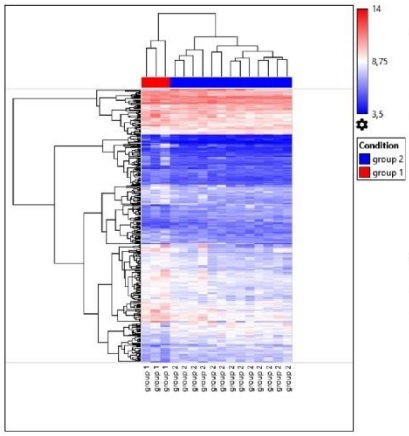


Figure S6. SEB-induced IL-31 production by memory T cells does not differ between group 1 and group 2 of patients. **(A)** IL-31 (pg/mL) produced by $CLA^{+/-}$ T-cell cocultures in basal conditions (M) or stimulated with SEB was compared between group 1 (n=16) and group 2 (n=36) of patients. **(B)** Correlation of CLA^{+}/Epi/HDM IL-31 production with CLA^{+}/Epi/SEB IL-31 production in group 1 of patients (n=16). CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated; SEB, staphylococcal enterotoxin B. ns: $p > .05$.

(A)



(B)

Term name	Term ID	adj.p
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

(C)

Term name	Term ID	adj.p
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 signaling	GO:0019935	0,038
multicellular organism development	GO:0007275	0,040

(D)

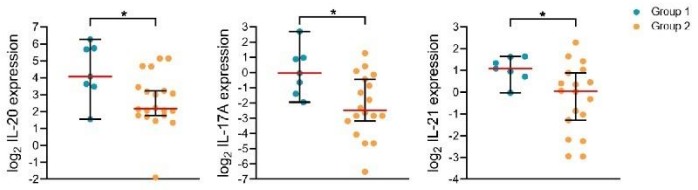


Figure S7. Group 1 lesional skin is enriched for immunological processes and group 2 lesional skin is enriched for developmental processes. **(A)** Hierarchical clustering of group 1 (n=3) and group 2 (n=13) samples based on DEG. Fold change based on linear data. Functional enrichment of all DEG **(B)** up-regulated in group 1 and **(C)** up-regulated in 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 (FDR < .05). **(D)** IL-17A, IL-20 and IL-21 mRNA expression in cutaneous lesions was compared between group 1 (n=7) and group 2 (n=17-20) of patients by qRT-PCR. GO, gene ontology. *: $p < .05$.

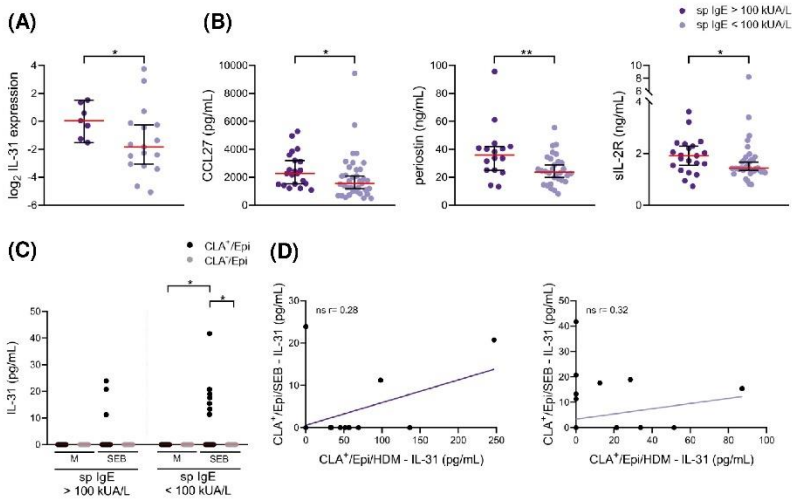


Figure S8. Patients with sp IgE > 100 kUA/L show increased IL-31 expression in lesional skin and plasma levels of CCL27, periostin and sIL-2R, but no difference in IL-31 response to SEB compared to patients with sp IgE < 100 kUA/L. **(A)** IL-31 mRNA expression (log_2) in cutaneous lesions (> 100 kUA/L n=7, < 100 kUA/L n=17), and **(B)** plasma CCL27 (pg/mL), periostin (ng/mL) and sIL-2R (ng/mL) (> 100 kUA/L n=16-21, < 100 kUA/L n=30-37) were compared between high and low sp IgE groups. **(C)** IL-31 (pg/mL) produced by $CLA^{+/-}$ T-cell cocultures in basal conditions (M) or stimulated with SEB was compared between high sp IgE (n=20) and low sp IgE (n=34) groups. **(D)** Correlation of CLA^+/Epi/HDM IL-31 production with CLA^+/Epi/SEB IL-31 production in the high sp IgE group (n=20) and low sp IgE group (n=34). CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated; SEB, staphylococcal enterotoxin B. ns: p >.05; *: p < .05; **: p < .01.

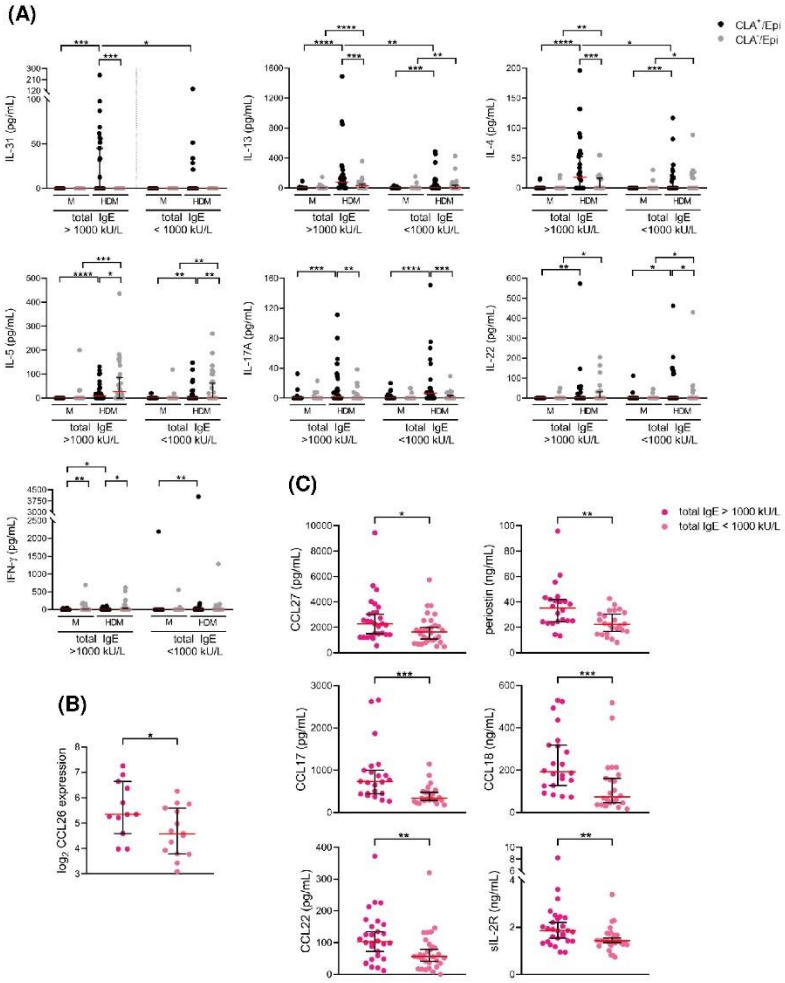


Figure S9. Patients with total IgE >1000 kU/L show increased HDM-induced CLA⁺ T-cell IL-31, IL-13 and IL-4 response, CCL26 expression in cutaneous lesions and plasma CCL27, periostin, CCL17, CCL18, CCL22 and sIL-2R compared to patients with lower total IgE levels. **(A)** Production (pg/mL) of mediators from AD culture supernatants separated according to total IgE levels (> 1000 kU/L n=21-28, < 1000 kU/L n=27-30). **(B)** CCL26 mRNA expression (log_2) in cutaneous lesions (> 1000 kU/L n=12, < 1000 kU/L n=15), and **(C)** plasma CCL27 (pg/mL), periostin (ng/mL), CCL17 (pg/mL), CCL18 (ng/mL), CCL22 (pg/mL) and sIL-2R (ng/mL) (> 1000 kU/L n=22-28, < 1000 kU/L n=23-30) were compared between high and low total IgE groups. CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HDM, house dust mite; M, untreated. *: $p < .05$; **: $p < .01$; ***: $p < .001$; ****: $p < .0001$.

Table S1. Patients' clinical data.

Clinical characteristics	Patients with AD	N
Age	35.00 (26.00-45.50)	57
Female gender, no (%)	30 (53.57)	56
Years since AD diagnosis	22.50 (12.50-30.50)	54
SCORAD	59.45 (41.63-65.04)	46
EASI	24.15 (17.38-32.00)	54
IGA	3.00 (3.00-4.00)	56
Pruritus (VAS)	7.00 (6.50-9.00)	57
Eosinophilia (x103/µL)	0.52 (0.24-0.78)	50
Total IgE (kU/L)	885.5 (236.7-3248)	58
Sp IgE response (OD)	11057 (2117-18474)	58
Sp IgE > 100 kUA/L, no (%)	21 (36.21)	58
LDH (U/L)	197.0 (125.0-237.0)	32
Atopic comorbidities, no (%)	40 (71.43)	56
Rhinitis	31 (56.36)	55
Asthma	22 (40.00)	55
Conjunctivitis	23 (41.82)	55
Food allergy	13 (24.53)	53

Categorical variables are presented as counts (percentages) and numerical variables are presented as medians (25th-75th percentiles). AD, atopic dermatitis; EASI, eczema area and severity index; IGA, investigator's global assessment; LDH, lactate dehydrogenase; OD, optical density; SCORAD, scoring atopic dermatitis; VAS, visual analogue scale.

Table S2. FAM-labelled Taqman probes for quantitative real-time PCR.

	Taqman probe
IL-13	Hs00174379_m1
IL-4	Hs00174122_m1
IL-31	Hs01098710_m1
IL-5	Hs01548712_g1
CCL17	Hs00171074_m1
CCL22	Hs01574247_m1
CCL26	Hs00171146_m1
TSLP	Hs00263639_m1
IL-22	Hs01574154_m1
IL-9	Hs00174125_m1
IL-17A	Hs00174383_m1
IL-17F	Hs00369400_m1
IL-20	Hs00218888_m1
IL-21	Hs00222327_m1
CXCL8	Hs00174103_m1
IFN-γ	Hs00174143_m1
CXCL10	Hs00171042_m1
TNF-α	Hs01113624_g1
IL-1α	Hs00174092_m1
LCN2	Hs01008571_m1
LOR	Hs01894962_s1
FLG	Hs00856927_g1
RPLP0	Hs99999902_m1

For tables S3, S4 and S5 please download files from https://www.frontiersin.org/articles/10.3389/fimmu.2023.1124018/full.

DIRECTOR'S REPORT

As supervisor of the present Doctoral Thesis, Dr. Luis F Santamaria Babí hereby state that the candidate Lídia Sans de San Nicolàs has actively participated in the peer-reviewed papers that conform this thesis. The candidate carried out the experiments, analysed the data and performed the graphs and tables, discussed the results, wrote the manuscripts and performed the required changes during revision. What follows below is a concise overview of the peer-reviewed papers included in the current Doctoral Thesis:

PAPER I

Citation: **Sans-De San Nicolàs L**, Figueras-Nart I, Bonfill-Ortí M, De Jesús-Gil C, García-Jiménez I, Guilabert A, Curto-Barredo L, Bertolín-Colilla M, Ferran M, Serra-Baldrich E, Zalewska-Janowska A, Wang YH, Howell MD, Pujol RM, Santamaria-Babí LF. SEB-induced IL-13 production in CLA^+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis. *Allergy*. 2022;77(11):3448-3451. Doi: 10.1111/all.15424

Impact Factor (2022): 14.71

Rank: Q1 Immunology

PAPER II

Citation: **Sans-de San Nicolàs L**, Figueras-Nart I, García-Jiménez I, Bonfill-Ortí M, Guilabert A, Curto-Barredo L, Bertolín-Colilla M, Ferran M, Serra-Baldrich E, Pujol RM and Santamaria-Babí LF. Allergen sensitization stratifies IL-31 production by memory T cells in atopic dermatitis patients. *Front Immunol*. 2023;14:1124018. Doi: 10.3389/fimmu.2023.1124018

Impact Factor (2022): 8.786

Rank: Q1 Immunology

SUMMARY OF RESULTS

The main purpose of this thesis has been to stablish a novel *ex vivo* model to study adult moderate-to-severe atopic dermatitis immunopathology and deepen in the effector function of skin-homing CLA^+ memory T cells to this pathogenesis. Fundamentally, this model is based on circulating CLA^+ T cells, which due to their memory phenotype and the ability to recirculate between skin and blood, allow obtaining translational readouts on the inflammatory mechanisms taking place in the skin during disease. The model also uses a suspension of autologous epidermal cells (Epi) obtained from lesional punch skin biopsies, which are a source of antigen presenting cells to process and/or present antigens or allergens for T-cell activation, thus resembling the *in situ* interaction between T cells and epidermal cells in the lesion. Then, the effector function from T cells was evaluated upon activation with SEB, since *S. aureus* is a relevant trigger in atopic dermatitis clinically associated with disease flare and severity, and HDM allergen, that is frequently related to disease exacerbation.

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

The effector T-cell response against SEB was characterized in our *ex vivo* model of coculture and we found that CLA^+/Epi cocultures from moderate-to-severe atopic dermatitis patients preferentially responded to SEB with production of Th2 cytokines IL-13 and IL-4, Th17 cytokine IL-17A, Th22 cytokine IL-22, and Th2 chemokines CCL17 and CCL22, over the CLA^-/Epi cocultures. There was a predominant IL-13 production among the Th2 cytokines (IL-4 and IL-5) by SEB-activated CLA^+/Epi cocultures, and no significant differences were observed in the amounts of IL-13 produced by atopic dermatitis-derived CLA^+/Epi cocultures compared to control-derived CLA^+/Epi cocultures. The SEB-specificity in the immune response produced by the CLA^+ T-cell subset was further supported by the fact that unspecific polyclonal stimulators of T cells, such as PMA/Ionomycin and CD3/CD28, induced a similar immune reaction in both CLA^+ and CLA^- T-cell subsets. Besides this, epidermal cells suspension promoted the T-cell response

to SEB, but this condition alone stimulated with SEB did not produce any of the measured cytokines, supporting the CLA-dependent cytokines production.

The heterogeneous SEB-induced IL-13 response *in vitro* by CLA^+/Epi atopic dermatitis cocultures was stratified according to the median: patients with IL-13 response over the median were arranged into Th2 high group, and patients with IL-13 response below the median were arranged into Th2 low group. Production of IL-13 and IL-4 was significantly increased in the SEB-activated CLA^+/Epi cocultures in the Th2 high group. In contrast, in the Th2 low group the CLA^+ T-cell immune response to SEB skewed towards Th17, Th22 and Th1, although not significant. When comparing the clinical characteristics between both groups no significant differences were found, suggesting that Th2 high and Th2 low groups were clinically homogeneous. Interestingly, in the Th2 high group, in contrast to the Th2 low group, CLA^+ T-cell-induced IL-13 response *in vitro* positively correlated with severity (EASI) and plasma levels of CCL17, sIL-2R, and *S. aureus*-specific IgE. Even though mRNA expression of several genes in the cutaneous lesions was similar in both groups, in the Th2 high group the SEB-induced CLA^+ T-cell IL-13 response positively correlated with CCL26 and inversely correlated with LCN2. Additionally, the IL-13/IL-17A and IL-13/IFN-γ ratios in the SEB-activated CLA^+/Epi cocultures were higher in the Th2 high than the Th2 low groups.

2. Allergen sensitization stratifies IL-31 production by memory T cells in atopic dermatitis patients

The study of the IL-31 role in adult moderate-to-severe atopic dermatitis was based on the CLA^+ T-cell response to HDM. Using the *ex vivo* model with HDM as a trigger, IL-31 was only reported in CLA^+/Epi cocultures from atopic dermatitis, compared to atopic dermatitis CLA^-/Epi cocultures and control CLA^+/Epi cocultures, and it was produced in a time-dependent manner. Because epidermal cells suspension promoted IL-31 production, their potential role as antigen presenting cells was assessed, and we found that HLA class II blockade

significantly reduced IL-31 production, whilst HLA class I blockade did not affect IL-31 production. Additionally, blocking of IL-33 and CD1a molecules did not affect IL-31 production, but cell-cell contact between CLA^+ T cells and epidermal cells was necessary for IL-31 production as demonstrated by transwell cultures.

There was a bimodal IL-31 response by HDM-induced CLA^+/Epi cocultures that allowed to define two subsets of patients according to the presence (Group 1) or absence (Group 2) of IL-31 response. In Group 1 of patients, CLA^+ T-cell-induced IL-31 response *in vitro* positively correlated with patient's pruritus intensity (Visual Analogue Scale, VAS), which was assessed the day before sample collection, and levels of CCL27 and periostin in plasma. Interestingly, a positive correlation was found between severity (SCORing Atopic Dermatitis, SCORAD) and plasma levels of CCL27 for Group 1 of patients, but not for Group 2 of patients. The simultaneous quantification of other mediators in the supernatants revealed that IL-13, IL-4, IL-5, IL-17A and IL-22 production by HDM-activated CLA^+/Epi cocultures was increased in Group 1 of patients, and positive correlations were found for IL-31 with IL-13 and IL-4 within this group. Furthermore, pathway enrichment analyses using differentially expressed genes from a gene-array analysis with Group 1 and Group 2 lesional skin revealed that Group 1 lesional skin was enriched for biological processes related to the immune system and the inflammatory response, while Group 2 of patients lacked a transcriptomic dysregulation of inflammatory genes. Of note, Group 1 of patients showed increased plasma levels of HDM-specific IgE and total IgE compared to Group 2 of patients, but Group 2 of patients still showed increased levels compared to control subjects (see Figure S1 in the APPENDIX I). Thus, Group 2 of patients were allergic to HDM (in a lower degree than Group 1 of patients) although no IL-31 response *in vitro* was observed in this group.

Next, focusing on the clinical differences between Group 1 and Group 2 of patients, patients were stratified according to levels of HDM-specific and total IgE. Those patients with HDM-specific IgE > 100 kUA/L showed higher HDM-

induced CLA^+ T-cell IL-31 response, as well as IL-31 mRNA expression in cutaneous lesions and plasma levels of CCL18, CCL22, CCL27, periostin and sIL-2R, than patients with HDM-specific IgE < 100 kUA/L. Finally, when stratifying patients based on the total IgE levels, we found that the IL-31 response by HDM-activated CLA^+/Epi cocultures, together with CCL26 mRNA expression in cutaneous lesions and plasma levels of CCL18, CCL22, CCL27, periostin and sIL-2R were increased in patients with total IgE > 1000 kU/L.

3. General summary

This translational approach based on the coculture of skin-homing CLA^+ memory T cells and autologous lesional epidermal cells from adult non-treated moderate-to-severe atopic dermatitis patients and its activation with clinically relevant triggers such as *S. aureus* and HDM, permits the description of differential effector profiles based on the clinical characteristics of patients. As peripheral cellular biomarkers, studying circulating CLA^+ memory T cells has provided functional comprehension of atopic dermatitis endotypes based on the SEB-CLA^+-IL-13 axis by identifying Th2 high and Th2 low responders from a clinically homogeneous population. Additionally, it allowed defining IL-31 producer and non-producer patients in relation with the degree of IgE sensitization to HDM by functionally analysing the CLA^+ T-cell immune response to HDM and its association with clinical features. To sum up, the CLA-dependent coculture model enables the functional stratification of adult moderate-to-severe atopic dermatitis patients into Th2 high / Th2 low responders and IL-31 producers / non-producers.

GENERAL DISCUSSION

Atopic dermatitis is an immune T-cell mediated skin disorder with relevant contributions of epidermal barrier dysfunction, cutaneous dysbiosis, environmental factors and pruritus. The Th2 immune axis dysregulation is considered central to the heterogeneous inflammatory response present in adult moderate-to-severe patients, demonstrated by biological therapies targeting this axis (Trier and Kim 2023). At the present time, the study of effector T-cell function in human atopic dermatitis is of descriptive nature and mainly based on polyclonal activation of peripheral blood mononuclear cells (Bakker et al. 2021; Czarnowicki et al. 2020) and skin transcriptome studies (He et al. 2020; Möbus et al. 2021). Although the usage of blood has the benefit of being easily accessible, activation of lymphocytes by unspecific non-physiologic polyclonal stimulators of the TCR does not grant the activation of specific lymphocytes that are relevant for the disease. On the other hand, skin transcriptome data is highly informative regarding general disease mechanisms, but it does not allow neither the detection nor the quantification of products specifically produced by effector T lymphocytes due to relevant disease triggers. Besides them, serum proteomic profiling allows for identification of T-cell mediators associated with patient's clinical status without providing information on the functionality of the cutaneous immune system during disease (Bakker et al. 2021; Thijs et al. 2017).

Given that circulating CLA^+ memory T cells represent a subset of memory T cells closely related to the cutaneous immune system due to their skin affinity, skin-blood recirculation capacity and specific response to skin-associated antigens, they constitute cellular peripheral biomarkers and provide translational knowledge on the mechanisms underlying T-cell-mediated cutaneous diseases such as atopic dermatitis (Sans-de San Nicolàs et al. 2023). In this regard, we stablished a novel *ex vivo* model of adult non-treated moderate-to-severe atopic dermatitis made of CLA^+ memory T cells isolated from peripheral blood cocultured with autologous lesional epidermal cells to resemble the interactions taking place in the lesions. Then, the coculture was activated with relevant disease

microbes (*S. aureus*) and allergens (house dust mite), which allowed to functionally stratify atopic dermatitis patients based on the specific CLA^+ T-cell responses.

S. aureus skin colonization is observed in most atopic dermatitis patients and it favours disease pathogenesis in many ways, including activation of the Th2 immune response and epidermal barrier disruption (Kim et al. 2019). Interestingly, a functional relation between SEB and CLA^+ memory T cells (but not systemic CLA^- memory T cells) has been reported in atopic dermatitis due to a preferential expression of SEB-reactive TCR Vβ regions in this T-cell subset (Davison et al. 2000; Strickland et al. 1999). Despite the relevant role of *S. aureus*, skin-homing CLA^+ memory T cells and IL-13 in atopic dermatitis, their relative interaction and relationship with the clinic was underexplored. In our model, atopic dermatitis-derived CLA^+/Epi cocultures response to SEB led to a predominant IL-13 response over Th2 cytokines, which goes in line with the dominance of IL-13 pathways in the lesion (Tsoi et al. 2019) and the central role of IL-13 in the inflammatory processes taking place in the skin facilitating *S. aureus* colonization and recruitment of CLA^+ $CD4^+$ T cells among others (Bieber 2020) (see Figure S2A in the APPENDIX I). Additionally, atopic dermatitis $CD4^+$ CLA^+ T cells present an epigenetic alteration that is translated into reduced methylation of the IL-13 promoter that correlates with increased IL-13 mRNA expression in this population (Acevedo et al. 2020). Considering that *S. aureus* is part of the normal cutaneous microbiome, it is not unusual that CLA^+ memory T cells from control subjects respond to SEB, which was reflected by similar IL-13 response between atopic dermatitis and control CLA^+/Epi cocultures, but it is of special interest in the context of atopic dermatitis due to the wide impact of IL-13 cytokine in cutaneous lesions.

The *ex vivo* model revealed the existence of Th2 high and Th2 low groups based on the SEB-CLA^+-IL-13 axis that were clinically homogeneous, since both groups belonged to the same moderate-to-severe population. However, deepen on the IL-

13 response reflected immunological, clinical, humoral and transcriptomic differences between both groups (see Figure S2B in the APPENDIX I). In the Th2 high group, correlation between IL-13 and severity, in terms of EASI, is supported by serum proteomic profiling where higher EASI score is found in the Th2-related clusters (Bakker et al. 2021), and increased EASI found in *S. aureus*-colonized patients (Simpson et al. 2018). Although correlation between severity, in terms of SCORAD, and IL-13 expression by CLA^+ T cells has previously been reported, it was based on unspecific stimulation of peripheral blood mononuclear cells and without patient stratification according to the immune signature (Czarnowicki et al. 2021; Czarnowicki et al. 2015b). An association between IL-13 response and levels of sIL-2R in plasma, which has been linked to a skin-homing chemokine cluster by serum proteomic profiling (Bakker et al. 2021) and is enhanced in *S. aureus*-colonized patients (Simpson et al. 2018), supports the relevance of addressing the *S. aureus*-induced IL-13 response by skin-homing CLA^+ T cells. Additionally, the association between IL-13 response and plasma levels of CCL17, which is the most reliable biomarker for atopic dermatitis (Renert-Yuval et al. 2021), may be consequence of the elevated CCL17 levels observed in *S. aureus*-colonized patients (Simpson et al. 2018) and the preferential expression of its receptor CCR4 on CLA^+ Th2 cells (Campbell et al. 1999; Imai et al. 1999). Therefore, a positive loop between *S. aureus* colonization, production of CCL17 by KCs and recruitment of $CCR4^+$ CLA^+ Th2 cells to the skin, which contribute to epidermal barrier dysfunction and facilitate *S. aureus* colonization, may promote disease exacerbation. Despite association between IL-13 and IgE levels against *S. aureus* has not previously been reported to our knowledge, increased Th2 markers and serum total IgE levels were reported in *S. aureus*-colonized patients (Simpson et al. 2018). We hypothesize that correlation between cellular (IL-13 response in SEB-induced CLA^+ T-cell subset) and humoral (specific IgE against *S. aureus*) response is stablished because of greater number of circulating $IL\text{-}13^+$ CLA^+ T cells responding to SEB in patients whose flare is associated with *S. aureus* colonization. In support of this, the content of IL-13 in the Th2 high

group correlated with the respective CCL26 mRNA expression in cutaneous lesions, which is a Th2 marker also enhanced in *S. aureus*-colonized patients (Simpson et al. 2018).

In atopic dermatitis, it is not clarified whether *S. aureus* colonization induces Th2 immune response, or an aberrant Th2 immune response facilitates *S. aureus* infection, or a bidirectional mechanistic function exists, but the interplay between *S. aureus* and Th2 immune response is clinically supported by biological therapies against IL-4Rα and IL-13 showing improvement in microbial diversity and decreased abundance of *S. aureus* (Beck et al. 2023; Callewaert et al. 2020). A high Th2/Th17 ratio may facilitate *S. aureus* colonization due to an imbalance of Th17-induced AMPs (Eyerich et al. 2009; Simpson et al. 2018) facilitated by IL-13-promoted decreased expression of AMPs (Howell et al. 2006; Nomura et al. 2003). Based on this, inverse correlation between IL-13 response and Th17-induced LCN2 mRNA expression in cutaneous lesions in the Th2 high group supported the Th2 signature and lowered Th17 immunity in this group, which was also underlined by the high SEB-induced CLA^+ Th2/Th17 ratio, that may perpetuate *S. aureus* infection. The relevance of these findings is further supported by the rapid reduction in *S. aureus* abundance together with increased CLA^+ Th17 cells in patients responding to the dupilumab (Simpson et al. 2023), as well as the reduced expression of IL-13 in CLA^+ $CD4^+$ T cells during dupilumab treatment (Bakker et al. 2021).

Our findings sustain, in a more functional approach based on the SEB-CLA^+-IL-13 axis in a clinically homogeneous adult moderate-to-severe population, the Th2 high and Th2 low endotypes suggested by serum proteomic profiling (Bakker et al. 2021; Thijs et al. 2017), and report 51.4% of patients as the Th2 high group, due to an enhanced production of IL-13 and IL-4, and 48.6% of patients as the Th2 low group with an immune response skewed towards IL-17A, IL-22 and IFN-γ. The identification of two clinically homogeneous groups based on the SEB-CLA^+-IL-13 axis provides valuable insights into the heterogeneous nature of the

disease and may explain the partial response to dupilumab treatment (Trier and Kim 2023) as well as it could be considered for categorizing patients for more personalized treatment strategies. Supporting this idea, a mathematical model has identified IL-13 as a potential predictive biomarker for patient stratification in dupilumab treatment (Miyano et al. 2022).

IL-31 is a clinically relevant neuroimmune cytokine with relevant contribution to atopic dermatitis pathogenesis involving inflammation, barrier dysfunction, tissue remodelling and pruritus (Nemmer et al. 2021) (see Figure S3A in the APPENDIX I). CLA^+ T cells are the main producers of IL-31 (Bilsborough et al. 2006) and they colocalize in the lesion (Cevikbas et al. 2014). Remarkably, allergen-specific and CLA^+ T cells share the same TCR repertoire as T cells infiltrating the lesion (Roesner et al. 2022), supporting their participation in local cutaneous inflammation. Sensitization to HDM, which is one of the most common allergen sources, is commonly observed in atopic dermatitis and it is associated with high levels of total IgE and disease exacerbation (Mittermann et al. 2016; Sonesson et al. 2013), as well as it induces the Th2 immune response including IL-31 production. The association between HDM and IL-31 expression has been poorly characterized in canine models (McCandless et al. 2014; Tamamoto-Mochizuki and Olivry 2021) and humans (Sonkoly et al. 2006; Szegedi et al. 2018). Additionally, the T-cell-derived IL-31 production in humans is basically explored through intracellular flow cytometry of peripheral blood mononuclear cells and quantitative real-time PCR, and correlations with patient's clinical features have not been addressed. In our *ex vivo* model, the degree of IgE sensitization to HDM prompted a bimodal (present or absent) IL-31 production by CLA^+/Epi cocultures in response to HDM that was not observed for IL-13 and IL-4. This finding highlights that even though both groups of patients (producers and non-producers of IL-31) were allergic, the degree of sensitization to HDM may be a significant influencer in stimulating selectively IL-31 producing CLA^+ Th2 cells. In this respect, the link between allergen sensitization, CLA^+ T-cell IL-31 response and

levels of CCL27 in plasma was clinically and mechanistically supported (see Figure S3B in the APPENDIX I), since CCL27 has been proposed as a potential biomarker of clinical response to anti-IL-31RA therapy (Sidbury et al. 2022) and it also exerts a chemoattract effect over CLA^+ T cells expressing the CCR10 receptor (Homey et al. 2002; Homey et al. 2000). These data, together with the correlation between CCL27 and SCORAD solely in patients producing IL-31 suggest that KCs in the lesion may upregulate CCL27 production to promote the recruitment of CLA^+ memory T cells to the inflamed skin where, upon activation with HDM, would produce IL-31 and exacerbate the disease.

Pruritus is largely non-histaminergic in nature in atopic dermatitis, being Th2 cytokines IL-4, IL-13 and IL-31 the major drivers. Provided that allergens induce the release of type 2 cytokines, either by activation of Th2 cells through allergen presentation by APCs or allergen recognition by IgE bound to the high-affinity IgE receptor (FcεRI) in mast cells and basophils, itch is conceived as a behavioural extension of type 2 inflammation (Garcovich et al. 2021). In line with the stablished literature demonstrating clinical relevance of targeting IL-31RA in pruritus improvement (Sidbury et al. 2022; Silverberg et al. 2021; Silverberg et al. 2020), the *in vitro* findings from our coculture system support the role of IL-31 as a mediator of pruritus with a strong correlation between IL-31 response to HDM by CLA^+ T cells and patient's pruritus (measured the day before sample collection). Hence, a link between the main inducer of type 2 inflammation (HDM allergen), the production of IL-31 by CLA^+ T cells, and pruritus as a behavioural extension of type 2 inflammation, has been reported for the first time, what suggests an association between allergen sensitization and patient's pruritus through a mechanism restricted to CLA^+ T cells. In this sense, an altered epidermal barrier that allows HDM penetration and activation of CLA^+ Th2 cells may induce itch that evokes scratching and perpetuates epidermal barrier disruption with entrance of allergens through the skin. In this respect, a recent study suggested that allergen exposure may predispose atopic dermatitis patients to acute itch

flares (Wang et al. 2021). Of note, no significant differences in pruritus intensity were detected between patients producing and non-producing IL-31 in our study, which may be consequence of the broad range of pruritogens, such as IL-4 and IL-13 (Steinhoff et al. 2022), that could promote itch in patients that do not produce IL-31 in response to HDM. Likewise, we discovered in our model a novel correlation between IL-31 response to HDM and plasma levels of periostin, which is produced by fibroblasts downstream of Th2 inflammatory pathways and has been associated with allergic inflammation and pruritus (Masouka et al. 2012; Nunomura et al. 2023). In canine models, epicutaneous application of HDM to sensitized dogs induces an upregulation of the Th2 signature, including IL-31 and periostin (Olivry et al. 2016). Apart from this, a novel network involving IL-31$^+$ macrophages, basophils, TSLP and periostin has been associated with itch in a murine atopic dermatitis model (Hashimoto et al. 2023), but the relevance of this work is reduced to basic non-translational knowledge due to the lack of efficacy of TSLP blockade in atopic dermatitis (Simpson et al. 2019). Interestingly, the levels of periostin in serum of patients with atopic dermatitis increase with severity, and they are higher in patients with high levels of total IgE (extrinsic patients) (Kou et al. 2014). Based on this, we suggest that assessing the presence of periostin in clinical studies targeting IL-31RA would provide further understanding on the possible link between IL-31, periostin and pruritus in humans. Furthermore, considering the levels of IgE specific for HDM may be valuable for patient stratification to IL-31 targeted therapies.

The presence of B cells in atopic dermatitis lesions is lower than that of T cells (Czarnowicki et al. 2017a), which goes in line with the prominent role of T cells in the disease pathogenesis, but B cells might contribute to the disease through the production of IgE to relevant disease antigens such as HDM. In addition, atopic dermatitis patients frequently show enhanced presence of IgE in serum, although clinical trials targeting IgE with the mAb omalizumab were discontinued due to lack of clinical efficacy (Trier and Kim 2023), pointing out that B cell may have

a secondary role in atopic dermatitis pathogenesis. Through our study, patients producing IL-31 in response to HDM showed increased levels of HDM-specific and total IgE and a prominent inflammatory signature in the lesion, and patients with no IL-31 response were also allergic with lowered levels of HDM-specific and total IgE. This data suggests that increased presence of B cells may be found in patients producing IL-31, and HDM processing and presentation by APCs bearing the FcεRI receptor may be favoured in this group. Interestingly, stratifying patients based on the HDM-specific IgE may provide an explanation on the partial IL-31 mRNA expression in skin from HDM-allergic patients in previous studies (Sonkoly et al. 2006). Additionally, a T-cell/B-cell cooperation may exist between HDM-specific IL-31 producing CLA^+ T cells and B cells, based on higher IL-31 response to HDM by CLA^+ T cells in patients with high levels of HDM-specific and total IgE as well as the increased expression of IL-31RA on memory B cells in patients with high levels of total IgE (Unger et al. 2023), that may result in raised IgE production and extended allergic inflammation in atopic dermatitis patients.

The heterogeneous IL-31 response to HDM could only be attributed to the CLA^+ T cells, since despite epidermal cells suspension contains a small number of immune cells, no response resulted from the activation of CLA^-/Epi cocultures or epidermal cells suspension alone. However, herein it is demonstrated that direct contact between T cells and epidermal cells is necessary for IL-31 response. One of the interactions between T cells and epidermal cells would be the HDM presentation through the HLA molecules. The IL-31 levels were decreased by 59% upon HLA-class II molecule neutralization, suggesting a preferential production of IL-31 by $CD4^+$ T cells. Remarkably, neutralization of HLA class I molecule, which interact with $CD8^+$ T cells, did not result in any reduction of cytokine production, indicating a putative role of other non-$CD4^+$/$CD8^+$ T cells in IL-31 production. Following these observations, we investigated whether HDM activated CLA^+ T cells via Langerhans cells expressing the non-classical major

histocompatibility complex family member CD1a, which are enriched in atopic dermatitis lesions (Jarrett et al. 2016), or via the innate cytokine IL-33, which is released by KCs under HDM stimulation (Dai et al. 2020), but no clear IL-31 downregulation was observed. Therefore, further studies are required to elucidate into other cellular components producing IL-31 in our model.

In summary, this novel *ex vivo* model has allowed to functionally stratifying patients from a homogeneous population of adult non-treated moderate-to-severe atopic dermatitis based on clinically relevant triggers, such as *S. aureus* and HDM, and the cutaneous immune system represented by CLA^+ T cells. Collectively, the insights gained with this translational model expand the understanding of the heterogeneous inflammatory component of the disease that may be of help for potentially enhancing the effectiveness of targeted therapies.

CONCLUSIONS

The stablished *ex vivo* CLA-dependent coculture model of adult non-treated moderate-to-severe atopic dermatitis provides a functional and translational tool for understanding the heterogeneous effector T-cell immune response. Additionally, it allows stratifying atopic dermatitis patients clinically and in response to therapies based on:

SEB-CLA$^+$-IL-13 axis

- The IL-13 produced by CLA$^+$ T cells in response to SEB functionally stratifies patients into two groups (Th2 high and Th2 low) that are clinically homogeneous.
- In the Th2 high group, the IL-13 response associates with disease severity (EASI), IgE sensitization to *S. aureus* and plasma levels of CCL17, which is the most reliable biomarker in atopic dermatitis.
- In the Th2 low group, the CLA$^+$ T-cell response to SEB skews towards Th17, Th22 and Th1.

HDM-CLA$^+$-IL-31 axis and pruritus

- The IL-31 response to HDM is restricted to CLA$^+$ T cells from atopic dermatitis patients.
- The degree of IgE sensitization to HDM associates with a bimodal (present or absent) IL-31 response.
- The IL-31 *in vitro* response strongly correlates with patient's pruritus and plasma levels of periostin and CCL27, which is a potential biomarker of clinical response to anti-IL-31RA therapy.

BIBLIOGRAPHY

Acevedo N, Benfeitas R, Katayama S, Bruhn S, Andersson A, Wikberg G, et al. Epigenetic alterations in skin homing CD4+CLA+ T cells of atopic dermatitis patients. Sci. Rep. 2020;10(1):18020

Agace WW. Tissue-tropic effector T cells: Generation and targeting opportunities. Nat. Rev. Immunol. 2006;6(9):682–92

Akdis M, Akdis CA, Weigl L, Disch R, Blaser K. Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA- memory T cells. J. Immunol. 1997;159(9):4611–9

Akdis M, Simon HU, Weigl L, Kreyden O, Blase K, Akdis CA. Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. J. Immunol. 1999;163(1):466–75

Antúnez C, Torres MJ, López S, Rodriguez-Pena R, Blanca M, Mayorga C, et al. Calcitonin gene-related peptide modulates interleukin-13 in circulating cutaneous lymphocyte-associated antigen-positive T cells in patients with atopic dermatitis. Br. J. Dermatol. 2009;161(3):547–53

Bakker DS, Nierkens S, Knol EF, Giovannone B, Delemarre EM, van der Schaft J, et al. Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. J. Allergy Clin. Immunol. 2021;147(1):189–98

Bakker DS, van der Wal MM, Heeb LEM, Giovannone B, Asamoah M, Delemarre EM, et al. Early and Long-Term Effects of Dupilumab Treatment on Circulating T-Cell Functions in Patients with Moderate-to-Severe Atopic Dermatitis. J. Invest. Dermatol. 2021;141(8):1943–53

Beck LA, Bieber T, Weidinger S, Tauber M, Saeki H, Irvine AD, et al. Tralokinumab treatment improves the skin microbiota by increasing the microbial diversity in adults with moderate-to-severe atopic dermatitis: Analysis of microbial diversity in ECZTRA 1, a randomized controlled trial. J. Am. Acad. Dermatol. 2023;88(4):816–23

Beck LA, Cork MJ, Amagai M, De Benedetto A, Kabashima K, Hamilton JD, et al. Type 2 Inflammation Contributes to Skin Barrier Dysfunction in Atopic Dermatitis. JID Innov. 2022;2(5):100131

Bieber T. Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. J. Allergy Clin. Immunol. 2020;75(1):54–62

Bieber T. Atopic dermatitis: an expanding therapeutic pipeline for a complex disease. Nat. Rev. Drug Discov. 2022;21(1):21–40

Biedermann T, Schwärzler C, Lametschwandtner G, Thoma G, Carballido-Perrig N, Kund J, et al. Targeting CLA/E-selectin interactions prevents CCR4-mediated recruitment of human Th2 memory cells to human skin in vivo. Eur. J. Immunol. 2002;32(11):3171–80

Bilsborough J, Leung DYM, Maurer M, Howell M, Boguniewcz M, Yao L, et al. IL-31 is associated with cutaneous lymphocyte antigen–positive skin homing T cells in patients with atopic dermatitis. J. Allergy Clin. Immunol. 2006;117(2):418–25

Bissonnette R, Rulloda J, Lee N, Kassner P, Jankicevic J, Ho W, et al. RPT193, an oral CCR4 inhibitor: Efficacy results from a randomized, placebo controlled Phase 1b monotherapy trial in patients with moderate to severe atopic dermatitis. Exp. Dermatol. 2021;30(S2):40–1

Breuer K, Wittmann M, Bösche B, Kapp A, Werfel T. Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB). Allergy. 2000;55(6):551–5

Brunner PM, Pavel AB, Khattri S, Leonard A, Malik K, Rose S, et al. Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J. Allergy Clin. Immunol. 2019;143(1):142–54

Callewaert C, Nakatsuji T, Knight R, Kosciolek T, Vrbanac A, Kotol P, et al. IL-4Rα Blockade by Dupilumab Decreases Staphylococcus aureus Colonization and Increases Microbial Diversity in Atopic Dermatitis. J. Invest. Dermatol. 2020;140(1):191-202

Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature. 1999;400(6746):776–80

Cevikbas F, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. J. Allergy Clin. Immunol. 2014;133(2):448–60

Cho SH, Strickland I, Boguniewicz M, Leung DYM. Fibronectin and fibrinogen contribute to the enhanced binding of Staphylococcus aureus to atopic skin. J. Allergy Clin. Immunol. 2001a;108(2):269–74

Cho SH, Strickland I, Tomkinson A, Fehringer AP, Gelfand EW, Leung DYM. Preferential binding of Staphylococcus aureus to skin sites of Th2-mediated inflammation in a murine model. J. Invest. Dermatol. Elsevier Masson SAS; 2001b;116(5):658–63

Chong BF, Murphy J-E, Kupper TS, Fuhlbrigge RC. E-Selectin, Thymus- and Activation-Regulated Chemokine/CCL17, and Intercellular Adhesion Molecule-1 Are Constitutively Coexpressed in Dermal Microvessels: A Foundation for a Cutaneous Immunosurveillance System. J. Immunol. 2004;172(3):1575–81

Chovatiya R, Paller AS. JAK inhibitors in the treatment of atopic dermatitis. J. Allergy Clin. Immunol. 2021;148(4):927–40

Clark RA, Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. Blood. 2007;109(1):194–202

Czarnowicki T, Esaki H, Gonzalez J, Malajian D, Shemer A, Noda S, et al. Early pediatric atopic dermatitis shows only a CLA+ Th2/Th1 imbalance, while adults acquire CLA+ Th22/Tc22 subsets. J. Allergy Clin. Immunol. 2015a;136(4):941–51

Czarnowicki T, Esaki H, Gonzalez J, Renert-Yuval Y, Brunner P, Oliva M, et al. Alterations in B-cell subsets in pediatric patients with early atopic dermatitis. J. Allergy Clin. Immunol. 2017a;140(1):134-44

Czarnowicki T, Gonzalez J, Shemer A, Malajian D, Xu H. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. J. Allergy Clin. Immunol. 2015b;136(1):104–15

Czarnowicki T, He H, Canter T, Han J, Lefferdink R, Erickson T, et al. Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood. J. Allergy Clin. Immunol. 2020;145(1):215–28

Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. J. Allergy Clin. Immunol. 2019;143(1):1–11

Czarnowicki T, Kim HJ, Villani AP, Glickman J, Duca E Del, Han J, et al. High-dimensional analysis defines multicytokine T-cell subsets and supports a role for IL-21 in atopic dermatitis. J. Allergy Clin. Immunol. 2021;76(10):3080–93

Czarnowicki T, Krueger JG, Guttman-Yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations with implications for the atopic march. J. Allergy Clin. Immunol. 2017b;139(6):1723–34

Czarnowicki T, Malajian D, Shemer A, Fuentes-Duculan J, Gonzalez J, Suárez-Fariñas M, et al. Skin-homing and systemic T-cell subsets show higher activation in atopic dermatitis versus psoriasis. J. Allergy Clin. Immunol. 2015c;136(1):208–11

Czarnowicki T, Santamaria-Babí LF, Guttman-Yassky E. Circulating CLA+ T cells in atopic dermatitis and their possible role as peripheral biomarkers. J. Allergy Clin. Immunol. 2017c;72(3):366–72

Dahabreh D, Yamamura K, Lozano-Ojalvo D, Del Duca E, Murai-Yamamura M, Garcet S, et al. 371 The OX40/OX40L axis plays a key role in treg dysfunction in atopic dermatitis. J. Invest. Dermatol. 2023;143(5):S64

Dai X, Tohyama M, Murakami M, Shiraishi K, Liu S, Mori H, et al. House dust mite allergens induce interleukin 33 (IL-33) synthesis and release from keratinocytes via ATP-mediated extracellular signaling. Biochim. Biophys. Acta - Mol. Basis Dis. 2020;1866(5):165719

David E, Ungar B, Renert-Yuval Y, Facheris P, del Duca E, Guttman-Yassky E. The evolving landscape of biologic therapies for atopic dermatitis: Present and future perspective. Clin. Exp. Allergy. 2023;53(2):156–72

Davison S, Allen M, Vaughan R, Barker J. Staphylococcal toxin-induced T cell proliferation in atopic eczema correlates with increased use of superantigen-reactive Vβ-chains in cutaneous lymphocyte-associated antigen (CLA)-positive lymphocytes. Clin. Exp. Immunol. 2000;121(2):181–6

Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat. Immunol. 2004;5(7):752–60

Elsner JS, Carlsson M, Stougaard JK, Nygaard U, Buchner M, Fölster-Holst R, et al. The OX40 axis is associated with both systemic and local involvement in atopic dermatitis. Acta Derm. Venereol. 2020;100(6):adv00099

Eyerich K, Brown SJ, Perez White BE, Tanaka RJ, Bissonette R, Dhar S, et al. Human and computational models of atopic dermatitis: A review and perspectives by an expert panel of the International Eczema Council. J. Allergy Clin. Immunol. 2019;143(1):36–45

Eyerich K, Pennino D, Scarponi C, Foerster S, Nasorri F, Behrendt H, et al. IL-17 in atopic eczema: Linking allergen-specific adaptive and microbial-triggered innate immune response. J. Allergy Clin. Immunol. 2009;123(1):59-66

Feld M, Garcia R, Buddenkotte J, Katayama S, Lewis K, Muirhead G, et al. The pruritus- and TH2-associated cytokine IL-31 promotes growth of sensory nerves. J. Allergy Clin. Immunol. 2016;138(2):500-8

Ferran M, Romeu ER, Rincón C, Sagristà M, Giménez AM, Celada A, et al. Circulating CLA+ T lymphocytes as peripheral cell biomarkers in T-cell-mediated skin diseases. Exp. Dermatol. 2013;22(7):439–42

Florian P, Flechsenhar KR, Bartnik E, Ding-Pfennigdorff D, Herrmann M, Bryce PJ, et al. Translational drug discovery and development with the use of tissue-relevant biomarkers: Towards more physiological relevance and better prediction of clinical efficacy. Exp. Dermatol. 2020;29(1):4–14

Fuhlbrigge RC, Kieffer JD, Armerding D. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. Nature. 1997;389(6654):978–81

Fuhlbrigge RC, King SL, Sackstein R, Kupper TS. CD43 is a ligand for E-selectin on CLA+ human T cells. Blood. 2006;107(4):1421–6

Garcovich S, Maurelli M, Gisondi P, Peris K, Yosipovitch G, Girolomoni G. Pruritus as a Distinctive Feature of Type 2 Inflammation. Vaccines (Basel). 2021;9(3):303

Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQF, et al. Progressive activation of TH2/TH22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J. Allergy Clin. Immunol. 2012;130(6):1344–54

Gottlieb AB, Krueger JG, Wittkowski K, Dedrick R, Walicke PA, Garovoy M. Psoriasis as a model for T-cell-mediated disease: immunobiologic and clinical effects of treatment with multiple doses of efalizumab, an anti-CD11a antibody. Arch. Dermatol. 2002;138(5):591–600

Guttman-Yassky E, Pavel AB, Zhou L, Estrada YD, Zhang N, Xu H, et al. GBR 830, an anti-OX40, improves skin gene signatures and clinical scores in patients with atopic dermatitis. J. Allergy Clin. Immunol. 2019;144(2):482-93

Guttman-Yassky E, Simpson EL, Reich K, Kabashima K, Igawa K, Suzuki T, et al. An anti-OX40 antibody to treat moderate-to-severe atopic dermatitis: a multicentre, double-blind, placebo-controlled phase 2b study. Lancet. 2023;401(10372):204–14

Hamilton JD, Suárez-Fariñas M, Dhingra N, Cardinale I, Li X, Kostic A, et al. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. J. Allergy Clin. Immunol. 2014;134(6):1293–300

Harper EG, Simpson EL, Takiguchi RH, Boyd MD, Kurtz SE, Bakke AC, et al. Efalizumab therapy for atopic dermatitis causes marked increases in circulating effector memory CD4+ T cells that express cutaneous lymphocyte antigen. J. Invest. Dermatol. 2008;128(5):1173–81

Hashimoto T, Yokozeki H, Karasuyama H, Satoh T. IL-31-generating network in atopic dermatitis comprising macrophages, basophils, thymic stromal lymphopoietin, and periostin. J. Allergy Clin. Imunol. 2023;151(3):737-46

Hassan AS, Kaelin U, Braathen LR, Yawalkar N. Clinical and immunopathologic findings during treatment of recalcitrant atopic eczema with efalizumab. J. Am. Acad. Dermatol. 2007;56(2):217–21

He H, Bissonnette R, Wu J, Diaz A, Saint-Cyr Proulx E, Maari C, et al. Tape strips detect distinct immune and barrier profiles in atopic dermatitis and psoriasis. J. Allergy Clin. Immunol. 2021;147(1):199–212

He H, Suryawanshi H, Morozov P, Gay-Mimbrera J, Del Duca E, Kim HJ, et al. Single-cell transcriptome analysis of human skin identifies novel fibroblast subpopulation and enrichment of immune subsets in atopic dermatitis. J. Allergy Clin. Immunol. 2020;145(6):1615–28

Hirahara K, Liu L, Clark RA, Yamanaka K, Fuhlbrigge RC, Kupper TS. The majority of human peripheral blood CD4+CD25highFoxp3+ regulatory T cells bear functional skin-homing receptors. J. Immunol.

2006;177(7):4488–94

Homey B, Alenius H, Müller A, Soto H, Bowman EP, Yuan W, et al. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat. Med. 2002;8(2):157–65

Homey B, Wang W, Soto H, Buchanan ME, Wiesenborn A, Catron D, et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). J. Immunol. 2000;164(7):3465–70

Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. Clin. Immunol. 2006;121(3):332–8

Hudak S, Hagen M, Liu Y, Catron D, Oldham E, McEvoy LM, et al. Immune Surveillance and Effector Functions of CCR10+ Skin Homing T Cells. J. Immunol. 2002;169(3):1189–96

Hunger RE, Yawalkar N, Braathen LR, Brand CU. The HECA-452 epitope is highly expressed on lymph cells derived from human skin. Br. J. Dermatol. 1999;141(3):565–9

Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, et al. Selective recruitment of CCR4-bearing T(h)2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int. Immunol. 1999;11(1):81–8

Irvine AD, McLean WHI, Leung DYM. Filaggrin Mutations Associated with Skin and Allergic Diseases. N. Engl. J. Med. 2011;365(14):1315–27

Jack C, Allakhverdi Z, Dupuis F. 185 High-dimensional analysis identifies variably persistent skin-homing Th2/Tc2 populations in AD patients under remission with dupilumab. J. Invest. Dermatol. 2023;143(5):S31

Jacquemin C, Martins C, Lucchese F, Thiolat D, Taieb A, Seneschal J, et al. NKG2D Defines a Subset of Skin Effector Memory CD8 T Cells with Proinflammatory Functions in Vitiligo. J. Invest. Dermatol. 2020;140(6):1143–53

Jarrett R, Salio M, Lloyd-Lavery A, Subramaniam S, Bourgeois E, Archer C, et al. Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite-derived phospholipase. Sci. Transl. Med. 2016;8(325):325ra18

De Jesús-Gil C, Sans-de San Nicolàs L, García-Jiménez I, Ferran M, Celada A, Chiriac A, et al. The Translational Relevance of Human Circulating Memory Cutaneous Lymphocyte-Associated Antigen Positive T Cells in Inflammatory Skin Disorders. Front. Immunol. 2021;12:652613

Johnson-Huang LM, Pensabene CA, Shah KR, Pierson KC, Kikuchi T, Lentini T, et al. Post-therapeutic relapse of psoriasis after CD11a blockade is associated with T cells and inflammatory myeloid DCs. PLoS One. 2012;7(2):e30308

Kim J, Kim BE, Ahn K, Leung DYM. Interactions between atopic dermatitis and staphylococcus aureus infection: Clinical implications. Allergy, Asthma Immunol. Res. 2019;11(5):593–603

Klicznik MM, Morawski PA, Höllbacher B, Varkhande SR, Motlet SJ, Kuri-Cervantes L, et al. Human CD4+ CD103+ cutaneous resident memory T cells are found in the circulation of healthy subjects. Sci. Immunol. 2019;4(37):eaav8995

Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012;22(5):850–9

Koppes SA, Brans R, Ljubojevic Hadzavdic S, Frings-Dresen MHW, Rustemeyer T, Kezic S. Stratum corneum tape stripping: Monitoring of inflammatory mediators in atopic dermatitis patients using topical therapy. Int. Arch. Allergy Immunol. 2016;170(3):187–93

Kou K, Okawa T, Yamaguchi Y, Ono J, Inoue Y, Kohno M, et al. Periostin levels correlate with disease severity and chronicity in patients with atopic dermatitis. Br. J. Dermatol. 2014;171(2):283–91

Laggner U, Meglio P Di, Perera GK, Hundhausen C, Lacy KE, Ali N, et al. Identification of a Novel Proinflammatory Human Skin-Homing Vg9Vd2 T Cell Subset with a Potential Role in Psoriasis. J. Immunol. 2011;187(5):2783–93

Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet. 2020;396(10247):345–60

Laughter MR, Maymone MBC, Mashayekhi S, Arents BWM, Karimkhani C, Langan SM, et al. The global burden of atopic dermatitis: lessons from the Global Burden of Disease Study 1990–2017. Br. J. Dermatol.

2021;184(2):304–9

Lee HJ, Kim M. Skin Barrier Function and the Microbiome. Int. J. Mol. Sci. 2022;23(21):13071

Leung DYM, Bhan AK, Schneeberger EE, Geha RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. J. Allergy Clin. Immunol. 1983;71(1 PART 1):47–56

Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial Superantigens Induce T Cell Expression of the Skin-selective Homing Receptor, the Cutaneous Lymphocyte-associated Antigen, via Stimulation of Interleukin 12 Production. J. Exp. Med. 1995;181(2):747–53

Mack MR, Kim BS. The Itch–Scratch Cycle: A Neuroimmune Perspective. Trends Immunol. 2018;39(12):980–91

Marin MJ, Estravís M, García-Sánchez A, Dávila I, Isidoro-García M, Sanz C. Genetics and Epigenetics of Atopic Dermatitis: An Updated Systematic Review. Genes (Basel). 2020;11(4):442

Masouka M, Shiraishi H, Ochta S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. J. Clin. Invest. 2012;122(7):2590–600

McCandless EE, Rugg CA, Fici GJ, Messamore JE, Aleo MM, Gonzales AJ. Allergen-induced production of IL-31 by canine Th2 cells and identification of immune, skin, and neuronal target cells. Vet. Immunol. Immunopathol. 2014;157(1–2):42–8

Mittermann I, Wikberg G, Johansson C, Lupinek C, Lundeberg L, Crameri R, et al. IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis. PLoS One. 2016;11(5):e0156077

Miyano T, Irvine AD, Tanaka RJ. A mathematical model to identify optimal combinations of drug targets for dupilumab poor responders in atopic dermatitis. J. Allergy Clin. Immunol. 2022;77(2):582–94

Möbus L, Rodriguez E, Harder I, Boraczynski N, Szymczak S, Hübenthal M, et al. Blood transcriptome profiling identifies 2 candidate endotypes of atopic dermatitis. J. Allergy Clin. Immunol. 2022;150(2):385–95

Möbus L, Rodriguez E, Harder I, Stölzl D, Boraczynski N, Gerdes S, et al. Atopic dermatitis displays stable and dynamic skin transcriptome signatures. J. Allergy Clin. Immunol. 2021;147(1):213–23

Nemmer JM, Kuchner M, Datsi A, Oláh P, Julia V, Raap U, et al. Interleukin-31 Signaling Bridges the Gap Between Immune Cells, the Nervous System and Epithelial Tissues. Front. Med. 2021;8:639097

Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, et al. Cytokine Milieu of Atopic Dermatitis, as Compared to Psoriasis, Skin Prevents Induction of Innate Immune Response Genes. J. Immunol. 2003;171(6):3262–9

Nunomura S, Uta D, Kitajima I, Nanri Y, Matsuda K, Ejiri N, et al. Periostin activates distinct modules of inflammation and itching downstream of the type 2 inflammation pathway. Cell Rep. 2023;42(1):111933

Oetjen LK, Mack MR, Feng J, Whelan T, H N, CJ G, et al. Sensory Neurons Co-opt Classical Immune Signaling Pathways to Meidate Chronic Itch. Cell. 2017;171(1):217–28

Olivry T, Mayhew D, Paps JS, Linder KE, Peredo C, Rajpal D, et al. Early Activation of Th2/Th22 Inflammatory and Pruritogenic Pathways in Acute Canine Atopic Dermatitis Skin Lesions. J. Invest. Dermatol. 2016;136(10):1961–9

Ong PY, Leung DYM. The infectious aspects of atopic dermatitis. Immunol. Allergy Clin. North Am. 2010;30(3):309–21

Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. Am. J. Pathol. 1990;136(5):1053–68

Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, Terstappen LW. Control of lymphocyte recirculation in man. II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. J. Immunol. 1993;150(3):1122–36

Renert-Yuval Y, Thyssen JP, Bissonnette R, Bieber T, Kabashima K, Hijnen DJ, et al. Biomarkers in atopic dermatitis—a review on behalf of the International Eczema Council. J. Allergy Clin. Immunol. 2021;147(4):1174–90

Robert C, Kupper TS. Inflammatory skin diseases, T cells, and immune surveillance. N. Engl. J. Med. 1999;341(24):1817–27

Roesner LM, Farag AK, Pospich R, Traidl S, Werfel T. T-cell receptor sequencing specifies psoriasis as a systemic and atopic dermatitis as a skin-focused, allergen-driven disease. Allergy. 2022;77(9):2737–47

Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-owsiak D, Wang X, et al. A role for IL-25 and IL-33 – driven type-2 innate lymphoid cells in atopic dermatitis. J. Exp. Med. 2013;210(13):2939–50

Sans-de San Nicolàs L, Czarnowicki T, Akdis M, Pujol RM, Lozano-Ojalvo D, Leung DYM, et al. CLA+ memory T cells in atopic dermatitis. Allergy. 2023

Santamaria Babí LF, Moser R, Perez Soler MT, Picker LJ, Blaser K, Hauser C. Migration of skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte-associated antigen (CLA), the very late antigen-4 (VLA-4), and the lymphocyte function-associated antigen-1 (LFA-1). J. Immunol. 1995a;154(4):1543–50

Santamaria Babí LF, Picker LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, et al. Circulating Allergen-reactive T Cells from Patients with Atopic Dermatitis and Allergic Contact Dermatitis Express the Skin-selective Homing Receptor, the Cutaneous Lymphocyte-associated Antigen. J. Exp. Med. 1995b;181(5):1935–40

Sidbury R, Alpizar S, Laquer V, Dhawan S, Abramovits W, Loprete L, et al. Pharmacokinetics, Safety, Efficacy, and Biomarker Profiles During Nemolizumab Treatment of Atopic Dermatitis in Adolescents. Dermatol. Ther. (Heidelb). 2022;12(3):631–42

Silverberg JI, Pinter A, Alavi A, Lynde C, Bouaziz JD, Wollenberg A, et al. Nemolizumab is associated with a rapid improvement in atopic dermatitis signs and symptoms: subpopulation (EASI ≥ 16) analysis of randomized phase 2B study. J. Eur. Acad. Dermatology Venereol. 2021;35(7):1562–8

Silverberg JI, Pinter A, Pulka G, Poulin Y, Bouaziz JD, Wollenberg A, et al. Phase 2B randomized study of nemolizumab in adults with moderate-to-severe atopic dermatitis and severe pruritus. J. Allergy Clin. Immunol. 2020;145(1):173–82

Simpson EL, Parnes JR, She D, Crouch S, Rees W, Mo M, et al. Tezepelumab, an anti–thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: A randomized phase 2a clinical trial. J. Am. Acad. Dermatol. 2019;80(4):1013–21

Simpson EL, Schlievert PM, Yoshida T, Lussier S, Boguniewicz M, Hata T, et al. Rapid Reduction in Staphylococcus aureus in Atopic Dermatitis Subjects Following Dupilumab Treatment. J. Allergy Clin. Immunol. 2023;S0091-6749(23)00754-6

Simpson EL, Villarreal M, Jepson B, Rafaels N, David G, Hanifin J, et al. Patients with Atopic Dermatitis Colonized with Staphylococcus aureus Have a Distinct Phenotype and Endotype. J. Invest. Dermatol. 2018;138(10):2224–33

Skov L, Olsen J V., Giorno R, Schlievert PM, Baadsgaard O, Leung DYM. Application of staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. J. Allergy Clin. Immunol. 2000;105(4):820–6

Sonesson A, Bartosik J, Christiansen J, Roscher I, Nilsson F, Schmidtchen A, et al. Sensitization to skin-associated microorganisms in adult patients with atopic dermatitis is of importance for disease severity. Acta Derm. Venereol. 2013;93(3):340–5

Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: A new link between T cells and pruritus in atopic skin inflammation. J. Allergy Clin. Immunol. 2006;117(2):411–7

Steinhoff M, Ahmad F, Pandey A, Datsi A, AlHammadi A, Al-Khawaga S, et al. Neuroimmune communication regulating pruritus in atopic dermatitis. J. Allergy Clin. Immunol. 2022;149(6):1875–98

Streilein JW. Skin-associated lymphoid tissues (SALT): origins and functions. J. Invest. Dermatol. 1983;80(1):S12–6

Strickland I, Hauk PJ, Trumble AE, Picker LJ, Leung DYM. Evidence for superantigen involvement in skin homing of T cells in atopic dermatitis. J. Invest. Dermatol. 1999;112(2):249–53

Strobl J, Gail LM, Kleissl L, Pandey RV, Smejkal V, Huber J, et al. Human resident memory T cells exit the skin and mediate systemic Th2-driven inflammation. J. Exp. Med. 2021;218(11):e20210417

Suárez-Fariñas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J. Allergy Clin. Immunol. 2011;127(4):954–64

Szegedi K, van Lier A, Res PC, Chielie S, Bos JD, Kezic S, et al. House dust mite allergens Der f and Der p induce IL-31 production by blood-derived T cells from atopic dermatitis patients. Exp. Dermatol. 2018;27(4):393–5

Takiguchi R, Tofte S, Simpson B, Harper E, Blauvelt A, Hanifin J, et al. Efalizumab for severe atopic dermatitis: A pilot study in adults. J. Am. Acad. Dermatol. 2007;56(2):222–7

Tamamoto-Mochizuki C, Olivry T. IL-31 and IL-31 receptor expression in acute experimental canine atopic dermatitis skin lesions. Vet. Dermatol. 2021;32(6):631-e169

Tatsuno K, Fujiyama T, Yamaguchi H, Waki M, Tokura Y. TSLP directly interacts with skin-homing Th2 cells highly expressing its receptor to enhance IL-4 production in atopic dermatitis. J. Invest. Dermatol. 2015;135(12):3017–24

Tauber M, Balica S, Hsu CY, Jean-Decoster C, Lauze C, Redoules D, et al. Staphylococcus aureus density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. J. Allergy Clin. Immunol. 2016;137(4):1272-1274

Teunissen MBM, Yeremenko NG, Baeten DLP, Chielie S, Spuls PI, Rie MA De, et al. The IL-17A-Producing CD8 þ T-Cell Population in Psoriatic Lesional Skin Comprises Mucosa-Associated Invariant T Cells and Conventional T Cells. J. Invest. Dermatol. 2014;134(12):2898–907

Thijs JL, Strickland I, Bruijnzeel-Koomen CAFM, Nierkens S, Giovannone B, Csomor E, et al. Moving toward endotypes in atopic dermatitis: Identification of patient clusters based on serum biomarker analysis. J. Allergy Clin. Immunol. 2017;140(3):730–7

Torres MJ, Gonzalez FJ, Corzo JL, Giron MD, Carvajal MJ, Garcia V, et al. Circulating CLA+ lymphocytes from children with atopic dermatitis contain an increased percentage of cells bearing staphylococcal-related T-cell receptor variable segments. Clin. Exp. Allergy. 1998;28(10):1264–72

Totté JEE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans SGMA. Prevalence and odds of Staphylococcus aureus carriage in atopic dermatitis: a systematic review and meta-analysis. Br. J. Dermatol. 2016;175(4):687–95

Trier AM, Kim BS. Insights into atopic dermatitis pathogenesis lead to newly approved systemic therapies. Br. J. Dermatol. 2023;188(6):698–708

Tsoi LC, Rodriguez E, Degenhardt F, Baurecht H, Wehkamp U, Volks N, et al. Atopic Dermatitis Is an IL-13–Dominant Disease with Greater Molecular Heterogeneity Compared to Psoriasis. J. Invest. Dermatol. 2019;139(7):1480–9

Unger Z, Gomez-Casado C, van Lierop A, Pongratz G, Bleck D, Homey B. 003 Memory B cells of atopic individuals preferentially express IL-31RA: a putative role of IL-31 in B cell biology. J. Invest. Dermatol. 2023;143(5):S1

Vugmeyster Y, Kikuchi T, Lowes MA, Chamian F, Kagen M, Gilleaudeau P, et al. Efalizumab (anti-CD11a)-induced increase in peripheral blood leukocytes in psoriasis patients is preferentially mediated by altered trafficking of memory CD8+ T cells into lesional skin. Clin. Immunol. 2004;113(1):38–46

Wang F, Trier AM, Li F, Kim S, Chen Z, Chai JN, et al. A basophil-neuronal axis promotes itch. Cell. 2021;184(2):422-40

Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. Nat. Rev. Dis. Prim. 2018;4(1):1

Weidinger S, Cork M, Reich A, Bieber T, Lucchesi D, Rynkiewicz N, et al. Amlitelimab reduces serum IL-13 in a phase 2a clinical trial in atopic dermatitis without impacting T-cell expansion in a T-cell recall assay. Br. J. Dermatol. 2023;188(S2):ljac140.045

Werfel T, Heratizadeh A, Niebuhr M, Kapp A, Roesner LM, Karch A, et al. Exacerbation of atopic dermatitis on grass pollen exposure in an environmental challenge chamber. J. Allergy Clin. Immunol. 2015;136(1):96–103

Werfel T, Layton G, Yeadon M, Whitlock L, Osterloh I, Jimenez P, et al. Efficacy and safety of the histamine H

4 receptor antagonist ZPL-3893787 in patients with atopic dermatitis. J. Allergy Clin. Immunol. 2019;143(5):1830–7

Wollenberg A, Blauvelt A, Guttman-Yassky E, Worm M, Lynde C, Lacour JP, et al. Tralokinumab for moderate-to-severe atopic dermatitis: results from two 52-week, randomized, double-blind, multicentre, placebo-controlled phase III trials (ECZTRA 1 and ECZTRA 2). Br. J. Dermatol. 2021;184(3):437–49

Yawalkar N, Hunger RE, Pichler WJ, Braathen LR, Brand CU. Human afferent lymph from normal skin contains an increased number of mainly memory/effector CD4+ T cells expressing activation, adhesion and co-stimulatory molecules. Eur. J. Immunol. 2000;30(2):491–7

Yoshino T, Okano M, Chen H, Tsuchiyama J, Kondo E. Cutaneous Lymphocyte Antigen Is Expressed on Memory/Effector B Cells in the Peripheral Blood and Monocytoid B Cells in the Lymphoid Tissues. Cell. Immunol. 1999;197(1):39–45

APPENDIX I: SUPPLEMENTARY FIGURES

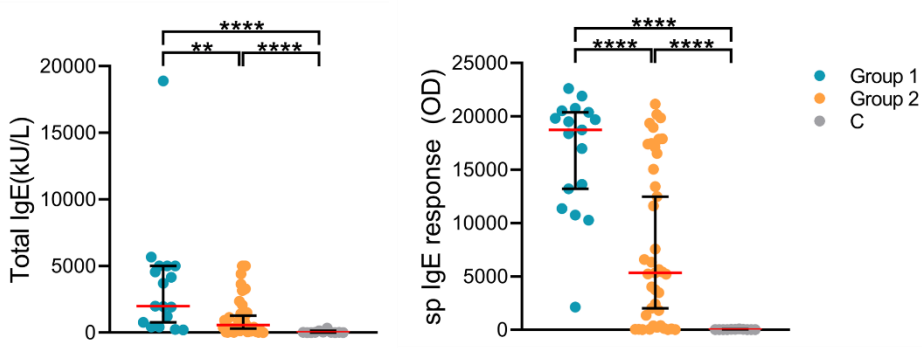


Figure S1. Group 1 (IL-31 producing) patients show increased total IgE and sp IgE levels compared to Group 2 (IL-31 non-producing) patients and controls, and Group 2 of patients still showed increased total IgE and sp IgE levels compared to controls. Plasma levels of total IgE and sp IgE were compared between Group 1 (n=17), Group 2 (n=41) and C (n=11) with Mann-Whitney test and represented with median ± 95% confidence interval. C, controls; OD, optical density; sp IgE, specific IgE to house dust mite. **p <.01; ****p <.0001.

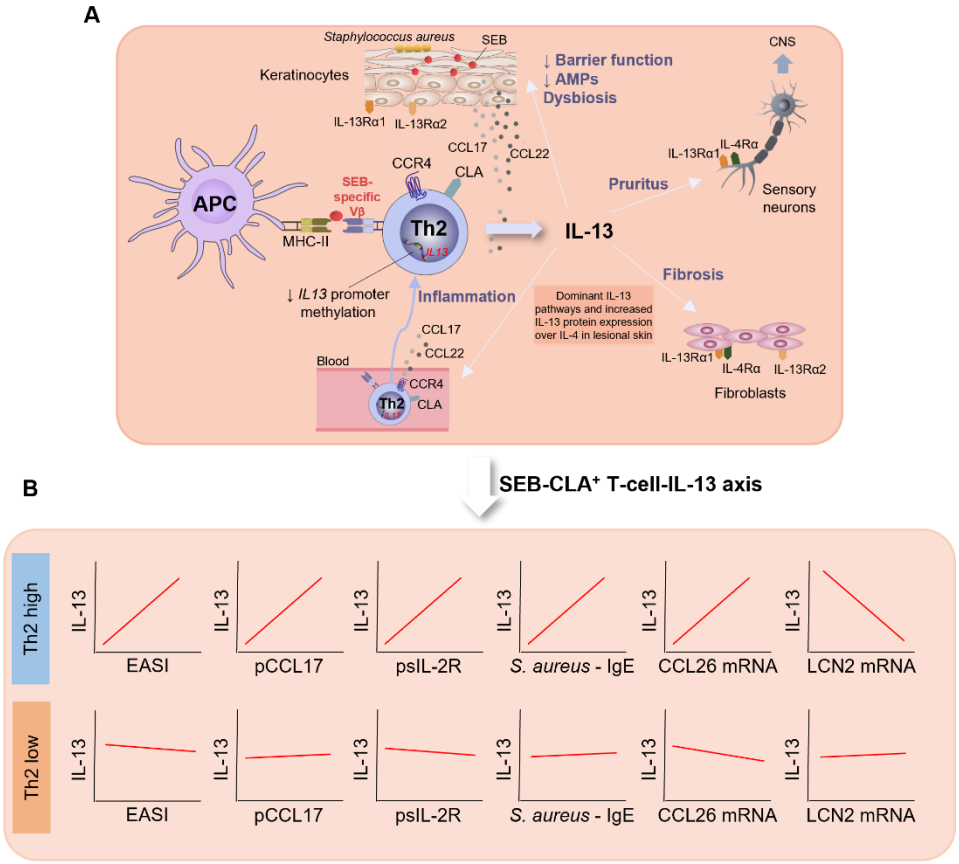


Figure S2. The SEB-CLA$^+$-IL-13 axis identifies Th2 high and Th2 low responders based on the IL-13 cytokine, which has a pivotal role in atopic dermatitis pathogenesis. (A) SEB-specific TCR Vβ regions are preferentially expressed by atopic dermatitis CLA$^+$ T cells that, upon activation, induce a predominant IL-13 response in the skin where abundant expression of IL-13Rα1 and IL-13Rα2 is found. Lesional skin presents dominant IL-13 pathways, with increased IL-13 expression over IL-4, that are involved in skin barrier dysfunction, dysbiosis, pruritus, inflammation, and fibrosis. Additionally, CLA$^+$ CD4$^+$ T cells from atopic dermatitis patients present an epigenetic alteration for IL-13. **(B)** In the Th2 high group, in contrast to the Th2 low group, the SEB-CLA$^+$ T-cell-IL-13 axis relates to patients' severity and plasma levels of IgE to *S. aureus* and sIL-2R. Only IL-13, but not other SEB-induced cytokines, correlates with levels of CCL17 in plasma, which is the most reliable biomarker for atopic dermatitis and it is a ligand for CCR4 that attracts circulating CLA$^+$ CCR4$^+$ Th2 cells to the skin. Additionally, IL-13 directly correlates with CCL26 and indirectly correlates with LCN2 mRNA expression in lesional skin. AMPs, antimicrobial peptides; APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; CNS, central nervous system; EASI, eczema area and severity index; MHC-II, major histocompatibility complex class II; *S. aureus, Staphylococcus aureus*; SEB, staphylococcal enterotoxin B. Adapted from Sans-de San Nicolàs et al. 2023.

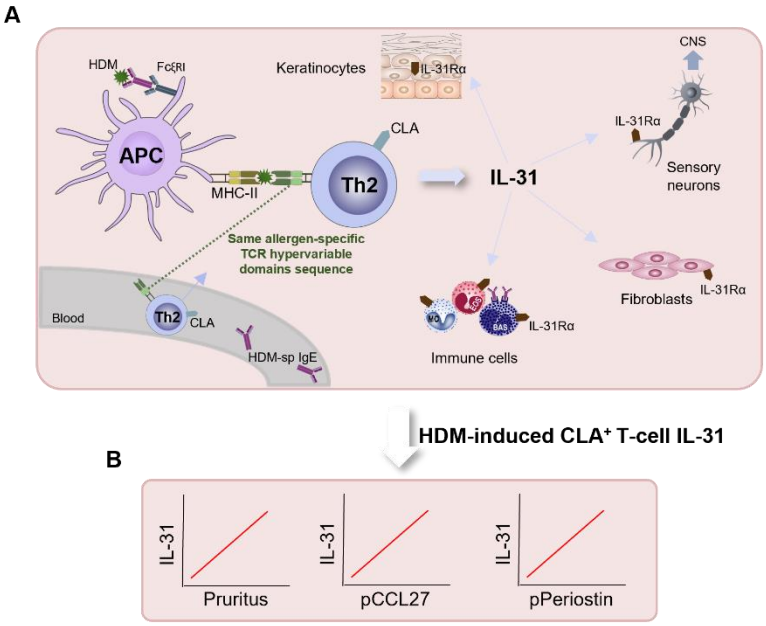


Figure S3. CLA$^+$ T cells produce IL-31 in response to HDM in relation to the degree of IgE sensitization to allergen, and it is involved in several aspects of atopic dermatitis pathogenesis. (A) Circulating CLA$^+$ T cells preferentially respond to HDM and share with infiltrating HDM-specific T cells the same TCRB CDR3 regions. CD4$^+$ CLA$^+$ T cells are the most abundant lymphocytic population in the lesions and major producers of IL-31, which is involved in inflammation, epidermal barrier dysfunction, pruritus, neuronal growth and fibrosis. **(B)** The IL-31 response *in vitro* by circulating CLA$^+$ T cells correlates with patient's pruritus intensity and levels of CCL27 and periostin in plasma. Interestingly, CCL27 in the stratum corneum is a biomarker of response to anti-IL-31Rα therapy, and it binds to CCR10 that is preferentially expressed on CLA$^+$ T cells to promote migration of circulating CLA$^+$ T cells to the skin. APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; CNS, central nervous system; HDM, house dust mite; HDM-sp IgE, IgE specific for house dust mite; MHC-II, major histocompatibility complex class II. Adapted from Sans-de San Nicolàs et al. 2023.

APPENDIX II: SUPPORTING PUBLICATIONS

I. The Translational Relevance of Human Circulating Memory Cutaneous Lymphocyte-Associated Antigen Positive T Cells in Inflammatory Skin Disorders

frontiers in Immunology

MINI REVIEW
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The Translational Relevance of Human Circulating Memory Cutaneous Lymphocyte-Associated Antigen Positive T Cells in Inflammatory Skin Disorders

Carmen de Jesús-Gil[†], *Lídia Sans-de SanNicolàs*[†], *Irene García-Jiménez*[1], *Marta Ferran*[2], *Antonio Celada*[3], *Anca Chiriac*[4], *Ramon M. Pujol*[2] *and Luis F. Santamaria-Babí*[1]*

[1] Translational Immunology, Department of Cellular Biology, Physiology, and Immunology, Faculty of Biology, Universitat de Barcelona, Parc Científic de Barcelona, Barcelona, Spain, [2] Department of Dermatology, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Universitat Autònoma de Barcelona, Barcelona, Spain, [3] Macrophage Biology, Department of Cellular Biology, Physiology, and Immunology, Faculty of Biology, Universitat de Barcelona, Barcelona, Spain, [4] Department of Dermatophysiology, Apollonia University, Iasi, Romania

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Edited by:
Daniel H. Kaplan,
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Reviewed by:
Julien Seneschal,
Université de Bordeaux, France
Koji Yasutomo,
Tokushima University, Japan

***Correspondence:**
Luis F. Santamaria-Babí
luis.santamaria@ub.edu

[†]These authors have contributed equally to this work and share first authorship

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Circulating memory T cells are heterogeneous in their tissue tropism. The skin-seeking T cell subset expresses the cutaneous lymphocyte-associated antigen (CLA) on their surface. CLA$^-$ memory T cells not only migrate from blood to skin but also recirculate between blood and skin. Studying CLA$^+$ memory T cells in cutaneous diseases has allowed a better understanding of immune-inflammatory mechanisms that take place. The analysis of the phenotypical features of these cells, their antigen specificity, cytokine production profile, and changes in relationship to clinical status and therapies among other characteristics have led to the concept that they constitute peripheral cellular biomarkers in T cell-mediated cutaneous conditions. CLA memory T cells are of relevance in the pathogenesis of several cutaneous diseases, such as psoriasis (PSO), atopic dermatitis, vitiligo, and drug-induced allergic reactions, to name a few. The interaction of circulating CLA$^-$ T cells with skin-resident cells has been investigated in different *ex vivo* coculture models made out of clinical samples. Interestingly, microbes that are present in the skin or related with human skin diseases are preferentially recognized by CLA$^+$ T cells. Thus, the interaction of *Streptococcus pyogenes* with CLA$^+$ T cells in PSO is providing novel concepts that help to understand disease immunopathogenesis. The goal of this review is to present latest results in the field of CLA$^+$ T cells in T cell-mediated inflammatory skin diseases and their translational relevance for human immunodermatology.

Keywords: skin-homing lymphocytes, cutaneous inflammation, CLA$^+$ T cell, human, translational, immunodermatology

INTRODUCTION

The existence of a cutaneous immune system in humans was postulated almost 50 years ago (1) and recently reformulated (2). In order to understand the adaptive immune response regionally, for those human memory T cells that belong to the cutaneous immunity, a skin-specific cell marker would be of great help. The cutaneous lymphocyte-associated antigen (CLA) constitutes a relevant marker that identifies the subset of memory T lymphocytes functionally related to skin physiology. CLA was discovered in 1990 by serendipity as a cell surface carbohydrate preferentially expressed by T cells present in cutaneous inflamed tissues, but no other organs (3). Since then, a comprehensive number of scientific evidence described in humans support the role of CLA as a relevant marker to identify skin-associated memory T cells involved in T cell-mediated cutaneous inflammation, that provide translational information in numerous different human skin diseases. CLA is more than a mere cell surface carbohydrate (3), preferentially expressed on $CD45RO^-$ T cells, which binds to endothelial E-selectin and mediates cell adhesion and transendothelial migration together with other molecules such as lymphocyte function-associated antigen-1 (LFA-1), very late activation antigen-4 (VLA-4), and C–C chemokine receptor type 10 (CCR10) (4). CLA^+ T lymphocytes are found in circulation and in inflamed and healthy skin but not infiltrating other non-cutaneous sites (3, 5). The fact that some CLA^- T cells are found in circulation deserves special attention due to the consequences of blocking LFA-1 in patients with atopic dermatitis (AD) and psoriasis (PSO) (6, 7). During treatment with efalizumab, patients present a circulating lymphocytosis of CLA^+ T cells. If the treatment is interrupted, a flare in disease occurs. One explanation to this effect is that CLA^+ T cells recirculate between blood and skin during cutaneous inflammation. Blocking their extravasation through LFA-1 leads to accumulation of those cells in blood, consequently when the blockade is released the cells enter abruptly into lesions and make flare. This mechanism of CLA^+ T cell recirculation has important implications for the translational relevance of studying circulating CLA^+ T cells in human inflammatory skin disorders (**Figure 1A**). CLA^+ memory T cells participate in pathological mechanisms of inflammatory disorders by recognizing key triggers of disease and producing cytokines that affect cells of the skin. The phenotype of those cells can reflect clinical status of the patients. The goal of this review is to update this information on circulating CLA^+ T cells in different human skin inflammatory diseases.

PSORIASIS

In PSO, CLA^+ T cells are contributing to understand the pathological mechanisms from a translational point of view using *ex vivo* studies with clinical samples. Although PSO is considered to be triggered by cathelicidin (LL-37) (8) and interferon (IFN)-alpha (9, 10), the translation of this mechanism into patients is complex since anti-IFN-alpha (11), anti-IFN-gamma (12), and anti-IL-22 do not induce clinical improvement, to name a few clinically invalidated mechanisms. *Streptococcus pyogenes* (*S. pyogenes*) infection is the best characterized clinically relevant trigger of PSO in patients (13). The molecular mechanism that links *S. pyogenes* and IL-17 response is starting to be clarified in PSO (14). Only CLA^+ memory T cells, but not CLA^-, preferentially respond to *S. pyogenes* in an autologous coculture of T cells and cutaneous epidermal cells from patients with PSO (15). Besides, patients with PSO who are negative for antistreptolysin O (ASO) antibody present increases levels of immunoglobulin (Ig) A, but not IgG, to *S. pyogenes*, which are directly associated with CLA^+ T-cell-dependent IL-17 response to *S. pyogenes in vitro* (16). These results suggest that increased exposure to *S. pyogenes*, as demonstrated by the presence of specific humoral immune response even in patients with ASO negative, upon recognition by CLA^+ T cells can fuel pathogenic IL-17 production. Remarkably, similar association has been recently described for *Candida albicans*, which is a potent IL-23/Th17 inducer too (17). Some other new mechanisms in PSO relating CLA^+ T cells and IL-17 have been recently revealed. The cytokine milieu present in psoriatic lesions can influence IL-17 response by CLA^+ T cells. IL-15 and IL-23, both present in psoriatic lesions, have been shown to synergize with CLA^+ T cells and autologous epidermal cells to produce IL-17A and IL-17F in PSO. This phenomenon occurs without the use of any exogenous stimulus, in a major histocompatibility complex (MHC)-dependent way, and independently of resident T cells (18), but it does not take place using CLA^- T cells or cells from healthy controls. This is an example of how psoriatic skin cytokine microenvironment interacts specifically with skin-related memory T cells generating the IL-17 response critical for PSO initiation and maintenance. IL-9 is another cytokine studied in the context of CLA^- T cells function in PSO in the *ex vivo* coculture model with autologous epidermal cells (19). *Streptococcus pyogenes* preferentially triggers IL-9 in CLA^+ T cells together with other mediators such IL-17A. In contrast to the previous studies where IL-9 is not induced by natural stimulus, IL-9 is produced in a time-dependent way and not transiently. Interestingly, the neutralization of *S. pyogenes*-induced IL-9 by CLA^+ T cells decreased IL-17A production by 50%.

Furthermore, the descriptive analysis of T cell subpopulations in patients with PSO revealed differential role for $CD4^-$ and $CD8^-$ T lymphocytes, whereas CLA expression is associated with skin recruitment of $CD4^+$ central memory T cells (T_{CM}), particularly those $CCR4^-$ and $CCR6^+$, suggesting a specific role for these cells in patrolling the skin compartment; $CD8^+$ T cells are more likely to accumulate in psoriatic skin and stay as resident memory T cells (T_{RM}) (20). Still, there is a need for psoriatic models, closely representing human disease, to be used as drug screening platforms. Recently, Shin et al. (21) developed psoriatic human 3D skin constructs (pHSCs) by incorporating T cells, over the classical approach based on the use of patient-derived keratinocytes or fibroblasts treated with PSO-related cytokines. As lymphocyte source, they tested *in vitro* polarized Th1/Th17 cells and $CCR6^+$ CLA^+ T cells from the patients with PSO, both of which showed psoriatic phenotype on epidermal cells along with disease-associated cytokine profile. Interestingly, when different psoriatic drugs were tested, those pHSCs with

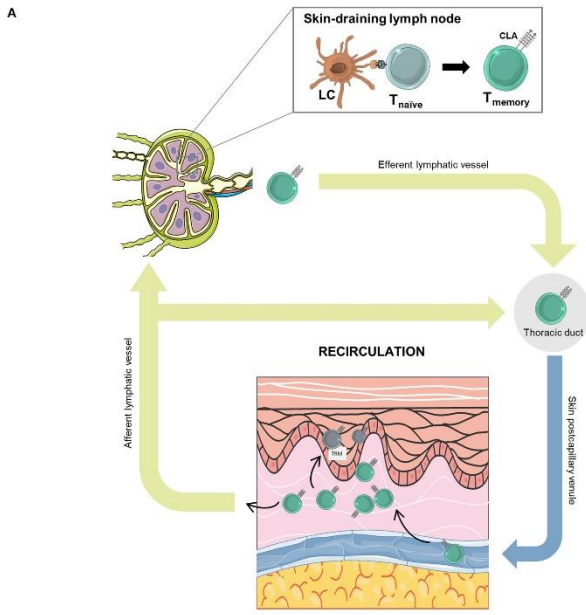

FIGURE 1 | Translational relevance of human circulating memory CLA+ T cells in inflammatory skin disorders. **(A)** In skin-draining lymph nodes, antigen-specific Langerhans cells encounter naive T cells generating memory T cells that express the skin-homing receptor CLA. CLA+ memory T cells enter the general circulation, through efferent lymphatic vessels, and will extravase to inflamed skin. Some CLA+ memory T cells leave cutaneous tissue through afferent lymphatic vessels,

(Continued)

FIGURE 1 | Recirculating back to the bloodstream, whereas, others are retained at the epidermal layer as resident memory T cells. **(B)** Principal antigen/allergen/autoantigen-induced effector functions of CLA+ memory T cells in human inflammatory cutaneous conditions. AA, alopecia areata; ACD, allergic contact dermatitis; AD, atopic dermatitis; CLA, cutaneous-associated lymphoid antigen; CLP, cutaneous lichen planus; DRESS, drug rash with eosinophilia and systemic symptoms; LC, Langerhans cells; OLP, oral lichen planus; PSO, psoriasis; T_{RM}, T-resident memory. This figure was created using images from Smart Servier (https://creativecommons.org/licenses/by/3.0/), WikiHow (https://creativecommons.org/licenses/by-nc-sa/3.0/) and Freepik.es (#bhrgf).

$CCR6^+$ CLA^+ T cells responded differently to the ones with *in vitro* polarized Th1/Th17 cells, highlighting the relevance of using patient-CLA^+ T cells to better address and even anticipate specific therapeutical responses *in vitro*, moving one step forward to personalized medicine in PSO field.

The CLA^+ lymphocytes have been extensively studied in the context of PSO disease, as last reviewed here (22). The importance of CLA^+ T cells in PSO resides not only in their contribution to lesion formation but also in their role as peripheral biomarkers of successful treatment, as it has been confirmed by recent studies. For example, a significant reduction of circulating CLA^+ T cell, as well as IL-6 and IL-22, has been associated with clinical efficacy after anti-tumor necrosis factor (TNF)-alpha treatment (23). Similarly, a reduction on the number of CLA^+ Th17/Tc17 and Th22/Tc22 cells was observed after 6 weeks of phototherapy and balneotherapy, which positively correlated with the reduction on PSO area and severity index (PASI) score (24).

ATOPIC DERMATITIS

The CLA^+ T cells are involved in initiation and perpetuation of AD (25), as they are functionally related to cutaneous inflammation (4, 26). Recent blood phenotyping studies on peripheral blood mononuclear cells from patients with AD and healthy controls (HC) have illustrated increased percentage of CLA^+ memory T cells in moderate-to-severe patients with AD compared with age-matched HC, but a decrease with increasing age only in patients with AD (27, 28). The expansion of CLA^+ T cells is accompanied by predominance of CLA^- Th2/Tc2 and Th22/Tc22 response in AD. Although CLA^+ Th2 cell counts are similarly increased across all ages and are significantly higher than HC, CLA^+ Th22 cell counts increase with age only in AD and its levels are also higher than that in HC (27–29). CLA^+ Th22 levels correlate with severity parameters [scoring atopic dermatitis (SCORAD) and eczema area and severity index], pruritus, and IL-17-producing cells (29, 30). CLA^+ IL-13^- T cells positively correlate with SCORAD, serum IgE levels, and IL-22 frequencies (27). Regarding Th1/Tc1 cell subsets, AD is characterized by decreased CLA^+ Th1/Tc1 frequencies in conjunction with negative correlations between CLA^+ IFN-gamma$^-$ T cells and SCORAD, and CLA^+ IL-13^+ and IL-22^+ populations. CLA^+ Th1 frequencies increase with age both in AD and HC, but do not reach the HC levels, and they are associated with disease duration. All these data support an imbalanced CLA^+ Th1:Th2 cell ratio that increases with age both in AD and HC, but remains decreased in AD (27–29).

The expression of the indicator of T cell mid activation, inducible costimulatory molecule (ICOS), has been reported to be enhanced both in CLA^- and CLA^- memory T cell subsets in patients with AD compared to HC and patients with PSO, predominantly in the skin-homing subset, and correlates with SCORAD. In contrast, the expression of human leukocyte antigen (HLA)-DR, the chronic T cell activation MHC class II antigen, is similar in infants regardless AD status, but increases with age reaching high levels in adult patients with AD vs. HC and patients with PSO, particularly among CLA^- T cell population, which correlates with SCORAD (28, 29, 31). Besides, frequencies of CLA^+ regulatory T cells (T_{regs}) are higher in AD compared with HC and patients with PSO, and they correlate with both ICOS and HLA-DR, and also with clinical parameters (SCORAD and IgE levels) (31). Intriguingly, OX40, another costimulatory molecule predominantly expressed on T cells and required for long-term memory responses, is mainly expressed by CLA^+ memory T cells in both patients with AD and HC (32). A recent epigenetic report has extended the knowledge on the CLA^+ T cells of male adult patients with severe AD and allergen-specific IgE sensitization (33). Peripheral blood mononuclear cells sorted into four different T cell populations ($CD8^+$, $CD4^+$, $CD4^+$ $CD45RA^+$, and $CD4^+$ CLA^+) have revealed differentially DNA methylations in 40 protein-coding genes in the $CD4^-$ CLA^+ subset in patients with AD vs. HC. Among them, IL-13 gene promoter shows decreased methylation levels, which negatively correlate with IL13 mRNA expression levels in this subset.

VITILIGO

Melanocyte-specific circulating memory $CD8^+$ CLA^- T cells induce melanocyte apoptosis, together with other mechanisms, such as cell detachment triggered by E-cadherin disruption (34), contribute to melanocyte loss and the development of depigmented skin lesions in vitiligo. Several blood endotyping studies have depicted that patients with vitiligo have low frequencies of circulating $CD4^+/CD8^-$ CLA^- T_{EM}/T_{CM} cells compared to patients with PSO, being similar to HC, supporting that CLA^+ T cells migrate to the skin (35, 36). Within T cell subsets, patients with vitiligo have the highest frequency of $CD4^-/CD8^+$ CLA^- T cells producing IFN-gamma compared with patients with AD, PSO, and alopecia areata (AA), and HC (35), supporting that vitiligo is caused by a type 1 T cell response (37, 38). Consistent with the literature describing $CD8^+$ T cell role in melanocytes death under IFN-gamma stimulation, $CD8^+$ CLA^+ T cell population producing IFN-gamma predominates over $CD4^+$ CLA^+ T cell subset in patients with vitiligo (35). Interestingly, the same study showed the highest frequency of $CD4^-/CD8^+$ CLA^+ T cells producing IL-9 in patients with vitiligo in comparison with patients with AD, PSO, and AA and HC, pointing out for the first time a possible role for IL-9 on the physiopathology of vitiligo. Furthermore, Th17 subset,

either CLA^+ or CLA^-, was also increased in vitiligo compared to patients with HC, AD and, surprisingly, with PSO, a disease associated to be driven by IL-17 activation. Regarding CLA^+ Th22 subset, it was also augmented in patients with vitiligo compared to patients with PSO and HC (35). In accordance to this fact, IL-22 has been reported to participate in the pathogenesis of vitiligo, as it promotes IL-1β secretion from keratinocytes what cause the suppression of melanogenesis and melanocyte migration as well as the induction of melanocyte apoptosis (39). The active participation of autorcactive $CD8^+$ T cells in vitiligo indicates that immune tolerance has been disrupted. Several studies have shown that patients with vitiligo have a reduced amount of infiltrating T_{regs} in non-lesional, perilesional, and lesional skin (40, 41). Patients with vitiligo also have fewer amounts of total circulating T_{regs} compared to patients with AD and PSO, the difference being not that obvious in the CLA^+ T_{regs} subset (35).

ALOPECIA AREATA

The CLA relevance in AA in humans was first revealed by Yano et al. (42), describing higher positivity in peripheral blood mononuclear cells, $CD4^-$ and $CD8^+$ lymphocytes from AA compared with HC, and that CLA positivity negatively correlated with clinical improvement. Later, phenotyping research on peripheral blood mononuclear cells described that $CLA^{+/-}$ Th2-cell frequencies are similar between patients with AA and AD, both higher than HC, and they correlate with AA severity; however, skin-homing Tc2 and Th22 are significantly higher in AA vs. HC (43). Also, the positive correlations have been reported not only between CLA^+ Th2/Tc2 and Th22/Tc22 but also between CLA^- Th17/Tc17 and Th22/Tc22, particularly in the $CD8^+$ subset. In addition to this, skin-homing $CD4^+/CD8^+$ T_{CM}-cell counts are higher in patients with AA, compared with HC and patients with AD, unlike $CD4^+/CD8^+$ T_{EM} frequencies, and $CD4^-$, but not $CD8^+$, subset shows HLA-DR activation in T_{CM} cells. Regarding T_{regs} cells, diminished frequencies of total and CLA^+ T_{regs} have been described in patients with AA compared to HC and patients with AD, which correlate with skin-homing Th9, Th2/Tc2, Tc17, and Tc22 counts.

OTHER INFLAMMATORY SKIN DISEASES

In lichen planus, there is an accumulation of CLA^- T cells in the epithelium of the buccal mucosa in oral lichen planus (OLP), and in the epidermis of skin biopsies in cutaneous lichen planus (CLP) (44). Increased E-selectin expression in lesional biopsies from OLP over perilesional tissue, together with a significantly higher proportion of $CD8^+$ CLA^+ T cells were observed by the immunohistochemical analysis. It has been shown cutaneous T cell-attracting chemokine (CTACK, also CCL27) secretion by oral keratinocytes, which increased in the presence of IFN-gamma and actively attracted CLA^- memory T cells to the oral epithelium. Although both normal oral mucosa and lesions from chronic OLP showed low levels of CTACK expression, it may still play a role in early recruitment of T cells and immunopathogenesis of OLP (45).

Although the role of CLA^+ T cells in non-immediate drug-induced cutaneous reactions was first reported two decades ago (46), there is still a lot to learn about them in drug allergy. Recently, it has been shown that in drug rash with cosinophilia and systemic symptoms (DRESS) (47), IL-4 and IL-13 producing $CD4^-$ T cells are increased during active disease and decline with recovery, pointing at the relevance of CLA^+ Th2 cells in the pathogenesis of DRESS. In β-lactam hypersensitivity, a new T cell subset has been proposed, in which blood- and skin-derived clones specific for piperacillin expressed high levels of skin-homing chemokine receptors and migrated in the presence of the ligands CCL27 (48).

DISCUSSION

The involvement of CLA^+ T cells in different inflammatory skin disorders, with diverse pathological immunological mechanisms, makes them interesting for human dermatology (**Figure 1B**). Also, circulating memory CLA^+ T cells specific for disease relevant antigens/allergens have reported, for example, *S. pyogenes* in PSO (22) or house dust mite and *Staphylococcus aureus* enterotoxin B in AD (4). Despite the functional relevance of memory CLA^+ T cells has been better described in PSO and AD (4, 22, 49); their potential role in other T cell-mediated skin conditions is still to be fully investigated. Various approaches that involve minimal manipulation of those cells are providing immunological information that relates to clinic in a translational way. Although animal models and complex *in vitro* models are providing important information to understand human inflammatory skin disorders, there is a need to use clinical human samples to gather genetic background and real diseased skin cells of patients (50). For example, animal models cannot reflect the immune response present in patients after several years of chronic cutaneous inflammation and numerous flares. Different aspects can influence the immune response in inflammatory skin diseases such as genetic background, disease endotype/patient heterogeneity, local antigen presentation by human epidermal cells, impact of environmental triggers of disease, or neurogenic inflammation. This integrated view is the approach that CLA^+ T cells exploration follows.

Nonetheless, the interplay between circulating and tissue-resident CLA^+ T cells should be further addressed. Particularly since the latter have been described to persist in resolved skin after treatment and to be involved in the recurrence of cutaneous lesions in PSO, vitiligo, or fixed drug eruptions (51).

We are clearly still at the top of the iceberg unraveling the information that those lymphocytes can provide for human inflammatory cutaneous conditions. For all these translational capacities, circulating memory CLA^+ T cells can be proposed as peripheral cellular biomarkers in human inflammatory skin disorders.

AUTHOR CONTRIBUTIONS

CJ-G and LS: equal contribution writing and experimental. LS-B: principal investigator, director, founding, and corresponding

author. RP: clinical, translational work, and founding. ACh: clinical and translational work. ACe: immunology expertise. ME: clinical and translational work. IG-J: contribution writing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Streilein JW. Lumphocyte traffic, T-cell malignancies and the skin. *J Invest Dermatol.* (1978) 71:167–71. doi: 10.1111/1523-1747.ep12547071
2. Egawa G, Kabashima K. Skin as a peripheral lymphoid organ: revisiting the concept of skin-associated lymphoid tissues. *J Invest Dermatol.* (2011) 131:2178–85. doi: 10.1038/jid.2011.198
3. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol.* (1990) 136:1053–68.
4. Czarnowicki T, Santamaria-Babí LF, Guttman-Yassky E. Circulating CLA+ T cells in atopic dermatitis and their possible role as peripheral biomarkers. *Allergy.* (2017) 72:366–72. doi: 10.1111/all.13080
5. Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K, Dowgiert RK, et al. The vast majority of CLA+ T cells are resident in normal skin. *J Immunol.* (2006) 176:4431–9. doi: 10.4049/jimmunol.176.7.4431
6. Gottlieb AB, Krueger JG, Wittkowski K, Dedrick R, Walicke PA, Garovoy M. Psoriasis as a model for T-cell-mediated disease: immunobiologic and clinical effects of treatment with multiple doses of efalizumab, an anti-CD11a antibody. *Arch Dermatol.* (2002) 138:591–600. doi: 10.1001/archderm.138.5.591
7. Harper EG, Simpson EL, Takiguchi RH, Boyd MD, Kurtz SE, Bakke AC, et al. Efalizumab therapy for atopic dermatitis causes marked increases in circulating effector memory CD4+ T cells that express cutaneous lymphocyte antigen. *J Invest Dermatol.* (2008) 128:1173–81. doi: 10.1038/sj.jid.5701169
8. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, et al. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nat Commun.* (2014) 5:5621. doi: 10.1038/ncomms6621
9. Funk J, Langeland T, Schrumpf E, Hanssen LE. Psoriasis induced by interferon-α. *Br J Dermatol.* (1991) 125:463–5. doi: 10.1111/j.1365-2133.1991.tb14774.x
10. Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, et al. Plasmacytoid predendritic cells initiate psoriasis through interferon-α production. *J Exp Med.* (2005) 202:135–43. doi: 10.1084/jem.20050500
11. Bissonnette R, Papp K, Maari C, Yao Y, Robbie G, White WI, et al. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. *J Am Acad Dermatol.* (2010) 62:427–36. doi: 10.1016/j.jaad.2009.03.042
12. Harden JL, Johnson-Huang LM, Chamian MF, Lee E, Pearce T, Leonardi CL, et al. Humanized anti-IFN-γ (HuZAF) in the treatment of psoriasis. *J Allergy Clin Immunol.* (2015) 135:553–6. doi: 10.1016/j.jaci.2014.05.046
13. Thorleifsdottir RH, Eysteinsdottir JH, Olafsson JH, Sigurdsson ML, Johnston A, Valdimarsson H, et al. Throat infections are associated with exacerbation in a substantial proportion of patients with chronic plaque psoriasis. *Acta Derm Venereol.* (2016) 96:788–91. doi: 10.2340/00015555-2408
14. de Jesús-Gil C, Ruiz-Romeu E, Ferran M, Chiriac A, Deza G, Höllo P, et al. CLA+ T cell response to microbes in psoriasis. *Front Immunol.* (2018) 9:1488. doi: 10.3389/fimmu.2018.01488
15. Ruiz-Romeu E, Ferran M, Sagristà M, Gómez J, Giménez-Arnau A, Herszenyi K, et al. *Streptococcus pyogenes*–induced cutaneous lymphocyte antigen–positive T cell–dependent epidermal cell activation triggers TH17 responses in patients with guttate psoriasis. *J Allergy Clin Immunol.* (2016) 138:191–9.e6. doi: 10.1016/j.jaci.2016.02.008
16. de Jesús-Gil C, Sans-de San Nicolás L, Ruiz-Romeu E, Ferran M, Soria-Martínez L, Chiriac A, et al. Specific IgA and CLA+ T-Cell IL-17 response to *Streptococcus pyogenes* in Psoriasis. *J Invest Dermatol.* (2020) 140:1364–70. doi: 10.1016/j.jid.2019.12.022
17. De Jesús-Gil C, Sans-de San Nicolás L, Ruiz-romeu E, Ferran M, Soria-martínez L, García-jiménez I, et al. Interplay between humoral and CLA+ T cell response against candida albicans in psoriasis. *Int J Mol Sci.* (2021) 22:1–14. doi: 10.3390/ijms22041519
18. de Jesús-Gil C, Ruiz-Romeu E, Ferran M, Sagristà M, Chiriac A, García P, et al. IL-15 and IL-23 synergize to trigger Th17 response by CLA+ T cells in psoriasis. *Exp Dermatol.* (2020) 29:630–8. doi: 10.1111/exd.14113
19. Ruiz-Romeu E, Ferran M, de Jesús-Gil C, García P, Sagristà M, Casanova JM, et al. Microbe-dependent induction of IL-9 by CLA+ T cells in psoriasis and relationship with IL-17A. *J Invest Dermatol.* (2018) 138:580–7. doi: 10.1016/j.jid.2017.08.048
20. Diani M, Galasso M, Cozzi C, Sgambelluri F, Altomare A, Cigni C, et al. Blood to skin recirculation of CD4+ memory T cells associates with cutaneous and systemic manifestations of psoriatic disease. *Clin Immunol.* (2017) 180:84–94. doi: 10.1016/j.clim.2017.04.001
21. Shin JU, Abaci HE, Herron L, Guo Z, Sallee B, Pappalardo A, et al. Recapitulating T cell infiltration in 3D psoriatic skin models for patient-specific drug testing. *Sci Rep.* (2020) 10:1–12. doi: 10.1038/s41598-020-60275-0
22. Ferran M, Romeu ER, Rincón C, Sagristà M, Giménez Arnau AM, Celada A, et al. Circulating CLA+ T lymphocytes as peripheral cell biomarkers in T-cell-mediated skin diseases. *Exp Dermatol.* (2013) 22:439–42. doi: 10.1111/exd.12154
23. Cordiali-Fei P, Bianchi L, Bonifati C, Trento E, Ruzzetti M, Francesconi F, et al. Immunologic biomarkers for clinical and therapeutic management of psoriasis. *Mediators Inflamm.* (2014) 2014:23606. doi: 10.1155/2014/236060
24. Eysteinsdóttir JH, Sigurgrímsdóttir H, Einarsdóttir HK, Freysdottir J, Agnarsson BA, Ólafsson JH, et al. Effective treatment with balneophototherapy and narrowband UVB monotherapy reduces skin homing Th17/Tc17 and Th22/Tc22 effector cells in peripheral blood in patients with psoriasis. *J Dermatol Sci.* (2019) 96:110–2. doi: 10.1016/j.jdermsci.2019.10.001
25. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nat Rev Dis Prim.* (2018) 4:1. doi: 10.1038/s41572-018-0001-z
26. Ferran M, Santamaria-Babí LF. Pathological mechanisms of skin homing T cells in atopic dermatitis. *World Allergy Organ J.* (2010) 3:44–7. doi: 10.1097/WOX.0b013e3181d675f8
27. Czarnowicki T, Gonzalez J, Shemer A, Malajian D, Xu H, Zheng X, et al. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. *J Allergy Clin Immunol.* (2015) 136:104–15.e7. doi: 10.1016/j.jaci.2015.01.020
28. Czarnowicki T, He H, Canter T, Han J, Lefferdink R, Erickson T, et al. Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood. *J Allergy Clin Immunol.* (2020) 145:215–28. doi: 10.1016/j.jaci.2019.09.031

29. Czarnowicki T, Esaki H, Gonzalez J, Malajian D, Shemer A, Noda S, et al. Early pediatric atopic dermatitis shows only a cutaneous lymphocyte antigen (CLA)+ TH2/TH1 cell imbalance, whereas adults acquire CLA+ TH22/TC22 cell subsets. *J Allergy Clin Immunol.* (2015) 136:941–51.e3. doi: 10.1016/j.jaci.2015.03.049
30. Farrera C, Melchiotti R, Petrov N, Weng Teng KW, Wong MT, Loh CY, et al. T-cell phenotyping uncovers systemic features of atopic dermatitis and psoriasis. *J Allergy Clin Immunol.* (2020) 145:1021–5.e15. doi: 10.1016/j.jaci.2019.11.030
31. Czarnowicki T, Malajian D, Shemer A, Fuentes-Duculan J, Gonzalez J, Suarez-Farinas M, et al. Skin-homing and systemic T-cell subsets show higher activation in atopic dermatitis versus psoriasis. *J Allergy Clin Immunol.* (2015) 136:208–11. doi: 10.1016/j.jaci.2015.03.032
32. Elsner JSH, Carlsson M, Stougaard JK, Nygaard U, Buchner M, Folster-Holst R, et al. The OX40 axis is associated with both systemic and local involvement in atopic dermatitis. *Acta Derm Venereol.* (2020) 100:1–5. doi: 10.2340/00015555-3452
33. Acevedo N, Benfeitas R, Katayama S, Bruhn S, Andersson A, Wikberg G, et al. Epigenetic alterations in skin homing CD4+ CLA− T cells of atopic dermatitis patients. *Sci Rep.* (2020) 10:1–18. doi: 10.1038/s41598-020-74798-z
34. Boukhedouni N, Martins C, Darrigade AS, Drullion C, Rambert J, Barrault C, et al. Type-1 cytokines regulate MMP-9 production and E-cadherin disruption to promote melanocyte loss in vitiligo. *JCI Insight.* (2020) 5:e133772. doi: 10.1172/jci.insight.133772
35. Czarnowicki T, He H, Leonard A, Kim HJ, Kameyama N, Pavel AB, et al. Blood endotyping distinguishes the profile of vitiligo from that of other inflammatory and autoimmune skin diseases. *J Allergy Clin Immunol.* (2019) 143:2095–107. doi: 10.1016/j.jaci.2018.11.031
36. Martins C, Darrigade AS, Jacquemin C, Barnetche T, Taieb A, Ezzedine K, et al. Phenotype and function of circulating memory T cells in human vitiligo. *Br J Dermatol.* (2020) 183:899–908. doi: 10.1111/bjd.18902
37. Van Den Boorn JG, Konijnenberg D, Dellemijn TAM, Van Der Veen JPW, Bos JD, Melief CJM, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J Invest Dermatol.* (2009) 129:2220–32. doi: 10.1038/jid.2009.32
38. Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, et al. Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of CD8+ cytotoxic T lymphocytes in vitiligo. *Acta Derm Venereol.* (2015) 95:664–70. doi: 10.2340/00015555-2080
39. Dong J, An X, Zhong H, Wang Y, Shang J. Interleukin-22 participates in the inflammatory process of vitiligo. *Oncotarget.* (2017) 8:109161–74. doi: 10.18632/oncotarget.22644
40. Zhang Q, Cui T, Chang Y, Zhang W, Li S, He Y, et al. HO-1 regulates the function of Treg: association with the immune intolerance in vitiligo. *J Cell Mol Med.* (2018) 22:4335–43. doi: 10.1111/jcmm.13723
41. Klarquist J, Denman CJ, Hernandez C, Wainwright DJ, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res.* (2010) 23:276–86. doi: 10.1111/j.1755-148X.2010.00688.x
42. Yano S, Nakamura K, Okochi H, Tamaki K. Analysis of the expression of cutaneous lymphocyte-associated antigen on the peripheral blood and cutaneous lymphocytes of alopecia areata patients. *Acta Derm Venereol.* (2002) 82:82–5. doi: 10.1080/000155502529 48077
43. Czarnowicki T, He HY, Wen HC, Hashim PW, Nia JK, Malik K, et al. Alopecia areata is characterized by expansion of circulating Th2/Tc2/Th22, within the skin-homing and systemic T-cell populations. *Allergy Eur J Allergy Clin Immunol.* (2018) 73:713–23. doi: 10.1111/all.13346
44. Walton LJ, Thornhill MH, Macey MG, Farthing PM. Cutaneous lymphocyte associated antigen (CLA) and αeβ7 integrins are expressed by mononuclear cells in skin and oral lichen planus. *J Oral Pathol Med.* (1997) 26:402–7. doi: 10.1111/j.1600-0714.1997.tb00239.x
45. Marshall A, Celentano A, Cirillo N, McCullough M, Porter S. Oral keratinocytes synthesize CTACK: a new insight into the pathophysiology of the oral mucosa. *Exp Dermatol.* (2018) 27:207–10. doi: 10.1111/exd.13471
46. Blanca M, Torres MJ, Leyva L, Mayorga C, Posadas S, Gonzalez L, et al. Expression of the skin-homing receptor in peripheral blood lymphocytes from subjects with nonimmediate cutaneous allergic drug reactions. *Allergy Eur J Allergy Clin Immunol.* (2000) 55:998–1004. doi: 10.1034/j.1398-9995.2000.00628.x
47. Teraki Y, Fukuda T. Skin-Homing IL-13-Producing T Cells Expand in the Circulation of Patients with Drug Rash with Eosinophilia and Systemic Symptoms. *Dermatology.* (2017) 233:242–9. doi: 10.1159/000475546
48. Sullivan A, Wang E, Farrell J, Whitaker P, Faulkner L, Peckham D, et al. β-Lactam hypersensitivity involves expansion of circulating and skin-resident Tc122 cells. *J Allergy Clin Immunol.* (2018) 141:235–49.e8. doi: 10.1016/j.jaci.2017.01.020
49. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet.* (2020) 396:345–60. doi: 10.1016/S0140-6736(20)31286-1
50. Florian P, Flechsenhar KR, Bartnik E, Ding-Pfennigdorff D, Herrmann M, Bryce PJ, et al. Translational drug discovery and development with the use of tissue-relevant biomarkers: towards more physiological relevance and better prediction of clinical efficacy. *Exp Dermatol.* (2020) 29:4–14. doi: 10.1111/exd.13942
51. Khalil S, Bardawil T, Kurban M, Abbas O. Tissue-resident memory T cells in the skin. *Inflamm Res.* (2020) 69:245–54. doi: 10.1007/s00011-020-01320-6

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II. CLA$^+$ memory T cells in atopic dermatitis

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REVIEW

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CLA$^+$ memory T cells in atopic dermatitis

Lídia Sans-de San Nicolàs[1] | Tali Czarnowicki[2] | Mübeccel Akdis[3] | Ramon M. Pujol[4] | Daniel Lozano-Ojalvo[5] | Donald Y. M. Leung[6] | Emma Guttman-Yassky[5] | 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]Shaare Zedek Medical Center, The Hebrew University of Jerusalem, Jerusalem, Israel

[3]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos-Wolfgang, Switzerland

[4]Departament de Dermatologia, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

[5]Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

[6]Department of Pediatrics, National Jewish Health, Denver, Colorado, USA

Correspondence
Luis F. Santamaria-Babí, Immunologia Translacional, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona (UB), Parc Científic de Barcelona (PCB), Baldiri i Reixac, 10, 08028, Barcelona, Spain.
Email: luis.santamaria@ub.edu

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Abstract

Circulating skin-homing cutaneous lymphocyte-associated antigen (CLA)$^+$ T cells constitute a small subset of human memory T cells involved in several aspects of atopic dermatitis: *Staphylococcus aureus* related mechanisms, the abnormal Th2 immune response, biomarkers, clinical aspects of the patients, pruritus, and the mechanism of action of targeted therapies. Superantigens, IL-13, IL-31, pruritus, CCL17 and early effects on dupilumab-treated patients have in common that they are associated with the CLA$^+$ T cell mechanisms in atopic dermatitis patients. The function of CLA$^+$ T cells corresponds with the role of T cells belonging to the skin-associated lymphoid tissue and could be a reason why they reflect different mechanisms of atopic dermatitis and many other T cell mediated skin diseases. The goal of this review is to gather all this translational information of atopic dermatitis pathology.

KEYWORDS
atopic dermatitis, biomarker, CLA$^+$ T cells, skin-homing, translational

Abbreviations: AD, atopic dermatitis; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CLA, cutaneous lymphocyte-associated antigen; CRTH2, chemokine receptor Th2; CTACK, T cell-attracting chemokine; EASI, eczema area and severity index; GM-CSF, granulocyte macrophage colony-stimulating factor; HDM, house dust mite; HLA, human leucocyte antigen; ICAM-1, intercellular adhesion molecule-1; ICOS, inducible T cell costimulatory; IFN, interferon; IgE, immunoglobulin E; IL, interleukin; ILC, innate lymphoid cells; LDH, lactate dehydrogenase; LFA-1, lymphocyte function-associated antigen-1; Mab, monoclonal antibody; MDC, macrophage-derived chemokine; PSGL-1, P-selectin glycoprotein ligand-1; *S. aureus*, *Staphylococcus aureus*; Sag, superantigen; SALT, skin-associated lymphoid tissue; SEB, staphylococcal enterotoxin B; TARC, thymus and activation-regulated chemokine; Tc, cytotoxic T cell; T_{cm}, central memory T cell; TCR, T-cell receptor; T_{em}, effector memory T cell; Th, helper T cell; TNF, tumor necrosis factor; T_{reg}, regulatory T cell; TSLP, thymic stromal lymphopoietin; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

1 | CLA EXPRESSION ON HUMAN T CELLS AND SKIN

The cutaneous lymphocyte-associated antigen (CLA) is a cell surface molecule preferentially expressed on human memory T cells infiltrating skin (over 90% of skin infiltrating T cells), in inflamed and non-inflamed situations, that it is not expressed on T cells infiltrating extra-cutaneous sites.[1] CLA is a carbohydrate, a modified form of sialyl Lewis X antigen,[2] and is an epitope of the surface protein P-selectin glycoprotein ligand-1 (PSGL-1).[3] It can be found on different human T-cell populations such as $CD45RO^+$ memory $CD4^+$ and $CD8^+$ T cells, effector/central T cells[4] and it is expressed on about 15% of peripheral blood T cells of healthy individuals.[1] In human $CDR45RO^+$ T cells CLA is upregulated during the naïve to memory transition by fucosyltransferase VII.[5] Other T-cell subsets such as Vγ9Vδ2 T cells[6] and $NKG2D^-$ $CD8^-$ T cells[7] express CLA. In addition, CLA is also expressed by regulatory T cells (T_{reg}).[8] Type 2 innate lymphoid cells (ILC)2[9] and ILC3,[10] and effector memory B cells.[11] Nevertheless, at present, the functional implications in atopic dermatitis (AD) of other T-cell types expressing CLA, besides the $CD45RO^+$ subset, have not been clarified. CLA has been shown to be induced by the effect of interleukin (IL)-12 on freshly generated helper T cells (Th)1/cytotoxic T cells (Tc)1 and Th2/Tc2 cells,[12] ex vivo in human Th2 cells,[13] as well as, by staphylococcal enterotoxin B (SEB).[14]

1.1 | CLA^+ T cells in skin migration and skin-blood recirculation

Most T cells that home to skin are of the $CD45RO^+$ phenotype and express CLA.[1] CLA functions as an adhesion molecule when is recognized by the lectin domain of the E-selectin present on endothelial cells.[2,15] Additional molecular interactions are required to mediate transendothelial migration of CLA^+ T cells through the superficial vascular plexus.[16,17] Adhesion interactions (lymphocyte function-associated antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1), and very late antigen-4 (VLA-4)/vascular cell adhesion molecule-1 (VCAM-1)) together with chemokines binding to their receptors are necessary (Figure 1). The keratinocyte-derived C-C chemokine ligand (CCL)27/CTACK (T cell-attracting chemokine) binds to C-C motif chemokine receptor (CCR)10, that is preferentially expressed on CLA^+ T cells.[18,19] Similarly, CCL17/TARC (thymus and activation-regulated chemokine), one of the best biomarkers of AD,[20] binds to CCR4, which is preferentially expressed by $CD4^+$ CLA^+ memory T cells.[21]

Efalizumab, a LFA-1 targeting monoclonal antibody (Mab) that blocks the LFA-1/ICAM-1 interaction, was formerly a candidate medication for AD that led to clinical improvement[22] and reduction of cutaneous CLA^+ memory T cells.[23] During treatment, patients developed a secondary CLA^- lymphocytosis that, after treatment discontinuation, led to disease exacerbation. Such phenomenon demonstrated normal T cell recirculation/turnover between peripheral tissues (e.g., skin) and blood. In that context, inflammatory tissue "resident" memory T cells can migrate back from the skin to the blood[24] and display a CLA^+ Th2 signature with increased expression of GATA3 and IL-13.[25] Thus, the relevance of circulating CLA^+ T cells in dermatology not only relies on their capacity to selectively migrate to skin, but also on their de-homing ability (Figure 1), implying that these circulating memory T cells might reflect cutaneous immune responses.[26] Consistently, it has been shown that CLA^+ memory/effector T cells can be found in draining lymphatics of the skin.[27–29] This feature, added to the positive correlation between the phenotype and amount of circulating CLA^+ T cells and AD severity, and the abundant infiltrates of CLA^+ T cells in AD lesional skin (compared to controls),[30] suggests that circulating CLA^+ T cells may serve as cellular peripheral biomarkers in AD.[31]

CLA^+ T cells also represent activated immune cells that can migrate to various tissues and induce an inflammatory response. Similar types of cellular migration have been demonstrated in the circulation of patients with various chronic inflammatory diseases.[32–34] Allergen-specific T cells have been reported at a frequency of one in 10^4–10^5 T cells; however, a Type 2 immune response in allergies and asthma is not solely confined to allergen-specific T cells. It harbors a wider skew in immune response including skin-homing CLA^+ Type 2 T cells, chemokine receptor Th2 (CRTH2)-expressing type T cells, ILC2, B cells and $CRTH2^+$ eosinophils.[32,35,36] The migration of activated T cells to other target organs of inflammation has been demonstrated in food allergen-specific and skin-homing T cells that are sensitized in the gut and can migrate into the skin causing AD.[34] Circulating T cells are highly active in polyallergic patients and express chemokine receptors for the migration to many different tissues.[37] Such a mechanism could be responsible for the atopic march of allergic diseases in the sequential order of AD, food allergy, asthma, and allergic rhinitis.[38,39]

These findings are in line with the epithelial barrier theory that proposes that environmental exposure to certain substances, such as detergents, surfactants, toothpastes, food emulsifiers and additives, cigarette smoke, particulate matter, diesel exhaust, ozone, nanoparticles and microplastics, might be toxic to our cells.[40–42] CLA^+ T cells have been proposed to be activated in the gut and migrate to skin. Disturbed gut barriers by environmental substances may lead to local T cells activation, which gain a skin-homing capacity and migrate to AD skin. The barrier theory describes that pathogen colonization, particularly *Staphylococcus aureus* (*S. aureus*), altered microbiota diversity, local inflammation, and incorrect regeneration and remodelling, take place in tissues with a compromised epithelial barrier. A myriad of chronic inflammatory diseases develop and worsen as a consequence of inflammatory cells migration to remote tissues, which also contributes to tissue damage and inflammation in distant organs.[43]

1.2 | CLA^+ T cells in the human cutaneous immune response

The skin-associated lymphoid tissue (SALT) was proposed by J. W. Streilein 40 years ago based on several pieces of evidence, among

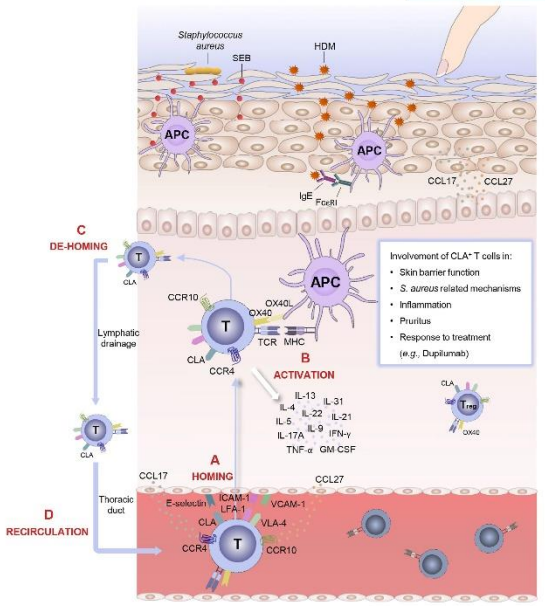

FIGURE 1 Circulating CLA$^+$ memory T cells in the pathological mechanisms of AD. Skin-homing CLA$^+$ T cells migrate into the skin (A) through a multistep process based on molecular interactions of CLA, LFA-1 and VLA-4 expressed on T cells, with E-selectin, ICAM-1 and VCAM-1 respectively, present on cutaneous endothelial cells. Additional interactions between CCR4 and CCR10 expressed on CLA$^+$ T cells, and their ligands CCL17 and CCL27 respectively, produced by keratinocytes, are required during this process. Local activation of CLA$^+$ T cells (B) implies their involvement in the cutaneous immune response and, by virtue of their de-homing (C) and skin-blood recirculation capacities (D), they reflect in the periphery the cutaneous abnormalities present in AD lesions, including *S. aureus* related mechanisms, abnormal Th2 immune response dominated by IL-13, and pruritogenic IL-31. Interestingly, an early effect of dupilumab in AD treated patients is only reflected on circulating CLA$^+$, but not CLA$^-$, CD4$^+$ CCR4$^+$ T cells. APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; HDM, house dust mite; MHC, major histocompatibility complex; *S. aureus*, *Staphylococcus aureus*; SEB, staphylococcal enterotoxin B; TCR, T-cell receptor.

TABLE 1 Similarities of skin-associated lymphoid tissues (SALT) and CLA$^+$ T cells properties.

SALT[44] properties	CLA$^+$ T cells properties
Only a subset of T cells displays skin affinity.	Selective skin homing.
Skin-related lymphocytes produce immunoregulatory molecules.	Memory phenotype with broad capacity for cytokine production.
Immune recognition of antigen in the skin.	Preferentially respond to antigens related to skin.

Abbreviations: CLA, cutaneous lymphocyte-associated antigen; SALT, skin-associated lymphoid tissues.

others, the existence of T cells with skin affinity and the ability to recognize skin-associated antigens.[44] Based on the skin tropism, recirculation, and specific responses of CLA$^-$ T cells, it may be considered that this population constitutes the subset of CD45RO$^+$ population that is closer to SALT features and may be contemplated representative of the skin-associated adaptive immune system (Table 1).[26]

Since the discovery of the CLA antigen numerous human studies have confirmed the implication of circulating CLA$^+$, but not CLA , memory T cells in diverse T cell-mediated cutaneous diseases with various pathological mechanisms. In this sense, circulating CLA$^+$ T cells respond to antigens, allergens, viruses, bacterial superantigens and drugs, and their phenotype has been reported to correlate with the clinical activity and response to treatment of cutaneous diseases (Table 2).[26] For instance, in cutaneous T-cell lymphoma, CLA is expressed on tumorigenic CCR4$^+$ CD4$^+$ T cells[48]; in dengue, CLA is upregulated in virus-specific effector CD4$^+$ and CD8$^+$ T cell populations the day before defervescence[59]; in herpes simplex, CLA is selectively expressed on circulating virus-specific CD8$^+$ T cells, which are involved in viral clearance in the skin[51]; in papuloerythroderma, circulating CD4$^+$ and CD8$^+$ T cells preferentially express the CLA marker and produce IL-4, IL-13, IL-22 and IL-31, that decrease after clinical remission[55]; and in plaque psoriasis, the response to *Streptococcus pyogenes* is confined to CLA$^+$ T cells with an IL-17 response that correlates with the clinical status of the patients.[56]

2 | CLA$^+$ T CELLS IN AD

AD is characterized by a compromised skin barrier, abnormal cutaneous immune responses, altered microbiota, and intense pruritus. Translational knowledge derived from the efficacy and mechanism of targeted therapies in AD patients has allowed identifying key disease pathways, such as Th2-derived cytokines IL-13, IL-4 and IL-31 and IL-22.[61–63] The majority of infiltrating cells in AD lesional skin are CD3$^+$ CD4$^+$ CD45RO$^+$ CLA$^+$ T cells,[30,64] which are related to different aspects of AD, including clinical features, response to treatment, and biomarkers (Figure 1).

2.1 | CLA$^+$ T cells in the clinical context of the AD patient

Circulating CD4$^+$ and CD8$^+$ CLA$^+$ T cells from AD patients express increased levels of CD25, CD40 ligand, human leucocyte antigen (HLA)-DR and inducible T cell costimulator (ICOS),[31,32,65] and spontaneously proliferate due to their in vivo activation phenotype. Additionally, long term T-cell HLA-DR activation in skin-homing cells is increased in adults with AD compared to psoriasis patients or controls.[65] Circulating CD4$^+$ and CD8$^+$ CLA$^+$ T cells also express the major Type 2 cytokines IL-4, IL-5, and IL-13,[66] as well as, IL-9, IL-17A, IL-21 IL-22, IL-31, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and granulocyte macrophage colony-stimulating factor (GM-CSF).[4,67–69]

CLA$^+$ T cells contribute to Th2 immune response by induction of immunoglobulin (Ig)E production by B cells and enhance eosinophil survival.[32,65,70] Production of IFN-γ by skin-homing T cells is one of the main mechanisms of eczema formation due to keratinocyte apoptosis. IFN-γ is mainly induced by IL-12, an important mediator for the direction of the immune response towards IFN-γ production. IL-12 is produced by keratinocytes and dendritic cells in the microenvironment.[71,72]

Patients with AD show increased frequencies of CLA expression and selective CLA$^+$ Th2/Tc2 and Th22/Tc22 expansion,

TABLE 2 Relationship between CLA$^+$ T cell biology and skin diseases besides atopic dermatitis.

Human skin diseases	Involvement of CLA$^+$ T cells
Acute graft versus-host disease	Upregulated expression of CLA on CD38^{bright} CD8$^+$ T cells.[45]
Allergic contact dermatitis	Response to nickel, cobalt, and chromium metal allergy.[46]
Alopecia areata	Th2/Tc2 activation.[47]
Cutaneous T-cell lymphoma	CLA expression on tumorigenic CCR4$^+$ CD4$^+$ T lymphocytes.[48]
Drug-induced allergic reactions	Response to drugs.[49]
Guttate psoriasis	*Streptococcus pyogenes* induces Th17 response.[50]
Herpes Simplex	CD8$^+$ T anti-viral response.[51]
Leprosy	Antigen-specific response.[52]
Lichen planus	CLA$^+$ T cells are present in lesions of oral lichen planus and oral lichenoid reactions.[53]
Melanoma	Skin metastasis and response to therapy.[54]
Papuloerythroderma	Higher proportion than CLA$^-$ of IL-4, IL-13, IL-22 and IL-31 production.[55]
Plaque Psoriasis	Response to *Streptococcus pyogenes* and relation with clinical status.[56]
Rosacea	Response to demodex[57]
Scleroderma	CD8$^+$ CLA$^+$ T cells producing IL-13 accumulate in lesions and produce cytotoxic granules.[58]
Skin dengue infection	Response to Dengue.[59]
Vitiligo	Response to autoantigens.[60]

Abbreviation: CLA, cutaneous lymphocyte-associated antigen.

accompanied by selective CLA^+ Th1/Tc1 reduction in blood.[68] Focusing on memory subsets, applying CLA positivity classification, AD immune activation involves not only of CLA^+ T cells but also of CLA^- or 'systemic' T-cell subset. Compared to psoriasis, another inflammatory skin disease,[73] 'systemic'/CLA^- and more prominently CLA^+ $CD45RO^+CCR7^+$ central memory (T_{cm}) and CLA^+ $CD45RO^+CCR7^-$ effector memory (T_{em}) T cells were significantly more activated in AD patients.[45] Additionally, frequencies of IL-13-producing CLA^+ T cells and circulating CLA^+ T_{em} and T_{cm} cells significantly correlated with disease severity and total IgE levels in serum of AD patients, exemplifying how CLA^+ frequencies may reflect several disease aspects. One such blood phenotyping study comparing adults and children with AD showed that in young children of less than 5 years old there is a dominant signature of CLA^+ Th2 cells, with CLA^+ Th1 reductions, while other immune changes build up with time and disease chronicity.[67] These results point to the Th2 dominance in early AD and support the importance of addressing this immune axis when treating young populations.

Exacerbations of AD are occasionally associated with exogenous environmental triggers.[74] The defective skin barrier prompts allergen/antigen penetration leading to specific responses of cutaneous T lymphocytes. The response to allergens such as house dust mite (HDM) is restricted to CLA^+ T cells in AD.[73] Intriguingly, a recent study has shown that the T-cell receptor (TCR) repertoire of circulating allergen-specific CLA^+, but no CLA^- T cells highly overlaps with that found in T cells infiltrating AD lesions,[75] raising evidence for circulating CLA^+ T cells as relevant T cells infiltrating the skin that respond to allergens locally.

Epigenetic modifications have been suggested as possible contributors to AD pathogenesis.[76] Acevedo et al. showed that in AD patients, $CD4^+$ CLA^+ memory T cells are characterized by dysregulated epigenetic signatures affecting key cytokine signaling pathways, with a reduced DNA methylation in the upstream region of *IL13* gene that correlates with increased *IL13* mRNA expression in these cells. Based on this, the epigenetic alteration in the *IL13* promoter may account for the augmented ability of $CD4^+$ CLA^+ T cells to produce IL-13 in AD.[30] Altogether these data suggest that CLA^+ T cells play a central role in the initiation and perpetuation of AD.[77]

2.2 | *S. aureus* and CLA^+ T cell interaction in AD

Staphylococcus aureus colonizes approximately 90% AD lesional and non-lesional skin compared to only 10% of healthy subjects[78] and is linked to AD flare up.[79] *Staphylococcus aureus* is involved in microbial dysbiosis, skin barrier abnormalities and T cell-mediated inflammation.[79] Importantly, *S. aureus*-colonized AD patients have a distinct phenotype and endotype with more severe disease.[80] SEB superantigen (Sag) is the most prevalent in AD[81] and it is associated with disease severity.[82] Application of SEB to intact AD skin induces dermatitis.[83] There is a strong mechanistic association between Sags and CLA^+ T cells, since *S. aureus*-reactive TCR Vβ skewing is found preferentially in circulating $CD4^+$ and $CD8^+$ CLA^+ T cells from AD patients and not controls,[84,85] and an increased percentage of CLA^+ T cells bearing TCR Vβ for *S. aureus* Sags is found in children with AD.[86]

Sags, compared to conventional antigens, induce T-cell expression of CLA via an IL-12 dependent mechanism[14] and contribute to AD skin inflammation by activating large numbers of T cells in lesional skin. This process is important in increasing the population of memory T cells that are capable of efficient extravasation to skin and maintaining continuous T-cell activation in the skin and thus perpetuate AD lesions even when the initiating allergen cannot be demonstrated or absent from the current environment. In a coculture model between circulating memory T cells and autologous epidermal cells from AD lesions, SEB induced preferential activation of CLA^+, rather than CLA^-, T cells leading to broad production of T-cell-derived mediators present in AD lesions (IL-13, IL-4, IL-17A, and IL-22), with IL-13 the highest Th2 cytokine produced.[87] This goes in line with the dominant IL-13 pathways,[88] as well as the increased IL-13 protein expression over IL4,[89] found in AD lesional skin. Furthermore, the same study reported that IL-13 was the only mediator that positively correlated with patients' eczema area and severity index (EASI), plasma levels of CCL17 and IgE against *S. aureus*, and CCL26 mRNA expression in cutaneous lesions (Figure 2).[87] Overall, these data are supported by the fact that IL-13 is the key Th2 cytokine with a wide impact on disease pathogenesis.[90] α-toxin, which is also produced by *S. aureus*, has also been reported to induce an enhanced IL-22 secretion by peripheral blood mononuclear cells and $CD4^+$ T cells from AD patients compared to patients with psoriasis and controls.[91]

2.3 | CLA^+ T cell relationship with AD biomarkers and targeted therapies

While AD diagnosis is still mostly based on clinical criteria, there is an ongoing search for reproducible, minimally invasive, reliable, and valid biomarkers.[20,92] Over 100 different markers have been suggested as biomarkers in AD. The most reliable biomarker reported is serum CCL17.[20]

The CLA^+ T cells and CCL17 functions are related mechanisms in AD (Figure 2), since CCR4, the receptor for CCL17, is preferentially expressed on circulating CLA^+ $CD4^+$ memory T cells[21] and T_{rcg}.[93] In support of this, CLA^+ memory Th2 cells from AD patients selectively migrate to human skin grafts transplanted onto severe combined immunodeficient mice in response to CCR4.[94] Two independent pediatric studies have shown that increased levels of skin CCL17 may predict AD development in infancy.[95,96] The preferential Th2 response by CLA^+ T cells,[4] along with the link between skin CCL17 and disease development and the positive correlation between serum CCL17 and disease severity,[97] is in line with the pathological role of CLA^+ T cells in pediatric AD population. In addition, in adults a recent phase 1b study has shown that the oral CCR4-antagonist RPT193 led to clinical improvement in

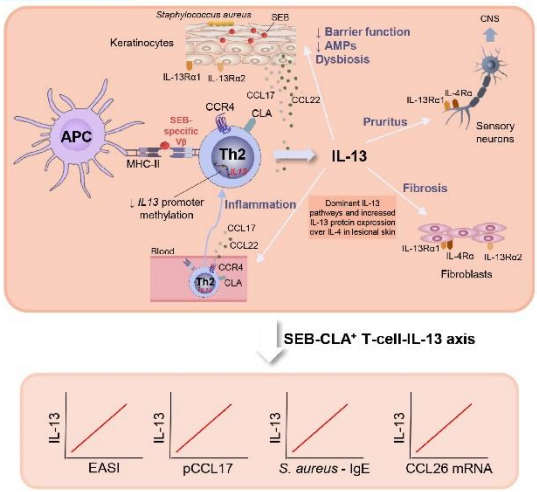

FIGURE 2 IL-13, SEB and CCL17 mechanisms meet in CLA$^+$ T cell biology in AD. SEB-specific TCR Vβ are preferentially expressed by CLA$^+$ T cells that upon activation induce a predominant IL-13 response in the skin where abundant expression of IL-13Rα1 and IL-13Rα2 is found. Lesional skin presents dominant IL-13 pathways, with increased IL-13 expression over IL-4, that are involved in skin barrier dysfunction, dysbiosis, pruritus, inflammation, and fibrosis. CLA$^+$ CD4$^+$ T cells in AD present an epigenetic alteration for IL-13, and the SEB-CLA$^+$ T-cell-IL-13 axis relates to patients' severity and plasma levels of IgE to *S. aureus*. Only IL-13, but not other SEB-induced cytokines, correlates with plasma levels of CCL17, one of the best biomarkers for AD, which is a ligand for CCR4 that attracts circulating CLA$^+$ CD4$^+$ CCR4$^+$ Th2 cells to skin. Additionally, IL-13 correlates with CCL26 mRNA expression in lesional skin. AMPs, antimicrobial peptides; APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; CNS, central nervous system; EASI, eczema area and severity index; MHC-II, major histocompatibility complex class II; *S. aureus*, *Staphylococcus aureus*; SEB, staphylococcal enterotoxin B.

moderate-to-severe AD.[98] On its behalf, CCL27, that is a CLA$^+$ T cells attracting chemokine, has been shown to be increased in the stratum corneum and associated with disease severity in pediatric AD.[99]

One potential issue for biomarkers in AD is that they differ among diverse populations. Circulating CLA$^+$ T cells have been shown to correlate with AD immune skewing across ages and ethnicities, and thus their applicability is not limited by disease chronicity and/or patient demographics. Other suggested biomarkers include E-selectin, CCL22/MDC (macrophage-derived chemokine), lactate dehydrogenase (LDH), IL-18, IL-13, among others.[20] Serum IgE, commonly measured in AD patients, was suggested as a disease biomarker, however it is only moderately correlated with AD severity, and while CLA is applicable in both intrinsic (normal IgE levels) and extrinsic (high IgE levels) AD patients, IgE measures and correlations with disease severity are mainly relevant in extrinsic AD patients,[100] a fact that limits its use as a biomarker.

Another consideration is the accessibility to the biomarker (blood, skin, tape stripping etc.), along with the requisite for repeated sampling. Biomarkers obtained from tape stripping or skin biopsies, as well as biomarkers that correlate with AD comorbidities, were investigated.[101] The fact that CLA$^+$ T cells are effortlessly extracted from peripheral blood tests puts them under the category of minimally invasive biomarkers.[20] Moreover, their ability to predict and monitor therapeutic responses reinforces their potential as cellular peripheral biomarkers in AD.[31] The fully human monoclonal IgG4 antibody dupilumab was shown to improve clinical, molecular and barrier measures in moderate-to-severe AD patients. Bakker et al. showed a significant reduction in the proliferation (Ki67 positivity) and decrease in production of IL-4, IL-5, IL-13, and IL-22 before and during treatment with dupilumab, limited to circulating CD4$^+$ CLA$^+$ T cells, supporting CLA$^+$ T-cell responses as a surrogate measure to dupilumab efficacy.[102,103] Besides this, recent multiparametric flow cytometry studies identified increased IL-13$^+$ CLA$^+$ cells in AD patients treated with dupilumab during clinical remission when compared to healthy controls.[104]

The OX40-OX40L axis has recently attracted attention in AD due to the improvements shown for both anti-OX40 depleting antibodies telazorlimab (GBR 830)[105] and rocatinlimab (KHK4083),[106] added to the non-depleting monoclonal antibody amlitelimab (KY1005)

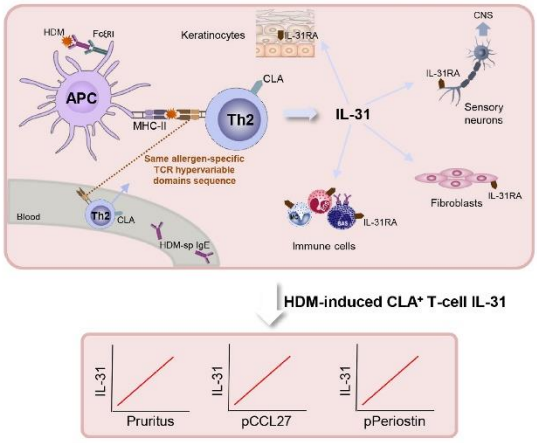

FIGURE 3 HDM specifically induces CLA$^+$ T cell IL-31, which correlates with patient's pruritus. Circulating CLA$^+$ T cells preferentially respond to HDM and share with infiltrating HDM-specific T cells same TCRB CDR3 regions. CD4$^+$ CLA$^+$ T cells are the most abundant lymphocytic population in AD lesions and major producers of IL-31. HDM-induced IL-31 by circulating CLA$^+$ T cells correlates with patient's pruritus intensity, and plasma levels of periostin, in patients with HDM-specific IgE. Additionally, plasma levels of the keratinocyte-derived chemokine CCL27, a ligand for CCR10 that is preferentially expressed by CLA$^+$ T cells, correlate with HDM-induced CLA$^+$ T cell IL-31 response. Interestingly, CCL27 in the stratum corneum is a biomarker of response to anti-IL31RA therapy in AD. APC, antigen presenting cell; CLA, cutaneous lymphocyte-associated antigen; CNS, central nervous system; HDM, house dust mite; HDM-sp IgE, IgE specific for house dust mite; MHC-II, major histocompatibility complex class II.

that binds to OX40L present on antigen presenting cells.[107] The OX40-OX40L interaction is involved in long-term and optimal cell activation of CD4$^+$ T cells and favors expansion and survival of Th2 cells.[105] OX40 is highly expressed by CLA$^+$ CD45RO$^+$ CD4$^+$ T cells in AD patients.[108] CLA$^+$ T_{reg} population from AD patients also express increased levels of OX40 compared to healthy controls and correlates with disease severity.[109,110]

IL-31 is a neuroimmune cytokine that was originally described as mainly produced by CLA$^+$ memory Th2 cells in AD,[64,111,112] with implications in pruritus, inflammation, fibrosis and epidermal barrier dysfunction.[113] Although there is an anti-IL31RA Mab (nemolizumab) in phase III for AD, the production of IL-31 and its relationship with the clinical status of the patients has not been characterized. A recent study has shown for the first time that in patients producing IL-31 by HDM-activated CLA$^-$ memory T cells, IL-31 directly correlated with patients' pruritus intensity, which was measured 24 h prior to sample collection, and plasma levels of periostin. The IL-31 response positively correlated with CCL27 plasma levels too (Figure 3), which is supported by the fact that stratum corneum CCL27 constitutes a biomarker of response to nemolizumab.[114] Additionally, it was suggested that plasma levels of HDM-specific IgE may stratify moderate-to-severe AD patients and hopefully be useful for identifying patients more probable to be responders for IL-31-directed therapies.[111] Interestingly, patients with elevated IgE levels (>1000 kU/L) display increased expression of IL-31RA on memory B cells,[115] supporting the association between IL-31 and IgE sensitization.

Th2 high and Th2 low endotypes have been hypothesized, supported by proteomic[116] and transcriptomic studies,[117] as well as differentiated responses to Th2-targeted therapies, and similarly to asthma. A recent coculture model defined the SEB-CLA$^+$ memory T-cell-IL-13 axis to functionally distinguish Th2 high and Th2 low responders within a clinically homogeneous adult moderate-to-severe AD population. Contrary to Th2 high group, where IL-13 response was associated with severity and Th2-related markers (CCL17, CCL26 and *S. aureus*-specific IgE), Th2 low group immune response skewed towards Th17, Th22, and Th1.[87]

3 | CONCLUSIONS

Translational research has bridged basic science with clinically relevant mechanisms of AD and provided a rationale for targeted therapies offering an integrated pathological view.[118] Current state of the art on the role played by circulating CLA$^+$ T cells in AD goes beyond their skin-homing capacities by clearly representing the Th2 immune

axis dysregulation found in the disease. Although some ILC2 cells express CLA, their role in adult moderate-to-severe AD is a complex matter, since ILC2 need to be activated by epithelial cytokines (alarmins) to induce Type 2 immune response and directed therapies against thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and IL-1α have not demonstrated clinical efficacy.[62]

In the clinical context of the patients, CCL17, a chemoattractant of CLA^+ Th2 cells, has been postulated as the most reliable biomarker for pediatric and adult populations, as discussed above. As for the relationship between *S. aureus* and AD, CLA^- T cells preferentially express specific TCR Vβ for *S. aureus* superantigens, such as SEB, leading to a broad cytokine-derived effector function (Th2, Th1, Th17, Th22), being IL-13 the most abundant Th2 cytokine produced. Regarding pruritus and IL-31, CLA^+ T cells are providing better understanding between clinical context of the patients and IL-31 production. From a therapeutic point of view, CLA^+ T cells are the subset of circulating memory T cells that reflects early effects of dupilumab on Th2 and Th22 responses in treated patients at Week 4.[102] All these different approaches suggest that CLA^+ T cells are at the core of AD pathogenesis. The study of SALT may provide a useful surrogate for investigating the immune-inflammatory cutaneous abnormalities present in AD.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Lídia Sans-de San Nicolás https://orcid.org/0000-0002-2828-2842
Daniel Lozano-Ojalvo https://orcid.org/0000-0002-3524-2929
Donald Y. M. Leung https://orcid.org/0000-0002-0177-3844
Emma Guttman-Yassky https://orcid.org/0000-0002-9363-324X
Luis F. Santamaria-Babí https://orcid.org/0000-0002-1674-6654

REFERENCES

1. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol*. 1990;136(5):1053-1068.
2. Berg EL, Yoshino T, Rott LS, et al. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. *J Exp Med*. 1991;174(6):1461-1466.
3. Fuhlbrigge RC, Kieffer JD, Armerding D. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature*. 1997;389(6654):978-981.
4. Czarnowicki T, He H, Canter T, et al. Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood. *J Allergy Clin Immunol*. 2020;145(1):215-228.
5. Erdmann I, Scheidegger EP, Koch FK, et al. Fucosyltransferase VII-deficient mice with defective E-, P-, and L-selectin ligands show impaired CD4+ and CD8+ T cell migration into the skin, but normal extravasation into visceral organs. *J Immunol*. 2002;168(5):2139-2146.
6. Laggner U, Di MP, Perera GK, et al. Identification of a novel Proinflammatory human skin-homing Vγ9Vδ2 T cell subset with a potential role in psoriasis. *J Immunol*. 2011;187(5):2783-2793.
7. Jacquemin C, Martins C, Lucchese F, et al. NKG2D defines a subset of skin effector memory CD8 T cells with proinflammatory functions in vitiligo. *J Invest Dermatol*. 2020;140(6):1143-1153.
8. Clark RA, Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. *Blood*. 2007;109(1):194-202.
9. Salimi M, Barlow JL, Saunders SP, et al. A role for IL-25 and IL-33–driven Type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med*. 2013;210(13):2939-2950.
10. Teunissen MBM, Yeremenko NG, Baeten DLP, et al. The IL-17A-producing CD8 þ T-cell population in psoriatic lesional skin comprises mucosa-associated invariant T cells and conventional T cells. *J Invest Dermatol*. 2014;134(12):2898-2907.
11. Yoshino T, Okano M, Chen H, Tsuchiyama J, Kondo E. Cutaneous lymphocyte antigen is expressed on memory/effector B cells in the peripheral blood and monocytoid B cells in the lymphoid tissues. *Cell Immunol*. 1999;197(1):39-45.
12. Akdis M, Klunker S, Schliz M, Blaser K, Akdis CA. Expression of a cutaneous lymphocyte-associated antigen on human CD4+ and CD8+ Th2 cells. *Eur J Immunol*. 2000;30(12):3533-3541.
13. Biedermann T, Lametschwandtner G, Tangemann K, et al. IL-12 instructs skin homing of human Th2 cells. *J Immunol*. 2006;177(6):3763-3770.
14. Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med*. 1995;181(2):747-753.
15. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature*. 1991;349(6312):796-799.
16. Sernicola A, Russo I, Silic-benussi M, Ciminale V, Alaibac M. Targeting the cutaneous lymphocyte antigen (CLA) in inflammatory and neoplastic skin conditions. *Expert Opin Biol Ther*. 2020;20(3):275-282.
17. Kunstfeld R, Lechleitner S, Gröger M, Wolff K, Petzelbauer P. HECA-452+ T cells migrate through superficial vascular plexus but not through deep vascular plexus endothelium. *J Invest Dermatol*. 1997;108(3):343-348.
18. Homey B, Wang W, Soto H, et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J Immunol*. 2000;164(7):3465-3470.
19. Homey B, Alenius H, Müller A, et al. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med*. 2002;8(2):157-165.

20. Renert-Yuval Y, Thyssen JP, Bissonnette R, et al. Biomarkers in atopic dermatitis—a review on behalf of the International Eczema Council. *J Allergy Clin Immunol*. 2021;147(4):1174-1190.
21. Campbell JJ, Haraldsen G, Pan J, et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature*. 1999;400(6746):776-780.
22. Takiguchi R, Tofte S, Simpson B, et al. Efalizumab for severe atopic dermatitis: a pilot study in adults. *J Am Acad Dermatol*. 2007;56(2):222-227.
23. Hassan AS, Kaelin U, Braathen LR, Yawalkar N. Clinical and immunopathologic findings during treatment of recalcitrant atopic eczema with efalizumab. *J Am Acad Dermatol*. 2007;56(2):217-221.
24. Klicznik MM, Morawski PA, Höllbacher B, et al. Human CD4+ CD103+ cutaneous resident memory T cells are found in the circulation of healthy subjects. *Sci Immunol*. 2019;4(37):eaav8995.
25. Strobl J, Gail LM, Kleissl L, et al. Human resident memory t cells exit the skin and mediate systemic th2-driven inflammation. *J Exp Med*. 2021;218(11):e20210417.
26. De Jesús-Gil C, Sans-de San Nicolàs L, García-Jiménez I, et al. The translational relevance of human circulating memory cutaneous lymphocyte-associated antigen positive T cells in inflammatory skin disorders. *Front Immunol*. 2021;12:652613.
27. Yawalkar N, Hunger RE, Pichler WJ, Braathen LR, Brand CU. Human afferent lymph from normal skin contains an increased number of mainly memory/effector CD4+ T cells expressing activation, adhesion and co-stimulatory molecules. *Eur J Immunol*. 2000;30(2):491-497.
28. Hunger RE, Yawalkar N, Braathen LR, Brand CU. The HECA-452 epitope is highly expressed on lymph cells derived from human skin. *Br J Dermatol*. 1999;141(3):565-569.
29. Akdis CA, Blaser K. Apoptosis in tissue inflammation and allergic disease. *Curr Opin Immunol*. 2004;16(6):717-723.
30. Acevedo N, Benfeitas R, Katayama S, et al. Epigenetic alterations in skin homing CD4+CLA+ T cells of atopic dermatitis patients. *Sci Rep*. 2020;10(1):18020.
31. Czarnowicki T, Santamaria Babí LF, Guttman-Yassky E. Circulating CLA+ T cells in atopic dermatitis and their possible role as peripheral biomarkers. *Allergy*. 2017;72(3):366-372.
32. Akdis M, Akdis CA, Weigl L, Disch R, Blaser K. Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA- memory T cells. *J Immunol*. 1997;159(9):4611-4619.
33. Jelcic I, Al Nimer F, Wang J, et al. Memory B cells activate brain-homing, autoreactive CD4+ T cells in multiple sclerosis. *Cell*. 2018;175(1):85-100.
34. Abemathy-carver KJ, Sampson HA, Picker LJ, Leung DYM. Milk-induced eczema is associated with the expansion of T cells expressing cutaneous lymphocyte antigen. *J Clin Investig*. 1995;95(2):913-918.
35. Walker JA, Mckenzie ANJ. TH2 cell development and function. *Nat Rev Immunol*. 2018;18(2):121-133.
36. Boonpiyathad T, Capova G, Duchna HW, et al. Impact of high-altitude therapy on Type-2 immune responses in asthma patients. *Allergy*. 2020;75(1):84-94.
37. David BA, Kubes P. Exploring the complex role of chemokines and chemoattractants in vivo on leukocyte dynamics. *Immunol Rev*. 2019;289(1):9-30.
38. Czarnowicki T, Krueger JG, Guttman-yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations with implications for the atopic march. *J Allergy Clin Immunol*. 2017;139(6):1723-1734.
39. Han H, Roan F, Ziegler SF. The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines. *Immunol Rev*. 2017;278(1):116-130.
40. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat Rev Immunol*. 2021;21(11):739-751.
41. Celebi Z, Betul S, Ozturk O, et al. Epithelial barrier hypothesis: effect of the external exposome on the microbiome and epithelial barriers in allergic disease. *Allergy*. 2022;77(5):1418-1449.
42. Pat Y, Ogulur I, Yazici D, et al. Effect of altered human exposome on the skin and mucosal epithelial barrier integrity. *Tissue Barriers*. 2022:2133877.
43. Tajik N, Frech M, Schulz O, et al. Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat Commun*. 2020;11(1):1995.
44. Streilein JW. Skin-associated lymphoid tissues (SALT): origins and functions. *J Invest Dermatol*. 1983;80(1):S12-S16.
45. Khandelwal P, Chaturvedi V, Owsley E, et al. CD38bright CD8+ T cells associated with the development of acute GVHD are activated, proliferating, and cytotoxic trafficking cells. *Biol Blood Marrow Transplant*. 2020;26(1):1-6.
46. Blom LH, Elrefaii SA, Zachariae C, Thyssen JP, Poulsen LK, Johansen JD. Memory T helper cells identify patients with nickel, cobalt, and chromium metal allergy. *Contact Dermatitis*. 2021;85(1):7-16.
47. Czarnowicki T, He HY, Wen HC, et al. Alopecia areata is characterized by expansion of circulating Th2/Tc2/Th22, within the skin-homing and systemic T-cell populations. *Allergy*. 2018;73(3):713-723.
48. Peru S, Prochazkova-carlotti M, Cherrier F, et al. Cutaneous lymphocyte antigen is a potential therapeutic target in cutaneous T-cell lymphoma. *J Invest Dermatol*. 2022;142(12):3243-3252.
49. Teraki Y, Fukuda T. Skin-homing IL-13-producing T cells expand in the circulation of patients with drug rash with eosinophilia and systemic symptoms. *Dermatology*. 2017;233(2-3):242-249.
50. de Jesús-Gil C, Sans-de San Nicolàs L, García-Jiménez I, Ferran M, Pujol RM, Santamaria-Babí LF. Human CLA+ memory T cell and cytokines in psoriasis. *Front Med*. 2021;8:731911.
51. Hensel MT, Peng T, Cheng A, et al. Selective expression of CCR10 and CXCR3 by circulating human herpes simplex virus-specific CD8 T cells. *J Virol*. 2017;91(19):e00810-17.
52. Dos Santos LN, Da Silva PHL, Alvim IMP, et al. Role of TEFFECTOR/MEMORY cells, TBX21 gene expression and T-cell homing receptor on type 1 reaction in borderline lepromatous leprosy patients. *PLoS One*. 2016;11(10):e0164543.
53. Werneck JT, Dias EP, Gonçalves LS, Junior AS. CLA and CD62E expression in oral lichen planus lesions. *J Oral Pathol Med*. 2016;45(3):218-223.
54. Jacquelot N, Enot DP, Flament C, et al. Chemokine receptor patterns in lymphocytes mirror metastatic spreading in melanoma. *J Clin Invest*. 2016;126(3):921-937.
55. Takamura S, Teraki Y. Interleukin (IL)-13/IL-22/IL-31 skewing within the skin-homing T-cell population in papuloerythroderma. *J Dermatol*. 2021;48(9):1357-1364.
56. De Jesús-Gil C, Sans-de San Nicolás L, Ruiz-Romeu E, et al. Specific IgA and CLA+ T-cell IL-17 response to *Streptococcus pyogenes* in psoriasis. *J Invest Dermatol*. 2020;140(7):1364-1370.
57. Gazi U, Gureser AS, Oztekin A, et al. Skin-homing T-cell responses associated with Demodex infestation and rosacea. *Parasite Immunol*. 2019;41(8):e12658.
58. Fuschiotti P, Larregina AT, Ho J, Feghali-Bostwick C, Medsger TAJ. IL-13-producing CD8+ T cells mediate dermal fibrosis in patients with systemic sclerosis. *Arthritis Rheum*. 2013;65(1):236-246.
59. Arora J, Opasawatchai A, Poonpanichakul T, et al. Single-cell temporal analysis of natural dengue infection reveals skin-homing lymphocyte expansion one day before defervescence. *iScience*. 2022;25(4):104034.
60. Frisoli ML, Essien K, Harris JE. Vitiligo: mechanisms of pathogenesis and treatment. *Annu Rev Immunol*. 2020;38:621-648.
61. Santamaria-Babi LF. Atopic dermatitis pathogenesis: lessons from immunology. *Dermatol Pract Concept*. 2022;12(1):e2022152.
62. Trier AM, Kim BS. Insights into atopic dermatitis pathogenesis lead to newly approved systemic therapies. *Br J Dermatol*. 2023;188(6):698-708.

63. David E, Ungar B, Renert-Yuval Y, Facheris P, del Duca E, Guttman-Yassky E. The evolving landscape of biologic therapies for atopic dermatitis: present and future perspective. *Clin Exp Allergy*. 2023;53(2):156-172.
64. Bilsborough J, Leung DYM, Maurer M, et al. IL-31 is associated with cutaneous lymphocyte antigen–positive skin homing T cells in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2006;117(2):418-425.
65. Czarnowicki T, Malajian D, Shemer A, et al. Skin-homing and systemic T-cell subsets show higher activation in atopic dermatitis versus psoriasis. *J Allergy Clin Immunol*. 2015;136(1):208-211.
66. Akdis CA, Simon D, Dibbert B, et al. T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis. *J Invest Dermatol*. 1999;113(4):628-634.
67. Czarnowicki T, Esaki H, Gonzalez J, et al. Early pediatric atopic dermatitis shows only a CLA+ Th2/Th1 imbalance, while adults acquire CLA+ Th22/Tc22 subsets. *J Allergy Clin Immunol*. 2015;136(4):941-951.
68. Czarnowicki T, Gonzalez J, Shemer A, et al. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. *J Allergy Clin Immunol*. 2015;136(1):104-115.
69. Czarnowicki T, Kim HJ, Villani AP, et al. High-dimensional analysis defines multicytokine T-cell subsets and supports a role for IL-21 in atopic dermatitis. *Allergy*. 2021;76(10):3080-3093.
70. Akdis M, Simon HU, Weigl L, Kreyden O, Blase K, Akdis CA. Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J Immunol*. 1999;163(1):466-475.
71. Muller G, Saloga J, Germann T, et al. Identification and induction of human keratinocyte-derived IL-12. *J Clin Invest*. 1994;94(5):1799-1805.
72. Stoll S, Jonuleit H, Schmitt E, et al. Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC- derived IL-18 enhances IL-12-dependent Th1 development. *Eur J Immunol*. 1998;28(10):3231-3239.
73. Noda S, Krueger JG, Guttman-yassky E. The translational revolution and use of biologics in patients with inflammatory skin diseases. *J Allergy Clin Immunol*. 2015;135(2):324-336.
74. Werfel T, Heratizadeh A, Niebuhr M, et al. Exacerbation of atopic dermatitis on grass pollen exposure in an environmental challenge chamber. *J Allergy Clin Immunol*. 2015;136(1):96-103.
75. Roesner LM, Farag AK, Pospich R, Traidl S, Werfel T. T-cell receptor sequencing specifies psoriasis as a systemic and atopic dermatitis as a skin-focused, allergen-driven disease. *Allergy*. 2022;77(9):2737-2747.
76. Marín MJ, Estravís M, García-Sánchez A, Dávila I, Isidoro-García M, Sanz C. Genetics and epigenetics of atopic dermatitis: an updated systematic review. *Genes (Basel)*. 2020;11(4):442.
77. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nat Rev Dis Prim*. 2018;4(1):1.
78. Beck LA, Bieber T, Weidinger S, et al. Tralokinumab treatment improves the skin microbiota by increasing the microbial diversity in adults with moderate-to-severe atopic dermatitis: analysis of microbial diversity in ECZTRA 1, a randomized controlled trial. *J Am Acad Dermatol*. 2023;88(4):816-823.
79. Kim J, Kim BE, Ahn K, Leung DYM. Interactions between atopic dermatitis and *Staphylococcus aureus* infection: clinical implications. *Allergy Asthma Immunol Res*. 2019;11(5):593-603.
80. Simpson EL, Villarreal M, Jepson B, et al. Patients with atopic dermatitis colonized with *Staphylococcus aureus* have a distinct phenotype and endotype. *J Invest Dermatol*. 2018;138(10):2224-2233.
81. Totté JEE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans SGMA. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol*. 2016;175(4):687-695.
82. Bunikowski R, Mielke M, Skarabis H, et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol*. 1999;103(1 I):119-124:119-124.
83. Skov L, Olsen JV, Giorno R, Schlievert PM, Baadsgaard O, Leung DYM. Application of staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J Allergy Clin Immunol*. 2000;105(4):820-826.
84. Strickland I, Hauk PJ, Trumble AE, Picker LJ, Leung DYM. Evidence for superantigen involvement in skin homing of T cells in atopic dermatitis. *J Invest Dermatol*. 1999;112(2):249-253.
85. Davison S, Allen M, Vaughan R, Barker J. Staphylococcal toxin-induced T cell proliferation in atopic eczema correlates with increased use of superantigen-reactive Vβ-chains in cutaneous lymphocyte-associated antigen (CLA)-positive lymphocytes. *Clin Exp Immunol*. 2000;121(2):181-186.
86. Torres MJ, Gonzalez FJ, Corzo JL, et al. Circulating CLA+ lymphocytes from children with atopic dermatitis contain an increased percentage of cells bearing staphylococcal-related T- cell receptor variable segments. *Clin Exp Allergy*. 1998;28(10):1264-1272.
87. Sans-De San Nicolàs L, Figueras-Nart I, Bonfill-Ortí M, et al. SEB-induced IL-13 production in CLA+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis. *Allergy*. 2022;77(11):3448-3451.
88. Tsoi LC, Rodriguez E, Degenhardt F, et al. Atopic dermatitis is an IL-13–dominant disease with greater molecular heterogeneity compared to psoriasis. *J Invest Dermatol*. 2019;139(7):1480-1489.
89. Koppes SA, Brans R, Ljubojevic Hadzavdic S, Frings-Dresen MHW, Rustemeyer T, Kezic S. Stratum corneum tape stripping: monitoring of inflammatory mediators in atopic dermatitis patients using topical therapy. *Int Arch Allergy Immunol*. 2016;170(3):187-193.
90. Bieber T. Interleukin-13: targeting an underestimated cytokine in atopic dermatitis. *J Allergy Clin Immunol*. 2020;75(1):54–62.
91. Niebuhr M, Scharonow H, Gathmann M, Mamerow D, Werfel T. Staphylococcal exotoxins are strong inducers of IL-22: a potential role in atopic dermatitis. *J Allergy Clin Immunol*. 2010;126(6):1176-1183.
92. Mastraftsi S, Vrioni G, Bakakis M, et al. Atopic dermatitis: striving for reliable biomarkers. *J Clin Med*. 2022;11(16):4639.
93. Hirahara K, Liu L, Clark RA, Yamanaka K, Fuhlbrigge RC, Kupper TS. The majority of human peripheral blood CD4+CD25highFoxp3+ regulatory T cells bear functional skin-homing receptors. *J Immunol*. 2006;177(7):4488-4494.
94. Biedermann T, Schwärzler C, Lametschwandtner G, et al. Targeting CLA/E-selectin interactions prevents CCR4-mediated recruitment of human Th2 memory cells to human skin in vivo. *Eur J Immunol*. 2002;32(11):3171-3180.
95. Rinnov MR, Halling AS, Gerner T, et al. Skin biomarkers predict development of atopic dermatitis in infancy. *Allergy*. 2023;78(3):791-802.
96. Halling A-S, Rinnov MR, Ruge IF, et al. Skin TARC/CCL17 increase precedes the development of childhood atopic dermatitis. *J Allergy Clin Immunol*. 2022;S0091-6749(22):2503-2509.
97. Bakker DS, de Graaf M, Nierkens S, et al. Unraveling heterogeneity in pediatric atopic dermatitis: identification of serum biomarker based patient clusters. *J Allergy Clin Immunol*. 2022;149(1):125-134.
98. Bissonnette R, Rulloda J, Lee N, et al. RPT193, an oral CCR4 inhibitor: efficacy results from a randomized, placebo controlled phase 1b monotherapy trial in patients with moderate to severe atopic dermatitis. *Exp Dermatol*. 2021;30(S2):40-41.
99. Andersson AM, Sølberg J, Koch A, et al. Assessment of biomarkers in pediatric atopic dermatitis by tape strips and skin biopsies. *Allergy*. 2022;77(5):1499-1509.

100. Suárez-Fariñas M, Dhingra N, Gittler J, et al. Intrinsic atopic dermatitis shows similar Th2 and higher Th17 immune activation compared to extrinsic atopic dermatitis. *J Allergy Clin Immunol*. 2013;132(2):361-370.
101. Yu L, Li L. Potential biomarkers of atopic dermatitis. *Front Med*. 2022;9:1028694.
102. Bakker DS, van der Wal MM, Heeb LEM, et al. Early and long-term effects of dupilumab treatment on circulating T-cell functions in patients with moderate-to-severe atopic dermatitis. *J Invest Dermatol*. 2021;141(8):1943-1953.
103. Dekkers C, van der Wal MM, El Amrani M, et al. Biological tipping point in atopic dermatitis patients treated with different dosing intervals of dupilumab. *J Invest Dermatol*. 2023;S0022-202X(23):1943-1947.
104. Jack C, Allakhverdi Z, Dupuis F. 185 high-dimensional analysis identifies variably persistent skin-homing Th2/Tc2 populations in AD patients under remission with dupilumab. *J Invest Dermatol*. 2023;143(5):S31.
105. Guttman-Yassky E, Pavel AB, Zhou L, et al. GBR 830, an anti-OX40, improves skin gene signatures and clinical scores in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2019;144(2):482-493.
106. Guttman-Yassky E, Simpson EL, Reich K, et al. An anti-OX40 antibody to treat moderate-to-severe atopic dermatitis: a multicentre, double-blind, placebo-controlled phase 2b study. *Lancet*. 2023;401(10372):204-214.
107. Weidinger S, Cork M, Reich A, et al. Amlitelimab reduces serum IL-13 in a phase 2a clinical trial in atopic dermatitis without impacting T-cell expansion in a T-cell recall assay. *Br J Dermatol*. 2023;188(S2):ii39.
108. Elsner JS, Carlsson M, Stougaard JK, et al. The OX40 axis is associated with both systemic and local involvement in atopic dermatitis. *Acta Derm Venereol*. 2020;100(6):adv00099.
109. Dahabreh D, Yamamura K, Lozano-Ojalvo D, et al. 371 The OX40/OX40L axis plays a key role in treg dysfunction in atopic dermatitis. *J Invest Dermatol*. 2023;143(5):S64.
110. Yamamura K, Dahabreh D, Garcet S, et al. 063 The role of OX40/OX40L axis in atopic dermatitis. *J Invest Dermatol*. 2023;143(5):S11.
111. Sans-de San Nicolàs L, Figueras-Nart I, García-Jiménez I, et al. Allergen sensitization stratifies IL-31 production by memory T cells in atopic dermatitis patients. *Front Immunol*. 2023;14:1124018.
112. Steinhoff M, Ahmad F, Pandey A, et al. Neuroimmune communication regulating pruritus in atopic dermatitis. *J Allergy Clin Immunol*. 2022;149(6):1875-1898.
113. Nemmer JM, Kuchner M, Datsi A, et al. Interleukin-31 signaling bridges the gap between immune cells, the nervous system and epithelial tissues. *Front Med*. 2021;8:639097.
114. Sidbury R, Alpizar S, Laquer V, et al. Pharmacokinetics, safety, efficacy, and biomarker profiles during Nemolizumab treatment of atopic dermatitis in adolescents. *Dermatol Ther (Heidelb)*. 2022;12(3):631-642.
115. Unger Z, Gomez-Casado C, van Lierop A, Pongratz G, Bleck D, Homey B. 003 memory B cells of atopic individuals preferentially express IL-31RA: a putative role of IL-31 in B cell biology. *J Invest Dermatol*. 2023;143(5):S1.
116. Thijs JL, Strickland I, Bruijnzeel-Koomen CAFM, et al. Moving toward endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis. *J Allergy Clin Immunol*. 2017;140(3):730-737.
117. Lefèvre-Utile A, Saichi M, Oláh P, et al. Transcriptome-based identification of novel endotypes in adult atopic dermatitis. *Allergy*. 2022;77(5):1486-1498.
118. Facheris P, Jeffery J, Del Duca E, Guttman-Yassky E. The translational revolution in atopic dermatitis: the paradigm shift from pathogenesis to treatment. *Cell Mol Immunol*. 2023;20(5):448-474.

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