Modulation of CD4+ T Helper Cell Memory Responses in the Human Skin

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Keywords

Immunological memory · Skin · Th17 · Tissue-resident memory T cells · Cutaneous lymphocyte antigen · CD103 · Monocyte/Macrophages · Psoriasis · Sodium salts · Neuropeptides

Abstract

Immunological memory is defined as the capacity to mount faster and more effective immune responses against antigenic challenges that have been previously encountered by the host. CD4+ T helper (Th) cells play central roles in the establishment of immunological memory as they assist the functions of other leukocytes. Th cells express polarized cytokine profiles and distinct migratory and seeding capacities, but also retain a certain functional plasticity that allows them to modulate their proliferation, activity, and homing behaviour upon need. Thus, in healthy individuals, T cell immunomodulation fulfils the task of eliciting protective immune responses where they are needed. At times, however, Th plasticity can lead to collateral tissue damage and progression to autoimmune diseases or, conversely, incapacity to reject malignant tissues and clear chronic infections. Furthermore, common immune players and molecular pathways of diseases can lead to different outcomes in different individuals. A mechanistic understanding of those pathways is therefore crucial for developing precise and curative medical interventions. Here, I focus on the skin microenvironment and comprehensively describe some of the cellular and molecular determinants of CD4+ T cell memory re-

sponses in homeostatic and pathological conditions. In discussing the cellular network orchestrating cutaneous immunity, I comprehensively describe the bidirectional interaction of skin antigen-presenting cells and mononuclear phagocytes with Th17 lymphocytes, and examine how the outcome of this interaction is influenced by endogenous skin molecules, including sodium salts and neuropeptides.

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Introduction

The human skin is a complex organ, divided into distinct anatomical compartments populated by discrete cell types of embryonic and haematopoietic origin (Box 1). The concept of the skin as an immunocompetent organ [1] can be related to pioneering surgical interventions on patients with psoriasis, a chronic relapsing skin disease characterized by irregular epidermal thickening, well-defined erythematous plaques, and T cell infiltration in the upper dermis. Those treatments showed that complete removal of the lesional epidermis by dermatome shaving induced prolonged states of disease remission, whereas recurrences were observed in patients with residual acanthosis [2–5]. In the late 1990s, it was shown that epidermal extracts from healthy skin could elicit polyclonal HLA-DR-restricted T cell responses in vitro, but lacked the capacity of activating T cell clones specific for tetanus toxoid [6, 7]. Those observations led to the concepts that specific antigens (Ag) or superantigens sequestered in the

Box 1. Anatomy of the human skin

The human skin represents an average of 1.8 m² of barrier tissue, accounting for 15% of the total body weight. This complex organ is divided into three main microanatomical compartments containing defined epithelial and immune cell types [136, 137]. Here, we focus on the architecture of the skin and briefly describe its innate cellular components of embryonic and haematopoietic origin [138, 139].

The outermost skin compartment, the epidermis, is a non-vascularized 0.1- to 1-mm-thick squamous epithelium of ectodermal origin interfacing the external environment. It displays a horny layer of flattened dead cells highly rich in lipids and keratins. This external layer originates from keratinocytes, which account for 85% of the total epidermal cells. Keratinocytes are firmly attached to each other by E-cadherin homotypic interactions and interconnected through desmosomes and hemidesmosomes by E-cadherin heterotypic interactions with $\alpha 6 \beta 4$ integrins. Nutrient delivery and waste disposal occur by diffusion through these intercellular connections. The lowest epidermal layer, the basal layer, consists of columnar epithelial cells that progressively differentiate into keratinocytes, while advancing from the spinous cell layer and the granular layer, towards the apical horny layer of the epidermis every 30 days. The healthy epidermis also contains other cells of embryonic origin. Melanocytes, producing the pigment melanin, originate from the neural crest and are found in the basal epidermal layer closely associated with a group of keratinocytes forming the so-called epidermal melanin unit. Merkel cells, which also originate from the neural crest, are granular cells that lie near the basal layer and are connected to neighbouring keratinocytes. Merkel cells are also connected to static endings of sensory fibres and are specialized in the perception of light touch [140]. LC are Langerin+EpCAM+CD1a+ cells of embryonic origin [141], representing about 1–2% of the total epidermal cells. They localize in the granular, basal, and spinous layers of the epidermis in close contact with keratinocytes and they are characterized by cytoplasmic organelles called Birbeck granules containing Langerin [138, 139, 141].

Epidermis-associated structures include hair follicles, sebaceous and sweat glands, which are distributed throughout the body and contribute differentially to barrier defence.

The epidermal basal layer attaches to the internal skin compartment, the dermis, through the epidermal-dermal junction, the function of which is to vehicle nutrients from the dermis to the epidermis. This intermediate compartment also contains endings of sensory neurons, including thermoreceptors that monitor heat, pain-sensing nociceptors, and pruriceptors, which transmit itching [140]. Notably, the stimulation of free nerve endings in the dermal-epidermal junction accounts for the sensation of itching and pain, a common symptom in skin diseases.

The dermis is a vascularized connective tissue of mesodermal origin rich in collagen fibres, which constitute about 70% of the weight of the dermis, as well as laminins and glycosaminoglycans, where dermal fibroblasts reside. Collagen fibres and glycosaminoglycans act as cell conduits and also absorb water, solutes, and mechanic stress. The dermis is irrigated by blood vessels controlled by the sympathetic nervous system and distributed above a network of lymphatic vessels detected as blind-ended capillary [24]. Both myelinated and non-myelinated sensory fibres, which have neuronal cell bodies sitting in the dorsal root ganglia, innervate Meissner corpuscles in the dermal papillae, Pacinian corpuscles in the deep dermis, and Ruffini corpuscles. These structures sense movement across the skin, high-frequency vibration, and skin stretch, respectively [140]. The middle layer, the dermis, is a connective tissue that hosts dermal DC, M ϕ , and mast cells, and becomes colonized by CD14+ myeloid cell precursors during inflammation. Three-dimensional studies of the cellular and vascular composition of the skin have shown that, at homeostasis, the human dermis contains two myeloid DC subsets expressing high levels of HLA-DR that are defined by their differential expression of CD1c and CD141, mainly distributed in the apical dermal layer, right below the dermal-epidermal junction [24]. The number of total myeloid DC is about 10⁸ DC in the entire skin area, corresponding to 10-fold more than in the blood. Although dermal DC are highly migratory cell subsets, only <1% of dermal DC is found in lymphatics of freshly isolated healthy skin [24]. Contrary to myeloid DC, pDC are not found in healthy human skin, but they are present in inflamed skin [142].

The dermis also hosts CD163+ in M ϕ and CD14+ cells. In comparison to DC, skin M ϕ and CD14+ cells are more sessile, they are not detected in lymphatic vessels, but they are found in the deep dermis, where they outnumber DC subsets. Interestingly, it has been shown that preparative cytotoxic therapy before haematopoietic stem cell transplantation (HSCT) causes the total loss of CD14+ blood-circulating and dermal-resident CD14+ cells, as well as a 50% reduction in dermal M ϕ . Skin CD14+ cells rapidly increase after HSCT, mirroring blood Mo reconstitution from donor-derived cell precursors, whereas reconstitution of skin M ϕ occurs at a significantly slower rate. Therefore, although the origin and differentiation capacity of human dermal CD14+ cells remain to be fully assessed, these cells may represent precursors of dermal M ϕ [75]. Hence, primary immunodeficiencies of IRF8 and GATA-2 result in the lack of dermal DC subsets and blood Mo, whereas dermal M ϕ are reduced and epidermal LC are not affected by the lack of IRF8 and GATA-2 [141, 143]. These in vivo observations underscore the heterogeneity of the cutaneous M ϕ population, which derives from both embryonic and blood Mo precursors [138].

The dermis sits on the last skin compartment, the hypodermis, a loose connective tissue, containing some fibroblasts and M ϕ , which connects the dermis to bone and muscles, and supplies the skin with blood vessels and nerves. The hypodermis forms about 50% of the whole body fat storage. Adipocytes constitute most of the cellular components of this compartment, which has recently been shown to actively contribute to skin immune defences in animal models [144].

skin microenvironment could activate cutaneous T cell responses when released upon wounding and in disease states, thus anticipating later findings [8–10].

The skin immune contexture has very recently witnessed a revival of interest as it has been recognized that functionally different T cell subsets and innate immune cells are compartmentally distributed in the human skin with their activity accounting for long-term immunoprotection. At homeostasis, the modulation of cutaneous T cell immunity fulfils the task of protecting the host. At times however, T cell plasticity can lead to collateral tissue damage and progression to inflammatory and autoimmune skin diseases or, conversely, to the incapacity of rejecting malignant tissues and clear infections. T helper cells producing IL-17 (Th17) are perhaps one of the most emblematic examples of this dichotomy [11] because, although Th17 responses are required for mucocutaneous immunity against *Candida* and *Staphylococcus* infections [12], deregulated IL-17 immunity is associated with skin pathology [13–15]. Furthermore, crucial recent findings have shown that, although the skin is the barrier organ most exposed to environmental cues, its molecular composition is rather stable over time and largely dependent on individual characteristics, including dietary habits, gender differences, and stress levels [16–18]. For instance, it has been recognized that high-salt diets derange immunohomeostasis and promote Th17 pathogenicity [19]. Moreover, some relevant studies have described how a significant gender bias in skin sodium deposition and in the perception of pain is associated with cutaneous diseases [18, 20], whereas it is now very well known that stress and mood disorders influence skin physiopathology [21, 22]. Thus, cutaneous immune surveillance may be seen as a skin-intrinsic process, whereby accessory cells and endogenous compounds act as modulators of T cell immunity. The main challenge in the study of cutaneous immunity is therefore to dissect the mechanisms that account for T cell immunomodulation, at homeostasis and in disease states, as this will offer the possibility of fine-tuning protective Th cell memory with personalized immunological interventions.

This review comprehensively describes some of the cellular and molecular determinants of CD4⁺ T cell memory responses in healthy human skin and how the deregulation of those determinants contributes to skin pathology. In discussing the cellular networks, which orchestrate skin immunosurveillance, I focus on the bidirectional interaction between skin-resident Ag-presenting cells (APC) and Th17 lymphocytes, and examine how skin sodium salts and neuropeptides influence the outcome of this interaction.

Heterogeneity of Skin-Tropic and Skin-Resident CD4⁺ T Lymphocytes

Healthy human skin contains about 10^6 T cells/cm², with 2×10^9 CD3⁺ cells in the first 30 μ m of the apical dermis, in comparison to the 10^{10} T cells in 5 L of blood in an average-sized individual [23, 24]. These figures are even higher in the deep dermis and remain rather constant throughout life [25]. Assessing the phenotype of T cells eluted from healthy skin biopsies or cell suspensions of digested healthy skin led to the observation that 95% of the cutaneous T cells were polyclonal TCR $\alpha\beta$ ⁺/CD45RO⁺ memory lymphocytes, with CD4⁺ T cells largely outnumbering CD8⁺ lymphocytes [23, 26, 27]. Cutaneous T cells expressed the skin-tropic molecule cutaneous lymphocyte Ag (CLA) [28] as well as CCR4 [29] and CCR6 chemokine receptors [23, 26], whereas a small fraction of CD4⁺CLA⁺ memory T cells eluted from the skin was CCR8⁺ and did not express CCR4 [30]. Thus, CLA in combination with CCR4, CCR6, and CCR8 constitute the combination code defining cutaneous memory T cells.

The evaluation of the heterogeneity of the cutaneous T cell pool was further strengthened by the identification of migratory and sessile T cell subsets in the peripheral blood and skin [31–33] of healthy donors as well as in the blood and skin of patients with leukaemic cutaneous T cell lymphomas (L-CTCL), who received a systemic injection of the T cell-depleting agent alemtuzumab [27, 34]. In those last studies, positive clinical responses to alemtuzumab in L-CTCL patients could be related to the capacity of the therapeutic to deplete malignant and benign CCR7⁺/CD62L⁺ central memory T cells (T_{cm}), and CCR7⁺/CD62L[–] skin-tropic migratory memory T cells (T_{mm}) from the skin. Both these cell subsets express the combination code CLA/CCR4. Notably, L-CTCL patients with malignant T_{cm} showed dermal T cell infiltration and a diffuse erythema that rapidly cleared with alemtuzumab treatment, whereas the disease mediated by malignant T_{mm} was characterized by expanding skin lesions with ill-defined borders and a slower response to treatment [27, 34]. Those in vivo observations proved, for the first time, that T_{cm} and T_{mm} constitute about one-third of the total cutaneous T cells and that these subsets circulate between blood and skin at different speeds, with their presence in the skin imprinting the macroscopic clinical symptoms of the disease. Contrary to the recirculating T_{cm} and T_{mm}, skin-resident CCR7[–]/CD69⁺ effector memory T cells (T_{em}), representing the majority of cutaneous T lymphocytes, were not affected by alemtuzumab treatment. Hence, the lack of cutaneous infections in T cell-depleted

L-CTCL patients provides indirect evidence that cutaneous Tem stay put and remain functional during the treatment [27, 34]. Notably, patients with mycosis fungoides, a clinically defined CTCL type characterized by the infiltration of malignant CLA+/CCR4+ Tem cells in the dermoepithelial junctions and manifesting with well-defined skin lesions, did not respond to alemtuzumab, thus indicating that sessile skin-resident Tem may also imprint a unique pathological pattern [34, 35].

The above findings also have implications for the understanding of other skin diseases characterized by morphologically different skin lesions, with different persistence and recurrence rates. For instance, sessile skin-resident Tem cells play major roles in the pathogenesis of psoriasis. This skin disease is in fact characterized by well-demarcated recurrent lesions that cannot be treated by inhibiting retention of CD69+ T cells into the skin through E-selectin blocking [36]. Furthermore, non-lesional skin of psoriatic patients was found to induce active psoriatic lesions after transplantation onto immunodeficient mice [9]. Contrary to psoriasis, the role played by Tcm, Tmm, and Tem in other skin pathologies, including atopic and contact dermatitis, remains to be understood [37]. Overall, the human skin is protected by recirculating tissue-tropic CD4+/CCR7+ Tcm and Tmm, and sessile tissue-resident (Trm) CD4+/CCR7– T cells, the latter representing the majority of the cutaneous T cell population.

Diversity of Skin-Resident Trm

The functional heterogeneity of cutaneous CD4+ Trm has recently been appreciated by the identification of two distinct subsets, characterized by a differential expression of CD69 and CD103 [27, 38, 39]. In this context, CD69 binds E-selectin [36] and sequesters the sphingosine-1-phosphate receptor (S1PR1) required for lymphocyte egress from tissues [38, 39], whereas CD103 mediates adhesive interactions with E-cadherin molecules expressed by skin keratinocytes [27]. The combined action of these molecules contributes to the tissue retention of cutaneous T cells. In the human skin, about 60% of the total Trm population is represented by dermal CD69+/CD103– CD4+ memory T cells, whereas CD69+/CD103+ CD4+ memory T lymphocytes constitute about 75% of the total skin Trm and are present at higher frequencies in the epidermis. Both subsets express higher effector functions in response to anti-CD3/CD28 mAb or microbial Ags in comparison to blood-circulating CD69– T cells. However, when compared to dermal CD69+/CD103– T cells, epidermal CD69+/

CD103+ lymphocytes produce a higher amount of IFN- γ , TNF- α , and IL-22, and they have a weaker proliferative capacity, as assessed by Ki-67 expression. Hence, CD69+/CD103+ Trm died rapidly when isolated from the skin [27], suggesting that their viability crucially depends on yet unknown microenvironmental signals present in the epidermis, which might modulate their functions.

Despite the advances described above, it remains unknown whether CD103+ and CD103– skin-resident Trm subsets also contain functional regulatory T cells (Treg), as suggested by the observation that CD4+/CD25high/CD69+ Treg isolated from human healthy skin express CLA and high levels of CCR4 and CCR6, and whether they lack CCR7 and have suppressive functions in vitro [40]. Interestingly, activated T cell clones isolated from bulk CLA+CCR8+CCR4– lymphocytes of healthy skin were found to produce high amounts of IL-10 and IL-4, in the absence of IFN- γ release [30]. The assessment of skin organ cultures and skin cell suspensions also revealed that about 20% of cutaneous CD4+ lymphocytes in the healthy human skin are CD45RO+FoxP3+ Treg cells expressing intermediate to high levels of CD25 [40, 41]. Those skin-resident memory Treg were characterized by high expression of CTLA-4, ICOS, CD27, and BCL-2, low levels of IL-2, IL-17, IFN- γ , and IL-10 cytokines, that they had poor proliferating capacity and that they were regulatory in vitro [40, 41]. Conversely, blood-circulating Treg cells were found to be cycling cells expressing lower levels of activation markers and higher levels of IL-2 and IL-17 cytokines compared to their skin counterpart [41, 42]. However, the developmental relationship between blood-circulating and skin-resident Treg remains to be defined. Future research would benefit from a consensus on cell phenotypes to enable a better understanding of how different cutaneous T cell subsets (Table 1) are put to work in the human skin.

CD4+ T Cells in the Skin Can Be Put to Work

Functional studies on bulk T lymphocytes eluted from healthy skin organ cultures or cell suspensions of digested skin, as well as purified cutaneous CLA+CCR4+ T cells documented the capacity of cutaneous T cells to secrete IFN- γ and IL-17 in response to mitogens or skin-tropic microbes including *Candida albicans* and *Staphylococcus aureus* [26, 43, 44], thus suggesting that cutaneous T cells can be put to work in vitro. In physiological settings, classical diagnostic tools to assess delayed-type hypersensitivity reaction (DTH) have proven useful in the study of

Table 1. Human skin-tropic and skin-resident memory CD4+ Tαβ cell subsets

Phenotype	Origin	Donor	Given name	Characteristics	Caveats	Ref.
CCR7+/CD62L+ and CD45RA-/- CCR7+/CD62L+	Cell suspension of digested skin and peripheral blood	L-CTCL patients and healthy donors	Tcm	Blood-recirculating skin-tropic (CLA+/CCR4+) migratory central memory T cells that accumulate in lesional L-CTCL skin. Tcm depletion in L-CTCL patients reduces cutaneous CD4+/IL-4+ T cell frequencies and enhances IFN-γ and TNF-α producing CD4+ T cell responses in the skin	Production of Th2 cytokines from cutaneous Tcm may be the result of activation in the skin. L-CTCL patients develop dermatitis, psoriatic plaques, and susceptibility to Staphylococcal infections; as these conditions depend on T cell effector functions, recirculating Tcm could influence the activity of Tcm responses in the skin. Ag specificity of skewed Th2 and Th1 responses was not investigated	[27, 31, 34]
CCR7+/CD62L-	Cell suspension of digested skin and skin biopsies	L-CTCL patients and healthy donors	CD69- T cells/ Tmm	Dermal skin-tropic (CLA+/CCR4+) migratory memory T cells (Tmm) including a larger migratory CD69- subset and a smaller more sessile population of CD69+ cells	Skin-tropic Tmm were not tested for Ag specificity. Tmm cytokine responses were not significantly different from Tcm and Tem, when tested on total peripheral blood T cells stimulated with PMA/ionomycin. No kinetic studies of functional responses were performed	[27, 34]
CD3+/CCR7-, CD45RA-/-CCR7-/-/CD62Ldim and CCR7-/-CD62L+/CD69+	Cell suspension of digested skin and peripheral blood	L-CTCL patients and healthy donors	Trm/ Tem	Sessile skin-resident memory T cells (Trm) and skin-tropic effector memory T cells (Tem)		[27, 31]
CD69+/CD103-	Cell suspension of digested skin	Healthy donors	CD103- Trm	Skin-resident Trm constituting about 20 and 40% of epidermal and dermal T cells, respectively; have high proliferative capacity and reduced effector functions in response to α-CD3/α-CD28 mAb and <i>C. albicans</i> and <i>S. aureus</i> , as assessed by intracellular expression of TNF-α, IFN-γ, IL-22, and Ki67	May represent precursors of CD103+ Trm	[27]
CD69+/CD103+	Cell suspension of digested skin	Healthy donors	CD103+ Trm	Skin-resident Trm constituting about 50 and 25% of epidermal and dermal T cells, respectively. Respond to α-CD3/α-CD28 mAb, <i>C. albicans</i> and <i>S. aureus</i> with low levels of Ki67 and high levels of TNF-α, IFN-γ and IL-22. Undergo rapid apoptosis once isolated from the skin	Ki67 detection measured after 2 weeks from stimulation in vitro might have underestimated CD103+ Trm proliferative capacity. No kinetic studies of functional responses were performed. CD103 expression may be a consequence of Ag-specific activation in the skin	[27]
CD69-/CD103-	Cell suspension of digested skin	Healthy donors	Tcirc	Skin-tropic blood-circulating memory T cells representing about 10 and 20% of epidermal and dermal T cells, respectively	Ki67 detection measured after 2 weeks from stimulation in vitro might have underestimated Tcirc proliferative capacity. No kinetic studies of functional responses were performed	[27]
CLA+/CCR4+/CCR6+	Cell suspension of digested skin, skin explant cultures and explants on 3D matrices with or without IL-2 and IL-15	Healthy donors and patients with psoriasis, CTCL, squamous cell carcinoma and melanoma	Heterogeneous population of Tcm and Tem	Skin-resident non-proliferating memory (CD45RO+) T cells including subsets expressing CXCR3, CCR7, CD62L, and CCR8. Respond to PMA/ionomycin stimulation by producing IL-2, IFN-γ, and TNF-α	Skin-eluted T cells were not tested for Ag specificity. Recovery of healthy memory T cell and malignant T cell clones depended on culture conditions; exogenous cytokines and chemokines released by dermal fibroblasts grown into 3D matrices stabilized CLA and CCR4 expression and enhanced T cell responses	[23, 26]
CD4+/CCR8+	Cell suspension of healthy digested skin	Healthy donors	None	Migratory (CLA+/CXCR3+/CCR4-/CCR6-/CCR7-) activated (CD25+/CD69+/CD27+) memory (CD45RO+) T cells that migrate towards CCL1 in vitro. Respond to PMA/ionomycin stimulation by expressing IL-2, IFN-γ, and TNF-α. T cell clones derived from skin CD4+/CCR8+ T cells produce TNF-α, IL-4 and very low amounts of IL-10	CD4+/CCR8+ T cells do not express the typical skin-resident combinatorial code as they lack CCR4 expression. CD4+/CCR8+ T cells are scarcely represented in inflamed skin, suggesting an exclusive functional role in healthy skin. Not tested for Ag specificity	[30]
CD4+/CD25hi and CD4+/CD25+/- FoxP3+	Short-term skin explant cultures with or without IL-2 and IL-15 and skin biopsies	Healthy donors	mTreg	Represent up to 10% of the total skin-resident T cells localized mainly within the epidermis in contact with LC and in the dermal-epidermal junction near dermal DC. About 5% of skin mTreg express Ki67 in situ and can express CD69 upon activation. Skin CD4+CD25+ T cells suppress proliferation of peripheral blood CD4+CD25- T cells in vitro	Phenotypic characterization limited to CD25 and FoxP3 expression; FoxP3 was not included as a phenotypic marker of suppressive CD4+CD25+ mTreg isolated from skin. Not tested for Ag specificity	[23, 40]

Table 1 (continued)

Phenotype	Origin	Donor	Given name	Characteristics	Caveats	Ref.
CD45RO+/FoxP3+	Hair follicles and cell suspension of healthy skin	Healthy donors	mTreg	Non-migratory, activated (CTLA-4hi/CD25hi/ICOShi/CD27hi/BCL-2hi/CD127lo/Ki67lo) memory (CD45RO+) T cells, containing a demethylated FoxP3 gene and expressing unique TCRVβ sequences. Anergic to PMA/ionomycin stimulation, as assessed by intracellular expression of IL-2, IL-17, IL-10, and IFN-γ. In lesional psoriatic skin FoxP3+ mTreg express Ki67 and IL-17 cytokine	mTreg were not tested for Ag specificity in physiological settings, despite their stable memory phenotype. Cutaneous mTreg from healthy skin did not express high levels of CD127, thus questioning the role of IL-7 as a putative maintenance factor of skin-resident T cells in humans; IL-17-producing mTreg in psoriasis might have derived from converted Tem, as clonality was not assessed	[38, 41]
CD4+/FoxP3+	Skin suction blister fluid following intradermal injection of PPD	Healthy donors	nTreg	CD4+/FoxP3+ T cells with phenotypic markers of nTreg (CD25+/CD27+/CD39+/CD127lo), 10% of which express Ki67. Unresponsive to ex vivo stimulation with PPD, as assessed by intracellular expression of IL-2 and IFN-γ	Technical limitations prevented the assessment of suppressive functions of cutaneous FoxP3+ T cells. Putative FoxP3+ nTreg from blister fluids may have originated from recently activated Tem. Suppressive FoxP3+ nTreg may have failed to accumulate in the blister fluid and remained in the skin tissue	[42]
CXCR3+/CCR4-/CCR6-	Peripheral blood	Healthy donors	Th1	Migratory (CLA+/CCR4-/CCR10-/CCR6-/CXCR3+) memory T cells that produce IFN-γ in response to PPD and cytomegalovirus		[77, 145, 146]
CXCR3+/CCR4-/CCR6+	Peripheral blood	Healthy donors	Th1*	Migratory (CLA+/CCR4+/CCR10-/CCR6+/CXCR3+) memory T cells that produce IFN-γ and IL-17 in response to <i>M. tuberculosis</i>		[77, 146]
CXCR3-/CCR4-/CCR6-	Peripheral blood	Healthy donors	Th2	Migratory (CLA+) memory T cells; a small subset of CCR4+ cells expresses CRTh2 and/or CCR3+. Produce IL-4 in response to the house dust mite allergen <i>D. pteronyssinus</i> and tetanus toxoid vaccine		[52, 145 147 - 149]
CLA+	Peripheral blood	Healthy donors and patients with plaque psoriasis and atopic dermatitis	Th9	Skin-tropic migratory and skin-resident memory T cells; produce IL-9 in response to <i>C. albicans</i> ; include small fractions of polyfunctional Ag-specific cells also producing TNF-α, IL-13, IL-17, IFN-γ, and granzyme B. They are found increased in lesional psoriatic skin, as compared to atopic dermatitis skin and healthy skin	The master regulator of Th9 differentiation remains to be identified	[44]
CXCR3-/CCR4-/CCR6+	Peripheral blood	Healthy donors	Th17	Skin-tropic migratory (CLA+/CCR10-/CCR6+/CXCR3-, a subset is CCR4+) memory T cells producing IL-17. Also produce IFN-γ in response to <i>C. albicans</i> and IL-10 in response to <i>S. aureus</i>		[77, 146]
CXCR3-/CCR4-/CCR10+	Peripheral blood	Healthy donors	Th22	Skin-tropic migratory (CLA+/CCR4+/CCR6+/CCR10+) T cells. Produce IL-22 in response to allogeneic feeder cells and PHA	Not tested for Ag specificity	[150]
CD4+/CD25hi, CD4+/CD25+/FoxP3+, CD45RO-/FoxP3+, CD4+CCR8+	Peripheral blood	Healthy donors	mTreg, nTreg, none	Migratory (CLA+/CCR4+/CCR6+) memory (CD45RO+) CD4+/FoxP3+ Treg, expressing low levels of activation markers and producing low amount of IL-2, IFN-γ, IL-17, IL-10 upon activation with PMA/ionomycin. CD4+/CD25+/CCR8+ T cells have suppressive functions in vitro	Phenotypic assessment of suppressive CD4+/CCR8+ lymphocytes was limited to CD25 expression. Not tested for Ag specificity	[23, 30, 40, 41]

Ag-specific T cell responses in human skin. In particular, the Montoux test (MT) is a very elegant *in vivo* model with which to study cutaneous T cell memory by provoking the formation of blisters at the site of intradermal injection of purified protein derivative (PPD) tuberculin in healthy individuals who had been previously vaccinated with BCG. With this method, PPD-specific and allergen-specific CD4⁺ CLA⁺ Tcm and Tem were found to accumulate in the skin and suction blister fluids of the MT reaction and at the site of repeated DTH challenge with PPD and keyhole limpet hemocyanin (KLH) [45, 46]. T cell infiltration occurred with specific kinetics, until resolution of the inflammatory reaction [47], thus underscoring that cutaneous immunity relies on the activity of distinct memory T cell subsets responding to recall Ag. In healthy conditions, protective memory responses can end by loss of CLA and consequent exit of memory T cells from the inflammatory site, and also by Treg immunosuppression *in situ* [42, 48]. In active diseases, however, prolonged Ag-specific immunity becomes deleterious. In psoriasis, for instance, T cells specific for the antimicrobial peptide LL37 [8, 49] can be regarded as a “pathological memory” leading to the recurrent Trm-dependent skin lesions characteristic of this disease, which is characterized by deranged Th17 and Th22 responses and a Treg to Th17 bias [41]. Notably, the clinical symptoms of psoriasis are relieved by IL-17 blockade, although the long-term effects of the treatments, also upon therapeutic withdrawal, remain to be fully appreciated [41, 50, 51]. Interestingly, rare patients with co-occurring psoriasis and atopic eczema were shown to display antagonistic Ag-specific Th17/Th1 and Th2 cells infiltrating skin lesions characteristic of each disease [52]. These very elegant *in vivo* observations support the notion that disease-specific molecular signatures and clinical symptoms might depend on phenotypically and functionally different Ag-specific T cell subsets present in the skin. Despite these advances however, the specific assessment of cutaneous memory Th cell responses in patients with psoriasis remains difficult, as the quest for novel psoriasis auto-Ag has slowed down in favour of the hypothesis that this disease is autoinflammatory rather than autoimmune.

Clonality of Cutaneous CD4⁺ T Cells

High-throughput TCR sequencing of skin-resident T cells in skin diseases has proven that unique cell clones, expressing individual TCR- β CDR3 sequences, expand in the skin lesions of patients with CTCL, MF, psoriasis, ec-

zematous dermatitis, and DTH [35, 53, 54]. As the common usage of CDR3 sequences is an indirect measure of TCR specificity, these results further argue for Trm to be poised for Ags recognition *in situ*. Hence, experimental models of DTH have revealed that unique clones develop and persist for up to 4 months in the skin of human volunteers previously sensitized and challenged with a contact allergen [54]. Furthermore, in healthy human subjects assessed by MT, CD4⁺ T cells isolated from blister fluids at site of PPD injection shared TCRV β elements with PPD-specific T cell clones isolated from autologous peripheral blood [45]. Interestingly, about 80% of the clones remained stable during the entire duration of the inflammatory response, whereas 20% of them were present either early or late. It was then proposed that some clones could be lost following a downregulation of CLA, whereas others could represent a population expanded at a later stage [45]. Considering that distinct T cell populations protect the human skin [27], the stability of Ag-specific T cell clones might depend on the developmental relationship among Tcm, Tmm, and Trm cell subsets. Notably, very recent animal studies have suggested that skin-resident Trm cells and blood-recirculating Tcm shared a common naïve T cell precursor of equal specificity and TCR usage [54]. Nevertheless, whether human skin-tropic Tcm play active roles in the maintenance of skin-resident Trm remains to be fully understood.

With regard to immune regulation, deep sequencing studies of the TCRV β have also revealed that CD4⁺ effector memory T cells (Tem) and Treg in the human skin share very few TCR sequences [41]. Therefore, it would be very interesting to know whether CD103⁺ and CD103[−] skin-resident Tem and Treg recognize different Ag [55] or different components of the same Ag [56, 57].

Although the assessment of T cell clonality supports the view of Ags being recognized by skin-resident T cells, as there is evidence that human cutaneous Trm cells co-localize and respond to Ag [58], different exogenous cues could contribute to Trm tissue maintenance. For CD8⁺ Trm [extensively reviewed in 59], inflammatory cytokines, in addition to Ag recognition, were shown to modulate the expression of tissue retention molecules [60]. For instance, CD69 expression is upregulated by IFN- γ , IL-33, and TNF- α *in vitro* [61, 62], whereas the expression of CD103 is upregulated by TGF β [63]. These processes could thus indirectly influence cutaneous CD4⁺ Trm tissue retention, independently of Ag recognition. The triggers that shape the cutaneous Trm landscape in humans is yet to be completely defined and might relate to the microbiological and metabolic cartography of the skin, as well as to individual characteristics [16, 64].

Innate Immune Cell Determinants of Cutaneous T Cell Responses

Despite the knowledge that cutaneous immunity depends on the expansion of Ag-specific T cell clones, recent findings in patients with genital herpes [58] and allergic contact dermatitis [10, 15], as well as from mouse models [65], have revealed that less than one-third of the skin-resident T cells involved in an Ag-specific response shared a clonal TCR, and only a minor fraction of those responded to the nominal Ag. In the light of these *in vivo* observations, it is likely that the modality of T cell activation and the type of accessory cells they interact with influence the function of skin-resident T cells [10, 55]. The compartmentalization of innate immune cells in skin microdomains, with stable dermal perivascular sheaths of dendritic cells (DC) and macrophages (Mø) [24], indeed argues that accessory cells might influence the quality of cutaneous T cell responses either directly, through the presentation of specific Ags and SAg *in situ*, or indirectly, through non-specific bystander signals [66].

It has long been known that T cells in the healthy human skin are found clustered in rows around postcapillary venules in the dermis and in proximity to cutaneous appendages [24, 67, 68], and that T cells conjugated to HLA-DR+ DC could be eluted from skin organ cultures [69]. More recently, dermal clusters of CD4+ T cells with DC and Mø were found in healthy skin at homeostasis [70], as well as in inflamed human skin, following infections [58, 71], cutaneous adverse reactions [72], and autoimmune inflammatory skin diseases [73]. Clustered CD4+ T cells in the skin lesions of patients with genital herpes and in patients with psoriasis expressed CCR5, CCR6, and CCR4, thus suggesting that recruitment and retention of skin-tropic T cells through chemokines contributed to the formation of clusters [58, 71, 73]. Whether the protective T cell pool contained CD69+ Trm was, however, not investigated. In HSV-2-infected patients and in allergic contact dermatitis patients, perivascular foci of virus- and allergen-specific IFN- γ -producing CD4+ T cells in the upper dermis remained detectable for 2 months even after complete skin healing [58, 65, 72], suggesting that cluster maintenance also depended on the retention of protective T cells *in situ* [54]. In murine models this Ag-specific response was inhibited by the blocking of LFA-1 and by the conditional depletion of cutaneous DC, whereas the lack of Mø, the blocking of Mø-derived CCL5 and of T cell-derived IFN- γ abrogated DC recruitment, cluster formation and, consequently, T cell activation [65, 72]. Thus, cutaneous immunity seems to rely on feed-

forward loops established by a division of labour between dermal Mø and DC subsets, which interact with T cells [65, 70, 72]. On the one hand, Ag presented by DC to T cells assures timely regulated protective T cell responses enabling Mø functions. On the other hand, the bidirectional interaction between skin-resident IFN- γ -secreting memory T cells and Mø enables the induction and retention of cellular aggregates *in situ* through CCL5/CCR5 chemotaxis. These findings, however, are yet to be confirmed in humans [58].

The cellular interactions described above could also maintain the homeostasis of the healthy human skin. For instance, dermal CD14+ DC have been shown to constitutively express IL-10 and induce hypo-responsiveness in naïve T cells *in vitro*. Despite these findings, the effect of DC on memory T cells has not been investigated in depth [70]. Also, it remains poorly understood how the bidirectional interactions of dermal Mø with memory T cells orchestrate T cell unresponsiveness in the human skin at homeostasis [74].

In addition to terminally differentiated myeloid DC and Mf, the skin dermis also hosts blood-derived monocyte (Mo) subsets, which can influence memory T cell responses in multiple ways [24, 75]. For example, classical CD14++CD16- Mo activated through TLR-2 and TLR-4 efficiently stimulate Th17 responses in comparison to TLR-activated non-classical CD14+CD16++ Mo and myeloid DC [76, 77]. Skewed Th17 memory responses *in vitro* were also induced by dermal DC eluted from human skin explants injected with IL-1 β , an endogenous pyrogenic factor abundantly produced by microbially activated Mo [78]. Th17 expansion depended on the capacity of DC to adhere to T cells and to produce IL-1 β , IL-6, and IL-23 cytokines [79], which is known to amplify and stabilize Th17 responses [77, 80]. Contrary to classical Mo and DC, non-classical Mo have been shown to limit Th17 responses by contact-dependent mechanisms that require functional LFA-1 [76]. Hence, given the high plasticity of peripheral blood Mo, once Th cells are put to work, the bidirectional interaction of polarized Th1, Th17, and Th2 cell subsets with Mo may generate positive feedback loops that amplify skewed Th cell memory responses in autoimmune inflammatory skin diseases, as shown by psoriasis and atopic dermatitis [81]. Interestingly, GM-CSF was shown to be part of a pathogenic Th cell response in multiple sclerosis [82], so that GM-CSF produced by Th cells might influence the differentiation of Mo-derived Mø and DC and feed forward the expansion of pathogenic Th cell subsets *in vivo* [83, 84]. Whether GM-CSF is also involved in the progression of cutaneous au-

toinflammatory skin diseases, however, remains to be understood.

Whereas DC and Mo/M ϕ subsets orchestrate T cell immunity in the dermis, Langerhans cells (LC) modulate T cell responses in the avascular epidermidis layer of the skin. At homeostasis, human LC have been shown to stimulate the proliferation of skin-resident memory T cells in the absence of exogenous Ags. Notably, proliferating T cells were found to express a memory Treg phenotype, CD4+CD25+FoxP3+CD127+, and had suppressive functions *in vitro* [55]. Memory Treg expansion required LC/Trm contact, functional HLA-DR/DP Ag-presenting molecules, and low Ag doses. Notably, human LC were found to display a poor capacity to internalize bacteria and to process and present HLA-II-restricted Ags [55, 85]. In addition, LC have been shown to negatively regulate Th cell responses to contact allergens through PDL-1 [86]. At the same time, however, human LC constitutively express high amounts of CD1a and efficiently stimulate CD1a-restricted immune responses that in turn amplify Th17 immunity *in vivo* [57]. Altogether, these heterogeneous observations argue for a dual contribution of LC in skin immunosurveillance, depending upon the context. At homeostasis, LC could maintain peripheral tolerance through the presentation of low doses of Ag and PD-L1-mediated immune regulation, whereas in skin diseases LC may indirectly enhance Th17-mediated skin inflammation through the presentation of CD1a-restricted Ags.

Sodium Salts as Skin-Intrinsic Modulators of T Cell Immunity

The outcome of T cell interaction with mononuclear phagocytes in cutaneous immunity is likely to be strongly influenced by the skin microenvironment, as the skin also has intrinsic metabolic functions and serves as a reservoir of immunomodulatory metabolites. Recent advances in this research field have identified sodium salts as modulators of T cell responses.

The dogma that interstitial sodium concentration is tightly regulated by the kidneys has been recently challenged by the observation that a relevant amount of Na⁺ is stored in the human skin, from where it can be mobilized by haemodialysis [87–91]. It has been suggested that the polarity of glycosaminoglycan within the extracellular matrix regulates the storage and the release of cutaneous Na⁺, while establishing a hypertonic microenvironment [92]. During infections, interstitial hypertonicity could

fulfil protective tasks, as it induces microbicidal responses in M ϕ [88]. M ϕ were also shown to act as osmosensors of electrolytes because they could eventually promote Na⁺ removal from the subcutaneous interstitium after pathogen clearance [93].

A hypertonic microenvironment could also bolster inflammatory T cell responses *in vivo*, in at least two ways. On the one hand, skin Na⁺ could indirectly impact on T cell immunity by influencing the physiology of skin mononuclear phagocytes. For instance, human Mo activated in high-salt media produced a high amount of IL-6 and IL-23, and a low amount of IL-10 [19], all cytokines that play crucial roles in the maintenance and regulation of Th17 responses [77, 94]. Moreover, in animal models Na⁺ salts enhanced lipopolysaccharide-driven pro-inflammatory M ϕ differentiation, while inhibiting the generation of anti-inflammatory M ϕ [88, 95]. It is therefore plausible that “salty” M ϕ and DC could modulate memory T cell responses in the skin in opposite directions, a possibility that needs to be investigated in depth. On the other hand, *in vitro* and *in vivo* observations have documented that Na⁺ salts directly promote pathogenic T cell responses and hamper immunoregulation. Hence, even a modest increase of NaCl in cell culture media containing exogenous polarizing cytokines could drive human naïve T cell differentiation into Th17 cells *in vitro* through mechanisms that require functional serum/glucocorticoid regulated kinase 1 (SGK1) expression in T cells [96]. Interestingly, secondary stimulation with high NaCl did not result in an enhanced expansion of IL-17-producing T cells. However, it could also modulate Treg responses, offering arguments in favour of the hypothesis that either IL-17 expression levels are already saturated after the first stimulation, or that differentiated Th cells are insensitive to a high concentration of NaCl. It is also possible that Na⁺-dependent Th17 expansion requires additional factors not included in the experimental settings. In line with this, animal studies have shown that sensing of high Na⁺ salt concentrations through SGK1 resulted in IL-23R expression on differentiating T cells and that IL-23, a cytokine produced by M ϕ and DC, was critical for maintaining the SGK1 expression on Th17 cells [97]. Although T cell responses were not analysed at clonal levels, human and animal studies have shown that Th17 responses elicited in the presence of a high concentration of NaCl were polyfunctional and pathogenic *in vivo* [96, 97]. High concentrations of NaCl were also found to induce the expression of pro-inflammatory cytokines in FoxP3+ Treg with loss of immunosuppressive capacity *in vitro*, whereas silencing of IFN- γ and SGK1 in Na⁺-conditioned Treg restored their regulatory functions [98].

Finally, T cell immunomodulation by sodium salts is likely to be influenced by individual characteristics. It is in fact known that women have an increased skin sodium storage in comparison to men and that the differences increase in elderly subjects [20, 99]. In addition, aging shows an increased proportion of CD4+ IL-23R+ T cells and an altered Th17/Treg balance [100]. These observations suggest a possible causal link between skin electrolytes and dysfunctional cutaneous immunosurveillance, depending on age and gender differences [18].

Neurogenic T Cell Immunomodulation

The skin is the largest sensory organ of the human body, densely innervated by myelinated and unmyelinated axons with their terminal endings in the epidermis and the dermal papillae. When they sense noxious stimuli, these free nerve endings release neuropeptides capable of conveying the sensation of pain [101, 102]. There is evidence, however, that derangements in the skin sensory nervous system also influences skin immunity in vivo. For instance, the healing of the lesional skin at the site of sensitivity loss, followed by gradual relapse upon improvement of the neurological conditions [103, 104], caused temporary nerve failure in patients with chronic plaque psoriasis. Interestingly, about 30% of psoriatic patients with traumatic spinal cord injury or surgical denervation developed local skin and nail fungal infections visible on the site below the neurological level of damage [103, 105]. Since psoriasis is an IL-17-mediated disease and IL-17 immunity is required for antifungal immunity [12, 106], the above clinical observations suggest that protection against fungal infections could also depend on neurogenic immunomodulation, as indirectly shown by animal studies demonstrating the function played by nociceptive sensory fibres in co-ordinating IL-17-dependent resistance to *C. albicans* infections [107]. Further evidence supporting the role played by neurogenic immunomodulation in human psoriasis and atopic dermatitis has been documented in histological studies describing an increased skin innervation in patients in comparison to healthy subjects, as well as an increased expression of sensory neuropeptides and their receptors on immune cells [108, 109]. Among those neuropeptides, the calcitonine gene-related peptide (CGRP) and the substance P (SP) have been found to co-accumulate in free nerve endings in the superficial dermis and in the epidermis [110, 111] and to act as immune modulators of T cell responses [112, 113].

In the early 1990s, pioneer findings on human skin demonstrated that CGRP-containing nerve fibres originating from the dermis were intimately associated with epidermal LC, and CGRP accumulated on their surface [114], thus setting the stage for an exploration of how the nervous system influences skin immunity. Since then, the few available studies have documented the capacity of CGRP to modulate human T cell responses in vitro by influencing APC functions. Human DC were found to express CGRP receptors and stimulation with CGRP resulting in increased intracellular Ca^{++} as well as proving effective sensing of the neuropeptide [115]. Furthermore, Mo, Mo-derived Mø and DC, and blood-circulating DC activated by microbial stimuli or inflammatory cytokines in the presence of CGRP expressed a reduced amount of Ag-presenting and costimulatory molecules and released a higher amount of IL-10 [115–117]. In line with this phenotypic modulation, CGRP-conditioned APC turned out to be poor stimulators of T cell proliferation in allogeneic and Ag-specific settings, an effect which was reverted by blocking IL-10 [115, 116]. CGRP was also found to influence T cells and Mo migration and adhesion [118, 119]. Moreover, IFN- γ release was diminished in the peripheral blood mononuclear cells (PBMC) of healthy donors activated with CGRP in vitro [116], whereas CGRP enhanced IL-13 release from the CLA+ memory T cells of some patients with atopic dermatitis [120]. Although the physiological relevance of these observations remains to be thoroughly investigated, the clinical benefit of phototherapy in human psoriasis, possibly associated with the release of intracellular CGRP storage following UV irradiation [121], could be considered indirect evidence of the anti-inflammatory effect of CGRP in vivo. Interestingly, however, the CGRP receptor-mediated signal results in elevated or attenuated intracellular cAMP and iCa^{++} levels, with downstream effects on multiple targets, depending upon the G-protein adaptors recruited to the signalling complex [111]. Thus, CGRP could also sustain pro-inflammatory functions in leukocytes. A multiparametric assessment of CGRP-conditioned CD4+ memory Th cell responses, and a systematic analysis of the effects of CGRP on skin-resident T cells and mononuclear phagocytes is therefore necessary to provide further insights on how CGRP influences cutaneous immunosurveillance.

SP is a neuropeptide found in sensory nerve fibres predominantly associated with the dermis and dermo-epidermal junction [112, 122] sensed by neurokinin receptors also expressed by immune cells [123]. There is evidence that SP modulates memory T cell responses di-

Fig. 1. A model of CD4⁺ Th cell immunomodulation in the human skin. **a** Four phenotypically and functionally distinct CD4⁺ T cell subsets have been shown to play active roles in skin immunosurveillance. These include skin-tropic blood-recirculating CCR7⁺ Tcm and Tmm, and sessile skin-resident CD103⁺ and CD103[−] Trm, differentially distributed throughout the epidermal and the dermal layers. **b** In those compartments, cutaneous T cells are often found in close connection with innate immune cells. In the avascular epidermis, LC accounts for the maintenance of Treg at a

steady state, whereas they promote Th responses in inflammatory conditions. In the vascularized dermis, DC and Mφ clusterize with Th cells and modulate their function. The outcome of those interactions can be further modulated by endogenous skin metabolites, such as sodium salts stored in the hypodermis (**c**), and neuropeptides, including CGRP and SP, released by nociceptive nerve fibres with their terminal endings in the epidermis (**d**). LC, Langerhans cells; DC, dendritic cells; Mø, macrophages; Mo, monocytes.

rectly and also indirectly, by acting on APC [123, 124]. SP doses as low as 10^{-8} M were found to induce the release of IL-1 β , IL-6, TNF- α , and IL-23 cytokines by human Mo in vitro [125], and SP-treated Mo were potent stimulators of IL-17-producing memory T cells [123]. In some studies, Mo pre-activation with lipopolysaccharide was necessary for SP sensing [126]. Interestingly, agonists of TLR and type I/II IFNs were shown to enhance the expression of neurokinin receptors on Mo-derived DC, and DC exposure to antagonists of those receptors at-

tenuated phenotypic maturation induced by TLR ligands [124]. When assessed for function, Mo-DC exposed to SP receptor antagonists had a lower capacity to activate Ag-specific Th cytokine responses [124]. Thus, functional neurokinin receptors in DC might be required for an optimal T cell activation by DC. In addition to its effect on APC, SP was also shown to bind specific receptors, rapidly and reversibly, on a subpopulation of resting, metabolically active peripheral blood CD4⁺ T cells. This process increased upon mitogen stimulation [127].

Box 2. Main concepts and open questions

1. The human skin is protected by phenotypically distinct subsets of recirculating skin-tropic and sessile skin-resident T cells. <i>What is the relative contribution of migratory and sessile T cells on long-term cutaneous immunity in humans?</i>
2. Different approaches and phenotypic markers have been used to identify, isolate, and study skin-tropic and skin-resident T cell subpopulations. <i>Is it possible to identify harmonized phenotypic and functional combinatorial codes of distinct Ag-specific T cell subsets present in the human skin?</i>
3. Skin physiopathology depends on a timely regulated activation of regulatory and effector T cell types. <i>To what extent does the bidirectional interaction among APC, mononuclear phagocytes, and T cells maintain this balance in the human skin?</i>
4. The human skin is recognized as a metabolically independent sensory organ. <i>Do skin metabolites and neuropeptides play effective roles in modulating cutaneous T cell memory responses?</i>
5. The cellular and molecular composition of the skin microenvironment depends on individual characteristics and is fundamental for maintaining skin homeostasis. <i>Can mononuclear phagocytes and endogenous skin factors be exploited for the personalized treatment of diseases that manifest with skin pathology?</i>

Moreover, SP had a mitogenic effect on CD4⁺ T cells in the absence of Ag and it modestly enhanced mitogen-induced T cell proliferation, regardless of the presence of SP antagonists [128], suggesting that other, yet unknown receptors could be involved. In fact, although SP per se did not induce cytokine secretion from T cells, it enhanced the release of IL-2 and IFN- γ from phytohaemagglutinin (PHA)-activated PBMC cultures [129, 130]. Interestingly, IL-2 production by PHA-stimulated PBMC in the presence of SP followed a bell-shaped time- and dose-dependent biphasic curve [129] that could depend on the use of different receptors expressed with different kinetics in activated T cell subsets. As SP cannot bind metabolically inactive lymphocytes [128], it will be interesting to assess the effect of the lymphocyte metabolism on the expression of neurokinin receptors.

Further evidence that skin homeostasis is influenced by the nervous system is indicated by the observation that stress can change skin physiology and that mood disorders exacerbate skin pathology [21, 22]. Recent advances have also shown the function of the hypothalamic-pituitary-adrenal axis in the regulation of skin physiopathology. For instance, sleep deprivation was found to delay skin healing and slightly decreased the numbers of Th cells in the peripheral blood of healthy women [21], whereas dysregulation in the cyclic release of the sleep hormone melatonin has been observed in patients with active psoriasis in comparison to healthy subjects [131]. Notably, melatonin was found to interfere with Th17 responses in vitro by blocking ROR γ t expression, and by promoting the expression of RORA

and the expansion of IL-10-secreting Tr1 cells, without any effect on Th1 responses [132]. Although a direct role of melatonin in the healing of lesional psoriatic skin remains to be investigated, the sleep hormone could indirectly influence the evolution of psoriatic lesions through the release of the melanocyte-stimulating hormone (MSH). In support of this possibility, studies have shown that exposure of peripheral blood DC to α -MSH in vitro induced a tolerogenic IL-10-secreting DC type capable of expanding functional Treg, which inhibited the activity of Th17 lymphocytes from individuals with psoriasis [133]. The latter findings are interesting as arguments in favour of MSH as an endogenous T cell immunomodulator that regulates IL-17 immunity in diseased human skin.

Conclusions

The recent revival of interest in a comprehensive understanding of the capacity of the skin to function as an immunocompetent organ has provided important insights into the role of T cells in the maintenance of skin homeostasis. Considering both the higher frequency of CD4⁺ T lymphocytes present in the human skin and their heterogeneity, it is likely that they play a major role in cutaneous immunity (Fig. 1). Despite important advances, however, our knowledge of the relative contribution of skin-tropic and sessile skin-resident T cell subsets in skin immunosurveillance is still poor (Box 2). This limitation needs to be taken into account because recent findings in

animal models have indicated a much more dynamic CD4⁺ T cell compartment in the skin [134, 135] in comparison to what has been described for the human skin [27, 34]. Further advances in this direction require harmonized protocols to identify distinct T cell subsets and a systematic analysis of their Ag specificity and clonality. More research endeavours are also needed to assess how Th memory responses are influenced by cellular and molecular components of the skin microenvironment. Therefore, in order to pin down the exclusive long-term contribution of blood-recirculating Tcm and Tmm and skin-resident Trm cells a multiparametric study is necessary, i.e., the isolation of phenotypically characterized CD4⁺ T cell subsets, the identification of their functional properties, and their modulation by endogenous skin factors. These research endeavours are indispensable with a

view to formulating new targeted and personalized immunotherapy concepts for the treatment of diseases that manifest with skin pathology.

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