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Impact of the innate and adaptive immune system in driving type 1 inflammatory skin disease

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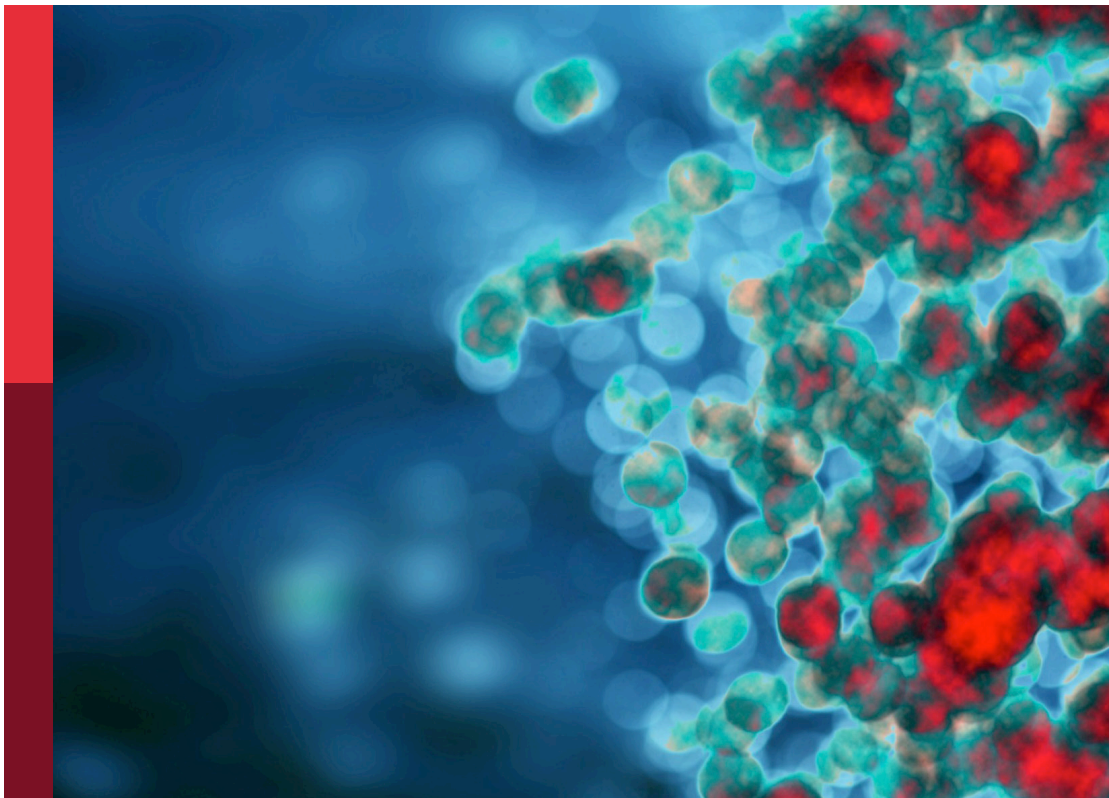

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Impact of the innate and adaptive immune system in driving type 1 inflammatory skin disease

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Editorial: Impact of the innate and adaptive immune system in driving type 1 inflammatory skin disease

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KEYWORDS

psoriasis, vitiligo, type 1 inflammatory skin disease, tissue-resident memory T cells (TRM cells), acne (acne vulgaris), atopic dermatitis (AD), juvenile dermatomyositis (JDM), Th1 cells

Editorial on the Research Topic

Impact of the innate and adaptive immune system in driving type 1 inflammatory skin disease

The last 20 years have witnessed a revolutionary change in how inflammatory skin diseases are treated. Immune profiling studies between lesional and non-lesional or healthy skin have provided crucial insights into the immune cell populations and culprit cytokines responsible for driving disease persistence or recurrence. These approaches have been bolstered by *in vivo* or *ex vivo* models, where cytokine neutralisation has been used to study the role of specific cytokine blockade on disease severity. Validation of the CD4+T helper (Th) subsets involved in the pathogenesis of skin disease has revealed numerous therapeutic targets such as polarising, maintenance and effector cytokines of T cell subsets. In particular, Th17 and Th2-associated cytokines, which include IL-17, IL-23, IL-22, IL-4 and IL-13 have been the target of intense pharmaceutical scrutiny and biologics targeting these cytokines have proven efficacious.

Type 1 inflammation associated with Th1 cells and Natural Killer cells, is primarily driven by TNFα and IFNγ. These protect against intracellular pathogens and tumour cells but aberrant activation is associated with a myriad of inflammatory skin conditions. While these diseases typically display a type-1 skewed immune bias, this Research Topic of two original research articles, one brief research report, five reviews and one mini-review underscores the role of both innate and adaptive immune cells in shaping the inflammatory milieu associated with type-1 inflammatory skin diseases. From acne, to eczema, psoriasis and vitiligo, a consistent theme emerges: these diseases are multifactorial processes with a dynamic interplay of multiple cellular and molecular players.

Yang et al. provide a comprehensive and timely review of rosacea that exemplifies this complexity of type 1 skin disease. They examine the molecular interactions that drive rosacea pathogenesis, in particular the role of LL37, the human Cathelicidin peptide that displays a wide range of immunomodulatory functions. In rosacea, LL37 exerts pleiotropic influence on effector cells - inducing the release of IL-8 from keratinocytes, VEGF from endothelial cells (mediated by mTORC1) and type I Interferons from plasmacytoid dendritic cells (pDCs). IL-8

serves as a chemoattractant for neutrophils and VEGF, in addition to promoting angiogenesis, stimulating Th1 cell differentiation. Through activation of the transient receptor potential vanilloid 4 (TRPV4) LL37 activates macrophages and mast cells.

Kallikrein 5 (KLK5) cleaves cathelicidin to the active LL37 peptide after Toll-like receptor 2 (TLR2) activation and this TLR2-KLK5-LL37-mTOR axis is a main therapeutic strategy for disease management. Additionally, new treatment strategies, such as targeting Th1/Th17 cells, the JAK/STAT pathway or the use of VEGF inhibitors to curtail angiogenesis are highlighted.

The brief research report from Raupov et al. provides insight into the role of type I interferon (IFN-I) signalling in juvenile dermatomyositis. Juvenile dermatomyositis is an idiopathic inflammatory myopathy characterised by muscle weakness and eczema. Raupov et al. show a significant correlation between elevated IFN-I scores and skin disease activity, highlighting the potential of serum IFN-I as a promising biomarker for skin involvement but also arthritis in these patients.

Two comprehensive reviews by Jin et al. and Huang et al. provide an in-depth look at the complexity of acne vulgaris, challenging the traditional perception of this condition as a mere superficial skin issue.

Jin et al. delve into the immune processes involved in the pathogenesis of acne vulgaris, detailing the role of microbiome dysbiosis and the innate and adaptive immune response to *Cutibacterium acnes* (*C. acnes*), *Staphylococcus* and *Malassezia*. Peptidoglycan, lipoteichoic acid and short-chain fatty acids of *C. acnes* induce pattern-recognition receptor activation on keratinocytes, sebocytes and monocytes, which release anti-microbial peptides and cytokines that tailor the adaptive immune response. Additionally, Jin et al. describe the role of neuropeptides, such as Corticotropin-releasing hormone and Substance P. Huang et al. build on this by detailing the differential cellular responses at different stages of disease development. Th1 and Th17 cells play an important early role in microcomedones when follicles rupture and neutrophils are attracted in large numbers to further drive inflammation. Interestingly, mast cells also play a role in early acne lesions, being recruited by keratinocyte-produced stem cell factor, and are a source of IL-17A in acne lesions.

These reviews underscore the need to study the temporal and spatial dynamics of immune activity in acne development, to facilitate more targeted treatments.

In an original article, Seiringer et al. use spatial transcriptomics to uncover the active role of sebaceous glands in psoriasis vulgaris and the pathogenesis of atopic dermatitis. Both diseases show altered lipid metabolism in the sebaceous gland transcriptome compared to non-lesional sebaceous glands and the upregulation of inflammatory mediators including serum amyloid A1. In atopic dermatitis, a number of genes associated with lipid skin barrier formation have been identified. Interestingly, genes such as *ALOX15B*, an important regulator of fatty acid metabolism, and *CCL17*, two of the spatially variable genes upregulated here are known to be induced by type-2 cytokines, IL-4 and IL-13 in macrophages, suggesting a potential role of the sebaceous gland in atopic dermatitis. In psoriatic tissue, sebaceous gland gene expression profiles showed an increase in type I interferon and anti-microbial peptide expression but also heightened differentiation and SUMOylation. These data present sebaceous glands as immunomodulatory structures that contribute to the shaping of the immune environment in skin disease.

Morelli et al. investigate the difference between anti-PD-1-induced psoriasis in three oncology patients with samples of chronic plaque psoriasis and paradoxical psoriasis (resulting from anti-TNFα treatment). Their original research article shows that this immune-related cutaneous adverse event is immunologically similar to plaque psoriasis. Conversely, the innate immune arm, i.e. the type I interferon response and myeloid cell involvement, plays a lesser role in anti-PD-1-induced psoriasis compared to paradoxical psoriasis. Interestingly, next-generation sequencing showed that all three patients harboured SNPs associated with an increased risk of psoriasis, including specific ERAP1 haplotypes that may be involved in the generation of certain autoantigens for HLA-class I presentation and autoimmune CD8+ T-cell activation. All three patients displayed enhanced expression of the psoriasis autoantigen ADAMTSL5, which is also found in melanoma tissue. The authors suggest that ADAMTSL5-specific T-cell responses that protect against the tumour may trigger the onset of psoriasis in these patients.

Liu G. et al. probe into the double-edged role of tissue-resident memory T (Trm) cells. Acting as sentinels, Trm cells remain in peripheral skin tissue for extended periods. The review discusses how CD4+ and CD8+ Trm cells play both protective and pathogenic roles. CD4+ Trm cells can circumvent the innate immune response and induce effector functions upon antigen recall. TGFβ, TNFα, IL-33 and IFNγ maintain CD103 and CD69 expression on CD4+ Trm cells, facilitating their retention in tissues, while IL-15 plays this role in CD8+ Trm cells. In psoriasis and vitiligo, CD4+ Trm and CD8+ Trm cells play a pathogenic role in disease recurrence, while in melanoma these CD8+ Trm cells actively hinder tumour progression and enhance immunotherapy responses.

Liu S. et al. highlight the multifaceted role of macrophages in numerous type-1 inflammatory skin diseases and also atopic dermatitis, melanoma and cutaneous T-cell lymphoma. In psoriasis, macrophages can express two important autoantigens, LL37 and ADAMTSL5 and produce important chemokines, including MIP-1α and –β. In atopic dermatitis, M2-like macrophages are a major source of IL-31, a main trigger of pruritus. M2-like macrophages also play a role in Bullous pemphigoid (BP), where these cells produce CCL18. Mouse studies show that macrophages may drive the blistering associated with BP. In melanoma, macrophages have been implicated in promoting metastases and angiogenesis via HIF-1α and HIF-2α. The plasticity of macrophages to adapt to different inflammatory environments underscores their importance in immune responses and potential therapeutic targeting.

Migayron et al. offer a compelling perspective on the role of type-2 immunity in type-1-associated skin diseases, vitiligo, localised scleroderma and alopecia areata. Their mini-review describes the complex role of Th2 cytokines and chemokines, IL-4, IL-13 and TSLP in these diseases and the cross-talk between mast cells and CD8+ T cells. Whether type-2 inflammation contributes to pathology or is a protective mechanism remains to be fully clarified in clinical subsets, which will reveal how to better stratify patients for therapeutic intervention.

To summarise, this Research Topic highlights new insights into our understanding of how we classify and characterise type 1 skin diseases. In particular it illuminates the rich but detrimental, immune cross-talk that drives disease persistence. We believe that this Research Topic will serve to inform and inspire further research in this field with the goal of identifying new therapeutic targets for a plenitude of type 1 skin diseases.

Author contributions

MM: Conceptualization, Writing – original draft, Writing – review & editing. JD: Conceptualization, Writing – original draft, Writing – review & editing. BM: Conceptualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The 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.

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A review of skin immune processes in acne

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Acne vulgaris is one of the most prevalent skin conditions, affecting almost all teenagers worldwide. Multiple factors, including the excessive production of sebum, dysbiosis of the skin microbiome, disruption of keratinization within hair follicles, and local inflammation, are believed to trigger or aggravate acne. Immune activity plays a crucial role in the pathogenesis of acne. Recent research has improved our understanding of the immunostimulatory functions of microorganisms, lipid mediators, and neuropeptides. Additionally, significant advances have been made in elucidating the intricate mechanisms through which cutaneous innate and adaptive immune cells perceive and transmit stimulatory signals and initiate immune responses. However, our understanding of precise temporal and spatial patterns of immune activity throughout various stages of acne development remains limited. This review provides a comprehensive overview of the current knowledge concerning the immune processes involved in the initiation and progression of acne. Furthermore, we highlight the significance of detailed spatiotemporal analyses, including analyses of temporal dynamics of immune cell populations as well as single-cell and spatial RNA sequencing, for the development of targeted therapeutic and prevention strategies.

KEYWORDS

acne vulgaris, immune response, microorganism, lipid mediators, neuropeptides, single-cell analysis

1 Introduction

Acne vulgaris is a dermatological condition that predominantly affects approximately 85% individuals in adolescence and early adulthood (1, 2). Acne tends to occur in regions characterized by a high concentration of sebaceous glands, such as facial and upper back regions (3). Pilosebaceous units (PSUs), composed of sebaceous glands and hair follicles, are the fundamental structures affected in acne lesions. In typical PSUs, the production and secretion of sebum (a mixture of lipids) are primarily carried out by the sebaceous glands. Secreted sebum travels through the sebaceous duct and enters the lumen of the hair follicle channel, where it coats the keratinocyte wall. The commensal microbiota within the hair follicle possesses the

ability to metabolize specific lipid species into free acids, resulting in an environment with a low pH, hindering the colonization or proliferation of harmful microorganisms.

Acne vulgaris manifests when the harmonious equilibrium within the PSU is disturbed. Considering the hair follicle duct as a conduit, hypercornification of the hair infundibulum combined with excess sebum, microorganisms, and keratin squamae can result in the development of microcomedones. These microcomedones subsequently develop into either white or black comedones (4), causing the obstruction of the hair follicle ducts.

Comedones coupled with the excessive production of sebum establish a relatively anaerobic environment, which facilitates the proliferation of specific species of microorganisms, ultimately resulting in dysbiosis of the skin microflora. The altered composition of microorganisms in the PSUs along with the virulence factors they release in conjunction with the enlarged comedones exert pressure on the wall of hair follicles, leading to their compression and subsequent rupture. This process ultimately compromises the structural integrity of the skin barrier within the hair follicles.

Subsequently, invading pathogens, their secreted virulence factors, and degraded sebum penetrate the dermis and activate immune cells, resulting in an intensified inflammatory response. This process results in the development of inflammatory lesions, including papules and pustules. In patients with severe acne, papules and pustules can lead to the development of nodules or cysts. Owing to the destruction of the dermis or hypodermis, certain lesions pose challenges in terms of restoration, ultimately leading to scar formation.

Prior studies have established that the immune system plays a critical role in all stages of acne development. This review provides a comprehensive overview of the immune processes involved in acne development, including a summary of the stimulators that activate the immune response, the mechanisms involved in both innate and adaptive immune responses, and the sequence of infiltrated immune cells in different types of acne lesions.

2 Stimulators triggering immune response

Substances that interfere with the regular functioning of PSUs generally stimulate innate immune responses. At present, these substances can be categorized into two distinct types: (1) exogenous substances originating from the external environment, including a diverse range of microorganisms and their virulent metabolites, and (2) autoantigens generated by the host, such as specific lipid mediators from sebum and blood and neuropeptides secreted by neuroendocrine cells. Stimulators with experimental evidence using human cells or tissues are listed in Table 1.

2.1 Skin microbiome

The skin microbiome consists of bacteria, viruses, fungi, and archaea that reside in or temporarily inhabit the skin or its appendages (41). The human skin provides diverse microhabitats (differing in thickness, moistness, gland and hair follicle density, and other parameters) for various microbial communities. The most frequently isolated microorganisms in hair follicles are *Cutibacterium acnes* and *Staphylococcus epidermidis* (42).

The capacity of *C. acnes* to elicit an immune response has been evaluated extensively (14). Previous *in vivo* and *in vitro* studies have demonstrated that certain strains of *C. acnes* as well as their toxic metabolites and cell wall components, such as peptidoglycan (PGN), lipoteichoic acid (LTA), and short-chain fatty acids (SCFAs) produced under lipid-rich hypoxic conditions, can induce a significant increase in cytokine expression in cultured keratinocytes (12, 31), sebocytes (16, 17), peripheral blood mononuclear cells (PBMCs) (9, 29), and monocytes (12, 31). The activation of the skin immune system in response to *C. acnes* has also been demonstrated *in vivo*. For instance, Ashbee et al. demonstrated that the levels of IgG1 and IgG3 antibodies targeting *C. acnes* were higher in individuals with severe acne than in those with normal skin, whereas IgG2 specific to *C. acnes* was higher in patients with moderate-to-severe acne than in those with mild acne (43). These *in vivo* results suggest that *C. acnes* plays a progressive role in acne of varying severity.

Despite evidence that *C. acnes* contributes to the development of acne, a consistent difference in the relative abundance of this bacterium between individuals with and without acne has not been detected (44–46). There is a widely accepted consensus that the dysbiosis of *C. acnes* at the strain level, the presence of virulent genetic elements, and altered transcriptional activity provide a more comprehensive explanation for the observed functional disparities between individuals with healthy skin and those with acne. This viewpoint is supported by several studies (15, 25, 42, 46–51).

In addition to the extensively studied *C. acnes*, other microorganisms, such as the most abundant skin commensal fungal genus *Malassezia* and species of *Staphylococcus*, are associated with acne (44, 52, 53). The potential impact of *Malassezia* on the pathogenesis of acne is supported by its positive response to antifungal agents in cases of refractory acne (54), its increased abundance in young individuals with acne (20, 55), increased levels of secreted lipases and stimulation of immune responses in PBMCs and keratinocytes (18, 48, 56).

There is evidence for associations between *Staphylococcus* species and acne. For example, they are highly abundant on the surfaces of comedones, papules, and pustules (45). Furthermore, the occurrence of *S. epidermidis* is higher in patients with acne than in heathy controls (57, 58). The NF-κB pathway is activated in keratinocytes upon treatment with *S. epidermidis* (21) and the mitogen-activated protein kinase (MAPK) is activated by *S. aureus* (59). However, Xia et al. claimed that LTA generated by *S. epidermidis* could inhibit the proliferation of *C. acnes* and reduce the protein expression of toll-like receptor (TLR)-2 in keratinocytes (60). Further studies are required to elucidate the functions of *S. epidermidis* in acne development and resolve inconsistencies.

Given that the immune responses of cultured cells to microorganisms or their secreted virulence factors depend on direct physical contact *in vitro*, it is crucial to determine whether pathogens co-localize with the same cells *in vivo*. To address this issue, Alexeyev et al. used fluorescent *in situ* hybridisation,

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TABLE 1 Experimentally evidenced stimulators and receptors of immune responses in acne development.

Stimulators	Receptors	Response cell/tissue	References
Microorganisms-related			
C. acnes	TLR2, TLR4, CD14, PAR-2	KCs	(5–8)
		PBMCs	(9–11)
		Monocytes	(12)
		$CD3^+$ T cells	(13)
		$CD4^+CD45R$ T cells	(9, 13)
		Explants	(14, 15)
		Sebocytes	(16, 17)
		THP-1 cells	(12)
Malassezia species		PBMCs	(18)
		KCs	(19)
		Acne lesions	(20)
Staphylococcus species	TLR2	KCs	(21)
		PBMCs	(11)
		Acne lesions	(22, 23)
Porphyrin III		KCs	(24)
		Acne lesions	(25)
CAMP1	TLR2	KCs	(26, 27)
		Acne lesions	(28)
Extracellular vesicles	TLR2	KCs	(29)
		THP-1 cells	(29)
HSP60		KCs	(30)
SCFAs		Monocytes	(31)
		KCs	(31)
Lipase		Acne lesions	(20, 22, 32)
Lipoprotein	TLR2	Monocytes	(33)
		KCs	(34)
Enterotoxin B		PBMCs	(9)
Lipid mediators			
Oleic acid	CD36	Sebocytes	(35)
Lauric acid	CD36	Sebocytes	(35)
Palmitic acid	CD36	Sebocytes	(35)
Squalene		KCs	(36)
		$TREM2^+$ macrophages	(37)
Neuropeptides			
Substance P		Sebocytes	(38, 39)
CRH	CRH-R	Sebocytes	(5, 40)
α-MSH	MC-1R	Sebocytes	(40)

CAMP, Christie-Atkins-Munch-Peterson; HSP, heat shock protein; SCFAs, short-chain fatty acids; CRH, Corticotropin-releasing hormone; TLR, Toll-like receptor; PAR-2, proteinase-activated receptor-2; CRH-R, corticotropin-releasing hormone receptor; α-MSH, alpha-melanocyte stimulating hormone; MC-1R, melanocortin-1 receptor; KCs, keratinocytes; PBMCs, peripheral blood mononuclear cells.

immunofluorescence microscopy, and immunohistochemistry. They observed that within the hair follicle, both microcolonies and biofilms of *C. acnes* were present on the follicular wall, indicating that there is a direct interaction between *C. acnes* and keratinocytes (32). Using quantitative PCR, Gram staining, immunofluorescence microscopy, and 16S ribosomal RNA sequencing, Nakatasuji et al. identified acne-associated *C. acnes* and *S. epidermidis* within dermal tissues. This finding provides evidence for direct physical interactions between bacteria and different cells in the dermal layer (61). Further studies are necessary to obtain more accurate observations of the direct interactions between particular microbial species as well as secreted virulence factors and skin cells in different acne lesions.

2.2 Lipid mediators

Lipids that function as immune-stimulating substances in acne mainly originate from sebum (62). Under typical circumstances, sebum contributes to the defense of the skin against pathogens and maintenance of moisture. However, several studies have shown that changes in the composition of lipid species as well as the oxidant/antioxidant and saturated/unsaturated ratios may convert lipids into immune stimulators during the development of acne (35, 36, 62–66). For instance, sebum free fatty acids, such as lauric acid, palmitic acid, and oleic acid, can enhance the innate immune defense of sebocytes by upregulating a human antimicrobial peptide (AMP), human β-defensin (hBD)-2 (35); Additionally, peroxidated squalene can upregulate the expression levels of inflammatory mediators, such as NF-κB, peroxisome proliferator-activated receptors (PPAR)α, and IL-6 (36); Furthermore, quantities of sebum oxidation-induced lipid peroxide (LPO) and interleukin (IL)-1α are higher in the inflammatory comedones than in non-inflammatory comedones, suggesting that a certain amount of LPO may be involved in inflammatory changes in early acne lesions (67).

Various lipid species come from not only sebum but also other skin surface lipids (62) and serum (68). Although several studies have reported characteristic differences of these lipids between patients with acne and healthy controls (66, 68–72), further research is needed to understand the mechanisms linking these lipids to immune activity.

2.3 Neuropeptides

Acne vulgaris is often exacerbated in individuals with mental stress or endocrine dyscrasia (58, 73, 74). This highlights the association between acne and the neuroendocrine system. As a crucial component of the peripheral neuroendocrine system, human skin not only acts as a recipient of signals from various neuropeptides secreted by the central nervous system and transported via the bloodstream but also produces neuropeptides that modulate skin cells via paracrine, juxtracrine, autocrine, and intracrine pathways (75–77).

Obligate immune cells, such as mast cells, Langerhans cells, and macrophages, as well as nonobligate immune cells, such as sebocytes, melanocytes, endothelial cells, and keratinocytes, have been identified as targets of neuropeptides in the cutaneous immune system (75, 78). For example, calcitonin gene-related peptide (CGRP) secreted by skin sensory nerve fibers stimulate the adhesion of leukocytes and monocytes to endothelial cells as well as the release of proinflammatory mediators, such as tumor necrosis factor (TNF)-α and IL-8, from mast cells (79).

However, limited studies have characterized the direct effect of neuropeptides on the immune response in acne. Corticotropin-releasing hormone (CRH), a neuropeptide, shows significantly stronger expression in sebaceous gland cells of acne-affected skin than in non-affected skin (40). It can be secreted by the hypothalamic-pituitary-adrenal axis, keratinocytes, melanocytes, dermal fibroblasts, or endothelial cells and targets one of its receptors, corticotropin-releasing hormone receptor 2 (CRH-R2), to stimulate the release of IL-6 and IL-8 in SZ95 sebocytes (5). Substance P (SP), another neuropeptide, is present at higher concentrations in the nerve fibers around sebaceous glands in patients with acne than in healthy controls (38). Cultured sebocytes treated with SP exhibit increased secretion of proinflammatory cytokines, including IL-6, IL-1, and TNF-α (39).

Further studies are needed to determine the secretory patterns of other neuropeptides in patients with acne and their potential to initiate an immune response.

3 Innate immune response

In response to aforementioned immunostimulators, immune-related cells in the skin show alterations in proliferation and/or differentiation as well as in signaling and/or metabolic pathways. This response results in the production of defensin substances or secondary signaling molecules that activate the immune systems.

The rapid and nonspecific immune response for the prevention of the rapid spread of antigens is referred to as innate immunity. In typical skin, a relatively low pH and low oxygen microenvironment, epidermal keratinocytes in the hair follicles serve as the initial barrier against a multitude of harmful microorganisms and external factors. When the integrity of this barrier is compromised due to imbalances in physiological activity or excessive stimulation from external factors, antigens can penetrate the epidermis and reach the dermis, thereby triggering a more intense and uncontrolled inflammatory response in the deeper layers of the skin. The components of the cutaneous innate immune system, including skin cells (follicular keratinocytes, sebocytes, melanocytes and Langerhans cells), haematopoietic cells, and soluble factors (e.g., cytokines and AMPs) have been comprehensively documented by Dreno et al. (80). In this section, we illustrate the intricate processes by which immune-related cells in acne identify immune stimulators, transmit signals within cells, and generate responses. A schematic of these immune processes is shown in Figure 1.

3.1 Recognition

TLRs, especially TLR2 and TLR4 in conjunction with CD14, are major receptors involved in the recognition of microorganisms or

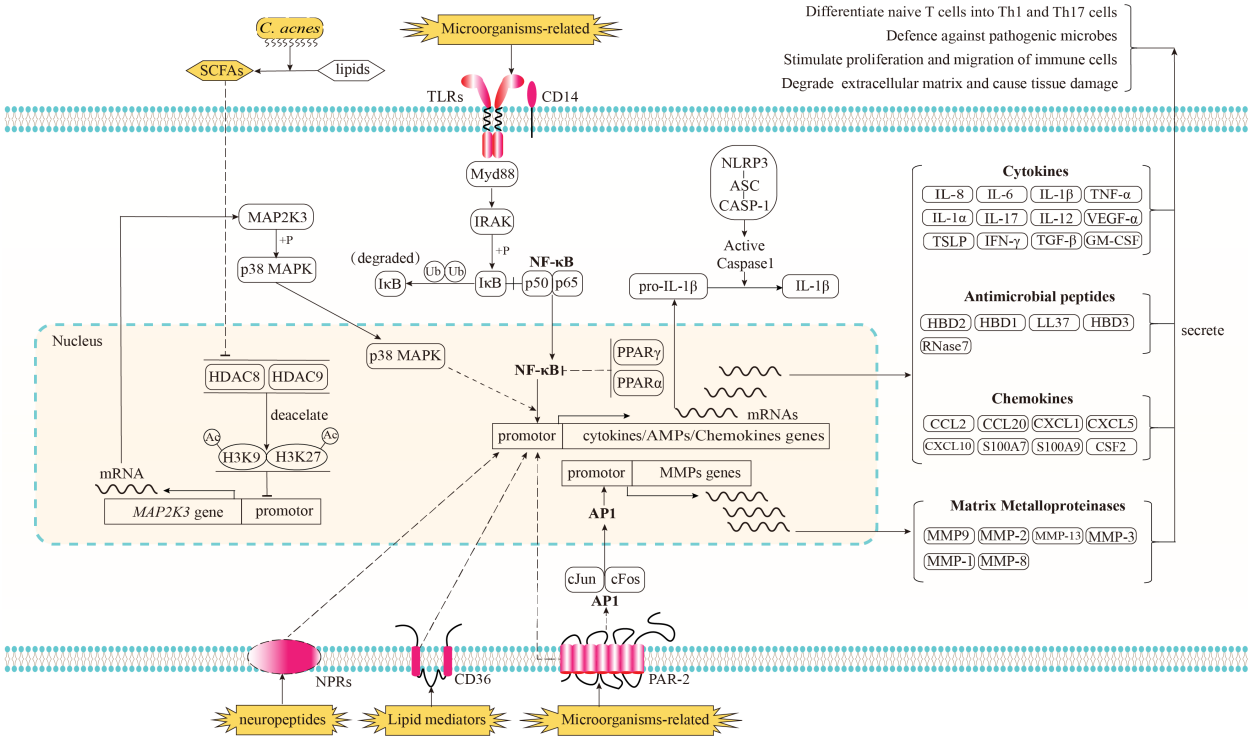

FIGURE 1
Recognition of immune stimulators and the signaling pathways implicated in the innate immune response of acne and its subsequent biological effects. Microorganisms-related stimulators can be detected by Toll-like receptors (TLRs) in conjunction with CD14 and proteinase-activated receptor-2 (PAR2). The activation of TLRs triggers the downstream NF-κB signaling pathway, resulting in the translocation of NF-κB into the nucleus and the upregulation of genes encoding cytokines, chemokines, and antimicrobial peptides (AMPs); The activation of PAR2 has been shown to elicit the transcriptional upregulation of genes encoding cytokines, chemokines, and AMPs via an unidentified pathway. Additionally, PAR2 activation triggers the downstream signaling pathway of activator protein-1 (AP-1), resulting in the translocation of AP-1 into the nucleus and an enhanced transcriptional expression of matrix metalloproteinases (MMPs). As potent anti-inflammatory factors, the nuclear receptors, peroxisome proliferator-activated receptors (PPARs) PPARα and PPARγ have the ability to inhibit the activation of NF-κB. When cultivated in an environment rich in lipids, the anaerobic fermentation of *C. acnes* can produce short chain free fatty acids (SCFAs). Certain species of SCFAs have the ability to inhibit the deacetylation function of histone deacetylase (HDAC) 8/9. The inhibition of HDAC8/9 consequently results in an amplification of the acetylation process on histone residues H3K9 and H3K27, which marker the promoter region of *MAP2K3*. This, in turn, leads to an enhanced transcription of *MAP2K3*. The heightened expression level of MAP2K3 then triggers the phosphorylation of p38 MAPK, ultimately resulting in the activation of p38 MAPK and an increase in the expression of genes responsible for cytokines and chemokines. Lipid mediators produced by sebaceous glands, such as certain species of free fatty acids (FFAs), have the potential to be identified by the lipid translocator CD36, while neuropeptide stimulators are believed to be recognized by their corresponding neuropeptide receptors (NPRs). Both of these mediators have the ability to enhance the expression of genes involved in immune responses, although the specific signaling pathways through which these receptors and immune response genes operate remain unclear. Prior to being released in an active state into the extracellular region, the inactive form of proinflammatory cytokines, such as pro-IL-1β, necessitates proteolytic processing. This processing is facilitated through the activation of the NLRP3 inflammasome complex. Subsequently, proteins of cytokines, AMPs, chemokines, and MMPs are secreted into the extracellular regions in order to regulate the functioning of neighboring cells, thereby resulting in a cascade of subsequent effects.

microbial-derived factors, such as PGN and LTA. These transmembrane proteins have been discovered in culture systems and within skin tissues. They are expressed in various skin cells, such as keratinocytes (81, 82) and sebocytes (16, 83), and in haematopoietic cells, such as PBMCs (10) and monocytic (12, 84). Their expression levels are positively regulated by *C. acnes* (81) and negatively regulated by retinoids (10). In addition to TLRs, proteinase-activated receptor-2 (PAR-2) is directly stimulated by proteases produced by *C. acnes*. Lee et al. found that PAR-2 levels are higher in keratinocytes and sebaceous glands of acne lesions than in non-lesional skin. Lee et al. found that PAR-2 levels are higher in keratinocytes and sebaceous glands of acne lesions than in non-lesional skin (85, 86). *In vitro* experiments using cultured keratinocytes and sebocytes further demonstrated the role of PAR-2 in mediating innate immunity and sebaceous lipogenesis.

Few studies have evaluated signal-receiving elements responsible for the recognition of various lipid mediators. Only free fatty acids, including lauric acid, palmitic acid, and oleic acid, have been shown to be transported by the transmembrane lipid translocator CD36 in cultured SZ95 sebocytes (35).

Neuropeptides stimulate immune responses through recognition by their corresponding neuropeptide receptors (40, 79, 87). However, studies of the roles of neuropeptide-mediated immune responses in acne are limited.

3.2 Signal transduction

When TL2 or TL4 binds to antigens with the assistance of its co-receptor, CD14, its cytoplasmic TIR domains interact with the TIR

domain of Myd88, an adaptor downstream of TLRs and IL-1 receptors. The death domain of Myd88 interacts with IL-1R-associated kinase (IRAK) family kinases via homotypic protein–protein interactions (88). Activated IRAK stimulates the NF-κB signaling pathway. In humans, NF-κB is a transcription factor in a complex consisting of p50 (NF-κB1) and p65 (rel-A) subunits (89). In normal conditions, NF-κB is sequestered in the cytoplasmic region by binding to the inhibitor of κB (IκB) in the cytoplasmic region. After receiving the upstream inflammatory signals, IκB kinase (IKK) is activated to phosphorylate IκB, and the phosphorylated IκB will undergo ubiquitylation and proteasomal degradation, resulting in the translocation of NF-κB members into the nucleus (90). The nuclear translocation of NF-κB positively regulates the mRNA expression of proinflammatory cytokines.

PAR-2 enables the activation of activator protein-1 (AP-1). Once activated, AP-1 is translocated into the nucleus and promotes the transcription of matrix metalloproteinases (MMPs) (85). However, the downstream effectors that mediate the PAR-2 pathway and stimulate cytokines and AMPs in acne have not been determined.

Before they are released into the extracellular region in an active form, the inactive form of proinflammatory cytokines, like pro-IL-1β, requires proteolytic processing. This process is dependent on the proteolytic activity of caspase-1. The inactive form of pro-caspase-1 is a part of the nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein (NLRP) inflammasome complex. Several NLRPs have been characterized according to the types of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) they are activated by (6). In acne vulgaris, NLRP3 contributes to the recognition of *C. acnes* in human monocytes, and its activation by *C. acnes* requires ROS, K^+ efflux, phagocytosis, and lysosomal destabilization (12). The activation of NLRP3 leads to proteolytic cleavage of the caspase recruitment domain (CARD) of pro-caspase-1, resulting in active caspase-1 and subsequent proteolysis of pro-IL-1β into mature and secreted active IL-1β (91).

PPARs act as anti-inflammatory factors. Dozsa et al. observed that patients with acne have lower expression levels of PPARγ and its target genes in sebocytes than those in healthy controls (92). Ottaviani et al. found that in cultured keratinocytes, peroxidated squalene could induce the secretion of the proinflammatory cytokine IL-6 through the activation of NF-κB. In this inflammatory environment, the PPARα expression level is increased, supporting the feedback reaction of PPARs to reduce inflammation via the inhibition of the NF-κB pathway (36).

Recently, histone deacetylases (HDACs) were identified as negative regulators inhibiting TLR-induced cytokine expression in keratinocytes. This regulation is crucial for maintaining immune tolerance under normal microbial conditions (31). Under hypoxic growth condition with lipid sources, *C. acnes* utilizes lipids to produce SCFAs, which in turn inhibit the activity of HDAC8 and HDAC9 (31). The inhibition of HADC8/9 increases the acetylation of histone residues H3K9 and H3K27, which mark the promoter region of *MAP2K3*. This increased level of acetylation opens the chromatin in *MAP2K3* and activates the facilitates chromatin transcription (FACT) complex, ultimately increasing the transcription of *MAP2K3*. The heightened expression of MAP2K3 is responsible for the phosphorylation of p38 MAPK and subsequent increased expression of IL-6, IL-8, TNF-α, thymic stromal lymphopoietin (TSLP), chemokine (C-C motif) ligand 5, and IFN-β (31, 93). IFN-β activates cutaneous immunity by promoting dendritic cell (DC) maturation and subsequent T cell proliferation (93).

3.3 Production of immune-related factors

After recognizing stimulators and modulating intracellular signaling pathways, skin immune cells produce and secrete immune-related soluble factors, including AMPs, cytokines, chemokines, and MMPs. AMPs are 12–50 amino acid, cationic, and amphiphilic peptides. In human skin, the best-characterized AMPs are cathelicidins and β-defensins (35, 94). They are produced in human keratinocytes and sebocytes in response to stimulators, like *C. acnes*, PGN, LPS, and *Malessezia furfur* (19, 95). AMPs directly inhibit *C. acnes* proliferation and immunomodulation by inducing angiogenesis and cytokine release (94).

Cytokines are regulators produced by host cells in response to infections and immune responses. IL-6, IL-8, IL-1β, and TNF-α are the most well-studied cytokines in acne research. IL-6 and IL-8 are secreted by monocytes (12), keratinocytes (31), PBMCs (29), and sebocytes (39) when stimulated by *C. acnes* or SP. Elevated expression levels of IL-6 and IL-8 in acne lesions also have been reported (33, 96). IL-1β expression is induced in PBMCs (10, 29), monocytes (12), and keratinocyte (31) when stimulated by *C. acnes* and SCFAs produced by *C. acnes*. IL-1 receptors (IL-1R) expressed on the membrane surface can transduce IL-1β signals into intracellular signals to activate NF-κB and AP-1 signaling pathways (89). These activated signals in effector cells promote cytokine production. TNF-α could be stimulated by *C. acnes* in cultured Th1 cells (29), infundibular keratinocyte (12), sebocytes (39), and monocytes (12) when stimulated by *C.acnes* or SP. Secreted TNF-α interacts with TNF-α receptors, stimulating the expression of vascular intercellular adhesion molecule 1 (ICAM-1) and increasing the activity of NF-κB and AP-1 (89). In addition to the aforementioned well-studied cytokines, a recent study has discovered that vascular endothelial growth factor α (VEGF-α), is secreted by a specific subset of type I conventional dendritic cells (cDC1s) during infection with either *C. acnes* or *S. aureus* in the mouse model of inflammatory acne. This secreted VEGF-α has the ability to attract neutrophils to the site of infection (97).

Chemokines are another important factor in the immune response. They act as critical mediators of immune cell migration during immune surveillance and immune development (98). In acne vulgaris, keratinocyte-secreted chemokines, including CCL2 and CCL5, $TREM2^+$ macrophage-secreted chemokines, such as CXCL16 and SPP1 (37), and sebocyte-secreted chemokines, like

CXCL8 (13), have the ability to attract immune cells to the acne region.

4 Adaptive immune response

Theoretically, adaptive immunity is activated upon exposure to antigens presented by the major histocompatibility complex (MHC) of antigen-presenting cells. The adaptive immune response is characterized by a slow speed, high specificity, and the ability to develop memory. Dendritic cells (DCs) are the key professional antigen-presenting cells activating T and B lymphocytes (3). However, specific DC subtypes responsible for the antigen presentation in acne remains understudied. In this section, we illustrate the immune cells that have been identified as critical players in adaptive immune response. And a model proposed based on these discoveries is presented in Figure 2.

Th1 and Th17 represent distinct subsets of $CD3^+$ $CD4^+$ T helper cells and are the predominant immune cell populations infiltrating the dermal papilla and around the perifollicular regions in early-stage acne. Th1 cells are recruited and activated in early acne lesions, as determined by Mouser et al. (99). who generated 14 T-cell lines from early papular inflammatory acne lesions with enhanced proliferative responses to antigens derived from *C. acnes*. Further, a Th1 cytokine pattern characterized by high IFN-γ production and low IL-4 production indicated the involvement of Th1 cells in the adaptive immune response in acne vulgaris, particularly in the early stage (99). This result was later verified by Agak et al. in a study of peripheral blood mononuclear cells treated with *C. acnes* (9).

The effector functions of Th17 cells differ from those of Th1 cells (99, 100). The differentiation and proliferation of Th17 cells are facilitated by various cytokines, such as IL-17, IL-1β, IL-6, TGF-β, and IL-23 (9). Agak et al. (9) found that Th17 can be differentiated from $CD4^+$ T cells rather than $CD8^+$ T cells when stimulated by *C. acnes*. This differentiation was indicated by the upregulation of IL-17 related genes, including IL-17, IL-17 receptor genes (IL-17RA and IL-17RC), and the downstream transcription factors (RORα and RORc). Moreover, Vitamin A (ATRA) and D (1,25D3), two commonly used immunomodulators in acne therapeutics, can inhibit *C. acnes*-induced Th17 differentiation (9).

Matti et al. demonstrated an accumulation of $CD4^+$ $IL\text{-}17^+$ cells in close proximity to the PSU, which suggests the interaction between sebocytes and these $CD4^+IL\text{-}17^+$ cells (13). Moreover, the chemoattractant process can be further enhanced by proinflammatory cytokines, such as IFN-γ, IL-17, and TNF-α. Although the $CD4^+$ $CD45RO^+$ effector T cell subset was the most abundant T cell subset attracted by sebocytes, functional activation was not observed. In contrast, the small number of $CD4^+CDRA^+$ naïve T cells attracted by sebocytes are targets for differentiation into Th17 cells. This differentiation is mediated by the sebocyte supernatant in a manner dependent on IL-1β, IL-6, and TGF-β as well as by the DCs generated in the presence of the SZ95 supernatant. This population of activated Th17 cells is characterized by elevated levels of IL-17 and IL-22. The supernatant of *C. acnes*-prestimulated SZ95 sebocytes has the

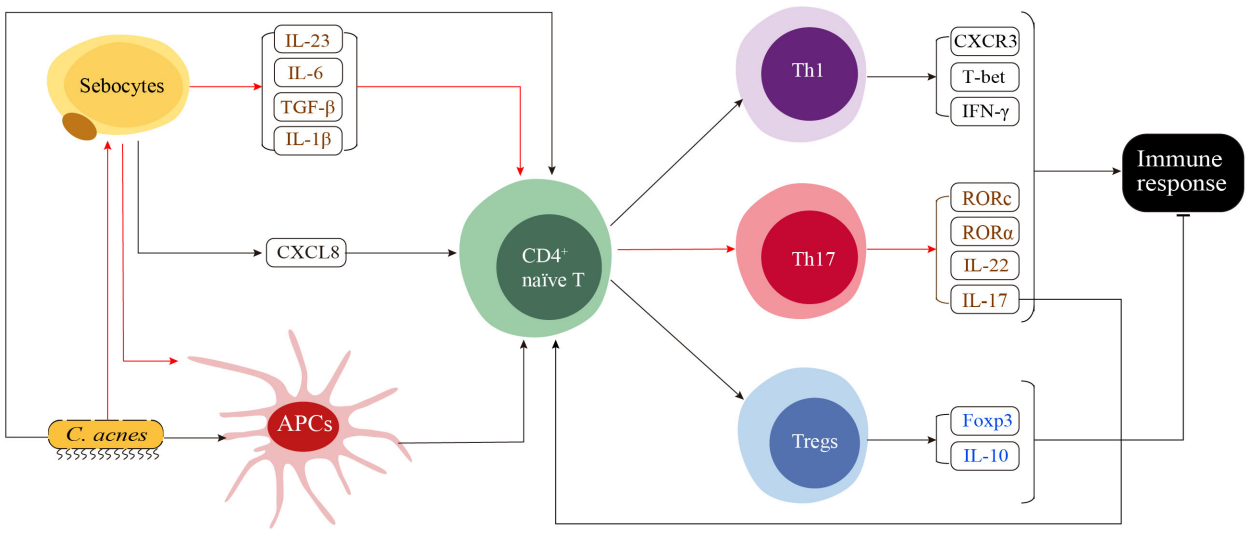

FIGURE 2
A proposed model of the adaptive immune response in acne. The innate immune response stimulates skin cells, such as sebocytes, to secrete CXCL8, which in turn recruits $CD4^+$ naïve T cells to the sites surrounding the pilosebaceous unit. $CD4^+$ naïve T cells in this region receive various stimulating signals, including cytokine signals from sebocytes, pathogen stress signals from *C. acnes* and major histocompatibility complex signals from *C. ances* and seboctes-stimulated antigen presenting cells (APCs). These signals determine the differentiation of $CD4^+$ naïve T cells into Th1, Th17 or Tregs. *C. acnes* directly induces the differentiation of $CD4^+$ naïve T cells into both Th1 and Th17 cells. Th17-related cytokines secreted by sebocytes, including IL-6, TGF-β and IL-1β, as well as the IL-23 secreted by currently unidentified cells, induce the differentiation of $CD4^+$ naïve T cells toward Th17. Additionally, the functional interaction between sebocytes and *C. acnes* induces the maturation of APCs, resulting in a preferential generation of Th17 cells over Th1 cells. The activation of Th1 and Th17 cells enhances both the innate and adaptive immune responses, while activated Tregs function as suppressors that negatively regulate the immune response.

potential to influence the primary capacity of DCs, leading to increased differentiation of naïve T cells toward Th17 rather than Th1 subsets (13).

T regulatory cells (Tregs) is characterized by high expression levels of IL-10 and FOXP3. IL-10 is an anti-inflammatory cytokine, and FOXP3 plays as a suppressive role in the immune system. Elevated expression levels of these molecules were observed in both the serum and papillary dermis of patients with acne. This finding suggests that Tregs cells may contribute to the prevention of autoimmunity and the suppression of excessive immune response in acne (7).

5 Sequential involvement of immune cells

In addition to characterizing the detailed functions of the aforementioned immune-associated factors, it is crucial to elucidate the order in which immune-related cells participate in acne development. Recently, Eliasse et al. used a multipronged approach that included flow cytometry, confocal microscopy, and bioinformatics to demonstrate that distinct cell populations play dominant roles at different stages of acne development (8). Combined with the findings of *in vivo* studies of inflammatory processes (8, 96, 101–103), a primary immune landscape of the acne process is beginning to emerge (Figures 2, 3). Disrupted homeostasis caused by the dysbiosis of microorganisms, sebum, neuroactivity, or environmental virulence factors triggers a response in the cells of PSUs. The initial immune response is triggered by keratinocytes and sebocytes, that secrete AMPs, cytokines and chemokines to attract immune cells, including $CD4^+$ helper T cells, $CD45RA^+$ memory/effector T cells, CLA^+ skin homing T cells, mast cells and $CD68^+$ macrophages. Although with immune cells infiltration, there are no clinical symptoms at this stage (non-lesional skin). Infiltrated immune cells in the comedone lesions include APCs ($CD14^+$ dermal DCs, $CD14^+CD163^+$macrophages, $CD1c^+$ conventional DC2s, conventional DC1s), $CD3^+CD4^+$ helper T subsets ($CD69^+$ resident T cells, regulatory T cells, naïve T cells, $CD161^+CXCR3^-$ Th17 cells, $CD161^-CXCR3^+$ Th1 cells, $CD161^+CXCR3^+$ Th1.17 cells) and $IL17^+$ mast cells. These cells are predominantly cluster in the papillary dermal, periductal or perivascular regions. As the comedones progress into papules and pustules, the number of $CD69^+$ resident T cells decreases, while neutrophils and B lymphocytes start to be recruited in large numbers within the lumen of pilosebaceous ducts (Figure 3).

However, little is known about the immune processes at the stages of pustules, nodules, cysts, and scars, and there are inconsistencies in study results (Figure 3). For instance, Demina et al. (104) revealed a decrease in anti-inflammatory cytokines (IL-4 and IL-10) in the serum of patients with acne. This suggests that an insufficient anti-inflammatory immune response may contribute to

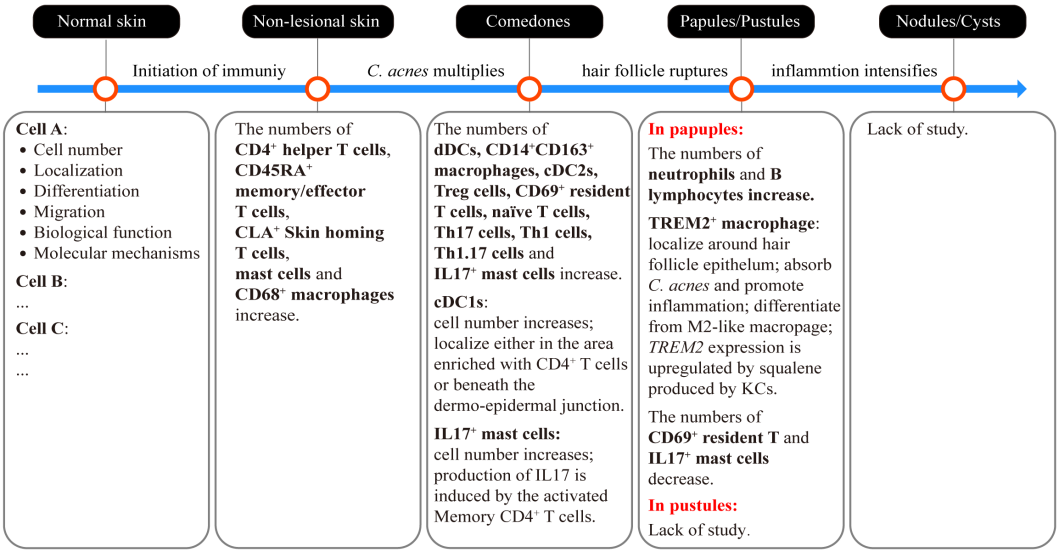

FIGURE 3
Sequential involvement of immune cells in different types of acne lesions and the critical events that drive the initiation and progression of acne. The dysbiosis of microorganisms, sebum, neuroactivity, or environmental virulence factors triggers the initiation of an immune response. At this stage, immune cells have already infiltrated the skin without any visible clinical lesions (non-lesional skin). This early-stage immune activity induces the hypercornification of the hair infundibulum and overstimulates sebocyte function, resulting in the formation of microcomedones and later comedones. *C. acnes* multiplies during the development of comedones. Continued stress from *C. acnes* and the enlargement of comedones on the hair follicles result in the rupture of the follicle wall. The contents of the comedones, including microorganisms, sebum and keratin squamae are released into the dermis, leading to the formation of papules or pustules. Ultimately, if the inflammation intensifies without control, papules or pustules may develop into more severe lesions, such as nodules and cysts. The sequential involvement of immune cells currently observed in different types of acne lesions is summarized in the corresponding text box. Further studies are needed to obtain more information, including the cell numbers, tissue localization, differentiation and migration trajectory, as well as the biological functions of specific immune cells involved in different acne lesions. Moreover, it is crucial to elucidate the molecular mechanisms that underlie these characteristics to facilitate the development of targeted treatment and prevention strategies for acne.

immunodeficiency. However, Kelhala et al. (96) found higher levels of IL-10 and Foxp3$^+$ Tregs, which can prevent autoimmunity and suppress the immune response in acne. Further investigations focused on various stages of acne are required to provide additional clarification regarding these contradictory findings and to construct a more comprehensive immune response process map at a more precise temporal-spatial scale.

Recently, single-cell sequencing technology has emerged as a powerful tool for acne research (37, 105). In an impressive example, Do et al. (2020) used single-cell and spatial RNA sequencing techniques to successfully identify a distinct subcluster of macrophages, known as TREM2$^+$ macrophages. These macrophages exhibit specificity and accumulate in early-stage acne lesions. Differentially expressed genes in TREM2$^+$ macrophages are involved in lipid metabolism and proinflammatory processes. A pseudo-time analysis revealed that TREM2$^+$ macrophages were differentiated from M2-like macrophages. Spatial RNA sequencing and ultra-high-resolution Seq-Scope have shown that TREM2 is localized in proximity to the hair follicle epithelium, which expresses squalene epoxidase. Wet experiments demonstrated that keratinocytes present in acne lesions exhibit an increased capacity for squalene synthesis. Squalene stimulates TREM2 expression in macrophages. Increased TREM2 expression enhances the phagocytic capacity of macrophages, allowing them to effectively absorb *C. acnes* and lipids. However, absorbed squalene inhibits the oxidative killing of *C. acnes*. Additionally, the upregulation of 25 proinflammatory genes was associated with the recruitment and activation of immune cells (37). These data provide a basis for identifying the specific cell types involved in the development of acne and provide information on their distribution, differentiation and migration trajectory, gene expression pattern, and biological function as well as interrelationships between different skin cells and microorganisms (Figure 3).

6 Conclusion

There has been substantial progress in our understanding of the mechanisms underlying the immune responses associated with acne development. The immune response in acne is intricately connected to the modified profiles of *C. acnes* phylotypes, related gene pools, and altered transcriptional activity. It is crucial to recognize that acne is not solely determined by the quantities of secreted sebum but also by the composition of diverse lipid species and the oxidant/antioxidant and saturated/unsaturated ratios, which ultimately determine whether these lipids exert beneficial or detrimental effects. The recognition of microbial pathogens and lipid mediators is attributed to TLRs, PAR-2, and CD36. The NF-κB, AP-1, and NLRP3 inflammasome signaling pathways play crucial roles in the expression and secretion of soluble factors associated with immune-inflammation. Conversely, the PPAR and HDAC8/9 pathways are responsible for the negative regulation of these immune-and inflammation-related soluble factors. Inflammatory events precede hyperproliferative alterations in keratinocytes within the pilosebaceous duct. Single-cell and spatial multi-omics techniques have provided key insights into the distribution, expression patterns, and functional characteristics of specific skin immune-associated cells in the context of acne and are important tools for further research.

Additional research is necessary to fully understand the influence of particular microbial phylotypes, genetic factors, lipid species compositions, and neuroendocrine activity on immune responses linked to acne *in vivo*. Obtaining comprehensive data is crucial to accurately portray immune activity in diverse lesion types with varying degrees of acne severity. Furthermore, it is imperative to determine whether these immune processes differ according to individual genetics, living conditions, and lifestyle choices. A thorough understanding of the immune processes involved in the development of acne can facilitate the implementation and advancement of targeted treatments and prevention approaches.

Author contributions

ZJ: Conceptualization, Writing – original draft, Writing – review & editing. YS: Writing – review & editing. LH: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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Association of different cell types and inflammation in early acne vulgaris

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Acne vulgaris, one of the most common skin diseases, is a chronic cutaneous inflammation of the upper pilosebaceous unit (PSU) with complex pathogenesis. Inflammation plays a central role in the pathogenesis of acne vulgaris. During the inflammatory process, the innate and adaptive immune systems are coordinately activated to induce immune responses. Understanding the infiltration and cytokine secretion of differential cells in acne lesions, especially in the early stages of inflammation, will provide an insight into the pathogenesis of acne. The purpose of this review is to synthesize the association of different cell types with inflammation in early acne vulgaris and provide a comprehensive understanding of skin inflammation and immune responses.

KEYWORDS

acne vulgaris, inflammation, immune system, *Cutibacterium acnes*, cytokines

1 Introduction

Acne vulgaris is a common inflammatory dermatosis, affecting approximately 650 million people worldwide (1, 2). Acne can negatively impact the quality of life of patients because of physical and psychosocial morbidities (3). Microcomedones and comedones are primary acne lesions that result from cystic formation in the infundibulum of the pilosebaceous unit (PSU) (4), and the majority of inflammatory lesions arise from comedones, including papule, pustule, nodule and cyst (5). The progression of acne vulgaris may not always occur in a linear manner from microcomedone to inflammatory lesions (6, 7). The etiology of acne is multifactorial and complex, mainly including hyperseborrhea and altered sebum composition, follicular hyperkeratinization, abnormalities of the microbial flora, inflammation and immune responses (8). These factors together can impair the PSU, leading to transformation of normal follicular canals into microcomedones and further progression into inflammatory lesions (9). It is now accepted that inflammation sets in early in the pathogenesis of acne (10).

Cutibacterium acnes (*C. acnes*; formerly known as *Propionibacterium acnes*) is a commensal microorganism that resides mainly in the anaerobic portions of the pilosebaceous follicles (11). Although *C. acnes* is observed in normal and acne skin, intense colonization likely causes inflammatory reactions and immune cell recruitment through dysbiosis of the skin microbiome and an imbalance of different *C. acnes* phylotypes (11–13). Based on the sequences of the recA and tly genes, *C. acnes* can be subdivided into phylotypes IA, IB, II and III (14, 15). Multilocus sequence typing (MLST) approaches further divide the type I strain into IA1, IA2, IB and IC clusters, some of which are acne-associated (IA1 and IC) (16, 17). Within microcomedones, which are usually barely visible clinically, *C. acnes* multiplies in the infra-infundibulum, resulting in bacterial colonization (18). *C. acnes* produces many enzymes and biologically active molecules to stimulate immune cells to secrete proinflammatory cytokines. The immune response to *C. acnes*, but not the bacteria itself, has a key role in the pathogenesis of acne (19).

The immune surveillance of the skin barrier is complex. Immune cells account for 7% of the cells in skin under normal conditions (20) and are involved in perceiving alarm signals and orchestrating immune responses when inflammation occurs. Because of the absence of the stratum corneum, the skin appendages become the points of entry for external pathogens, and skin commensal microbiota can extend within the dermis, establishing direct communication with the host immune system (21). The PSU is classified as a site of immune cell recruitment because alteration in microenvironments can impact skin immunobiology (22, 23). The anaerobic and lipophilic microenvironments of the PSU favor the growth of *C. acnes*, particularly in acne vulgaris.

2 Inflammation in early acne vulgaris

The early stage of acne is characterized by the subclinical microcomedones (5). The interior of microcomedones is mostly composed of lipids with clusters of bacteria, and their outer shell is made up of corneocyte layers (18). Due to increasing pressure from the expansion of the keratin layer in a confined space, hypoxia may facilitate the multiplication of *C. acnes* and lipid accumulation (24, 25). Increased sebum production supports *C. acnes* growth in the PSU. Moreover, the metabolites of bacteria can alter the sebum composition, which contributes to the inflammatory response (26). Eventually, the rupture of the follicular walls causes extrusion of the content and a rapid inflammatory response. Although both $CD4^+$ T lymphocytes and neutrophils infiltrate around acne inflammatory lesions (27), lymphocytes may play a more central role in early acne lesions than neutrophils, which are strongly attracted after the follicles have been disrupted (28). Additionally, other inflammatory cells, especially $CD4^+$ T cells and macrophages, are also observed in the perifollicular region and dermis in acne-uninvolved skin (10). This line of evidence suggests the involvement of innate and adaptive immune processes in the pathogenesis of acne vulgaris. Further studies indicate that acnes at early stage, 6-72 hours after the development of lesions, only show small papules with a minimal erythema, with neither rupture of the follicular walls nor neutrophilic infiltration. After 72 hours of the development of acne, neutrophils can be observed in 33% of lesions (28). This evidence indicates that acne vulgaris is featured by microcomedones and small papules in early stage, followed by neutrophilic infiltration. There is no agreed definition of the early stages of acne vulgaris. We defined microcomedones and small papules with no disruption of the follicle wall as the early stage of acne in our review (Figure 1).

3 Adaptive immune cells

3.1 T helper 1 cells

Epidermal T cells, mainly $CD8^+$ T cells, are distributed in the stratum basale and stratum spinosum, while dermal T cells are often situated beneath the dermal-epidermal junction or adjacent to cutaneous appendages (29). The number of $CD4^+$ T cells in the epidermis is comparable to that in the dermis, and they are only found around hair follicles. Under physiological conditions, 98% of cutaneous lymphocyte-associated antigen $(CLA)^+$ effector memory T cells reside in the skin and can initiate and perpetuate immune reactions without recruiting T cells from the blood (30). $CD4^+$ T helper (Th) cells regulate adaptive immune responses by secreting cytokines and chemokines to activate and recruit effector cells (31).

Previous studies showed that a subpopulation of *C. acnes*-specific Th1 cells is present in early acne lesions, while *C. acnes* can stimulate T cell proliferation (32, 33). Acne lesions exhibit high expression levels of Th1 effector cytokine interferon-γ (IFN-γ), Th1 polarizing key transcription factor T-bet, and the pivotal Th1 activating cytokine interleukin 12 (IL-12), suggesting the role of Th1 cells in acne. (33, 34). *C. acnes* induces production of IL-12 by monocytes via Toll-like receptor-2 (TLR-2) signaling. The innate immune system recognizes *C. acnes* via TLR-2, increasing the levels of IL-8 and IL-12 (35). In turn, IL-12 activates the transcription factor signal transducer and activator of transcription 4 (STAT4), inducing the production of IFN-γ by Th1 cells (36), while IFN-γ promotes the differentiation of Th1 cells and induces chemokine secretion to recruit immune cells. IFN-γ–stimulated sebocytes seem to foster the migration of $CD45RO^+$ T cells with no influence on cytokine secretion (37).

3.2 T helper 17 cells

In comparison to the skin of healthy individuals, acne-involved skin displays a high number of $IL\text{-}17^+$ cells near the PSU (34, 38, 39). The dermal $IL\text{-}17^+$ cells are lymphocytes, which affect epidermal keratinocytes in a paracrine manner (40). There is a significant elevation in Th17 lineage signature cytokines, including IL-1β, IL-6 and transforming growth factor-β (TGF-β), in acne lesional vs. nonlesional skin (34). *C. acnes* increases expression levels of key Th17-related genes in human peripheral blood mononuclear cells (PBMCs) (38). Correspondingly, an integrated bioinformatics study demonstrates increased infiltration of Th17

cells and Th17-related cytokines in acne lesions (41). Moreover, the number of Th17 cells is increased in the closed comedone stage of acne, indicating that Th17 cells are involved in the pathogenesis of acne, at least in early stage (42).

Sebocytes can drive a Th17 immune response via the production of IL-6, TGF-β and IL-1β. Sebocytes can recruit various subsets of T cells, including $CD4^+CD45RO^+$ effector and $CD4^+CD45RA^+$ naive T cells in a CXCL8-dependent manner. Although sebocytes do not alter the effector T-cell phenotype, they affect the migration of naive T cells and alter their developmental trajectory towards Th17 cells via the secretion of IL-6, TGF-β and IL-1β (37). In addition to its effects on Th17 cells, *C. acnes* can also promote mixed Th17/Th1 cell and Th1-like cell responses *in vitro* by inducing concomitant secretion of IL-17A and IFN-γ (39). These mixed Th17/Th1 cytokines are most likely derived from Th17 subsets displaying a degree of plasticity and acquiring functional characteristics of Th1 cells (43). Acne-associated *C. acnes* strains provide a microbial microenvironment, regulating the programs responsible for the differentiation of Th17 cells into Th17/Th1 cells (44).

Th17 cells are characterized by the production of IL-17A and IL-17F and potent inducers of tissue inflammation. IL-17 and IL-22, effector cytokines of Th17 cells, enhance the expression of antimicrobial peptides (AMPs), including cathelicidins and β-defensins (45). Human β-defensin-2 (hBD)-2 is elevated in acne. AMPs suppress excess cytokine release after minor epidermal injury to maintain inflammatory homeostasis. Other studies also showed that AMPs promote additional inflammatory responses in addition to their antibacterial activity (46, 47). Although Th17 cells can strengthen the body's defense against extracellular pathogens, the excessive Th17 responses can drive chronic inflammation, likely contributing to the development of acne (48). The role of Th17 response in acne cannot be dissociated from the local microenvironment, i.e., dysseborrhea and loss of *C. acnes* phylotype diversity.

While Th17-cell-derived IL-26 exerts direct antimicrobial activity against extracellular bacteria, it lacks antimicrobial potency against *C. acnes* (44, 49). *C. acnes* phylotypes directly influence the Th17 cytokine profile and differentially modulate the $CD4^+$ T cell responses involving the generation of Th17 cells. *C. acnes* phylotypes IA2, IB, and IC are increased in acne patients. The acne-related *C. acnes* subtypes increase secretion of IFN-γ and IL-17, while decreasing levels of IL-10 in PBMCs. In contrast, healthy skin-related *C. acnes* subtypes increase IL-10 levels (50). IL-10 can repress proinflammatory responses by downregulating IFN-γ and IL-17 (51). IL-10-producing Th17 cells are protective and exhibit microbicidal activity against *C. acnes*, whereas IFN-γ-producing Th17 cells are pathogenic without microbicidal activity (44). Acne-associated *C. acnes* strains promote the differentiation of a non-antimicrobial Th17 subpopulation ($n\text{-}_{AM}Th17$). Healthy skin-related *C. acnes* strains can specifically stimulate antimicrobial subpopulation of Th17 cells ($_{AM}Th17$) to secrete antimicrobial proteins and generate T-cell extracellular traps (TETs) capable of capturing and killing *C. acnes*. *C. acnes* is entangled in TETs in proximity to Th17 cells in acne lesional skin (52). Although TETs are involved in antimicrobial responses, whether TETs exacerbate inflammation is unclear.

3.3 Regulatory T cells

In inflammatory disorders, Th17 cells have intimate links with Foxp3-expressing regulatory T (Treg) cells in immune balance. Tissue-resident Treg cells are predominantly distributed near the hair bulge area in the steady state (53). Tregs are efficient suppressors of both innate and adaptive immune responses, which are well known to be involved in preservation of cutaneous homeostasis and in the regulation of skin immune response (54). Significantly high numbers of $Foxp3^+$ cells are observed in the papillary dermis in early acne lesions (34, 40). Treg cells in acne patients may have functional deficiency to suppress the abnormality persistent immune response in acne lesions. Treg cells lose their suppressive function and become IL-17-expressing cells under inflammatory conditions. The dysfunction of Treg cells might be a underlying mechanism accounting for chronic skin inflammation (55–57). Moreover, the number of Tregs is lower in acne lesions than in nonlesional skin of acne patients (41). However, whether an increase in the number of Treg cells alone can benefit acne remains to be determined.

Immunopathogenesis of acne vulgaris may be related to deviations of the Th17/Treg balance (41). Increases in the Th17/Treg ratio may contribute to the initiation of inflammatory processes and can negatively affect Treg-controlled homeostasis and integrity of hair follicles (58). Retinoids exert beneficial effects on acne, via inhibition of IL-17 and increase in Foxp3 expression, whereby regulating the balance between Treg and Th17 cell differentiation (59, 60). The effective drugs treatment should not only attenuate Th17/IL-17 signaling, but also improve Treg function in order to stabilize the hair follicles. Comparison of the ratio of Th17/Treg cells between acne lesional skin and healthy skin and clarification of Treg-related disturbances of homeostasis of hair follicle would be helpful to elucidate the pathogenesis of acne vulgaris.

4 Innate immune cells

4.1 Dendritic cells

Dendritic cells (DCs) are a family of antigen-sensing and antigen-presenting cells that link the innate and adaptive immune systems (61). Skin DCs can be classified into four types: epidermal Langerhans cells (LCs), conventional DCs (cDCs), plasmacytoid DCs (pDCs) and monocyte-derived DCs (62). DC subsets are developmentally imprinted and modulated by local microenvironmental and inflammatory state (63). LCs are the main DC subsets in the epidermis, taking up and processing antigens for presentation to skin resident memory T cells or effector T cells (64, 65).

Skin immunohistochemistry revealed that $CD1^+$cells (considered to be LCs) and $CD83^+$ dendritic cells were significantly higher in early acne stage than in nonlesional skin (10, 27, 28, 34). An analysis of skin biopsy samples also noted a clear increase in the number of LCs and DCs in the closed comedone stage. Interestingly, cDC2s are associated with perilesional $CD4^+$T cells (42). Bacterial peptidoglycan (PGN)-activated DCs selectively

produce IL-1 and IL-23, which efficiently activate protective Th17 cells (66, 67). It has been postulated that changes in the follicular microenvironment may increase the production of immunogenic *C. acnes* proteins. LCs process antigens and migrate to the local lymph node, where antigens are presented to $CD4^+$T cells (68).

4.2 Macrophages

Macrophages are usually regarded as terminally differentiated monocytic phagocytes. Monocytes are recruited to the tissue where they differentiate into macrophages. Macrophages are activated by different stimuli and exert heterogeneous effects in healthy and inflamed skin, and based on these effects, they can be classified into classically (M1) and alternatively (M2) activated subsets (69, 70).

Number of $CD68^+$ macrophages is significantly higher both in early acne lesions and uninvolved follicles in acne patients compared with healthy subject (10, 34, 42). *C. acnes* triggers inflammatory cytokine expression through the activation of TLR2 on macrophages, followed by the activation of the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome, Nuclear factor kappa-B (NF- κB) as well as mitogen-activated protein kinase (MAPK) signaling cascade (71–74). $TLR2^+$ macrophages are present in acne lesions and increased during the evolution of the disease (35). *C. acnes* can also stimulate type I interferon (IFN-I) synthesis via the wiring of a TLR2- TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway in human macrophages (75). In addition, IFN-I stimulates and amplifies the secretion of chemokines and other immune mediators, contributing to inflammatory responses (76).

Under normal conditions, M1 macrophages, also termed as skin-resident macrophages, surround the sebaceous glands (77, 78). Both M1 and M2 subsets can be found in acne lesions (79), and M1-like macrophages mount an antimicrobial response against *C. acnes* (80). Sebum can affect the polarization of macrophages favoring the generation of M2 macrophages (81). Lipids that accumulate in the PSU are oxidized by *C. acnes* lipase, and macrophages can phagocytose oxidized lipids, consequently becoming foam cells (82). These foam cells express TREM2 and infiltrate in acne lesions. The sebum of acne patients has a higher content of squalene (83), which can increase TREM2 expression on macrophages. TREM2 expression enhances the phagocytic capacity of the macrophages to uptake lipids and bacteria, but these macrophages are unable to kill the bacteria. Squalene-induced TREM2 macrophages contribute to inflammation by up-regulating expression of proinflammatory chemokines, cytokines, MMPs, and S100 proteins to recruit and activate immune cells (79). Accumulation of intracellular lipids and lipid metabolic products trigger the production of proinflammatory cytokines in macrophages, contributing to the immunopathology of early acne vulgaris. Notably, TREM2 macrophages are not typically present in other inflammatory skin diseases, such as psoriasis (84) and atopic dermatitis (85). However, the pathogenic role of macrophages in acne has not been fully elucidated yet and more studies are needed to characterize the functional of macrophage in acne.

4.3 Mast cells

Mast cells (MCs) are most abundant in the upper dermis and are located near blood vessels and nerve endings under physiological conditions. The MC number is not affected by age or sex (86). MCs are key effector cells that respond to allergic inflammation and innate immune responses against bacteria. A number of factors can activate MCs to release granule-stored mediators and synthesize other types of mediators, leading to the development of inflammatory dermatoses (87).

The high-affinity IgE receptor (FcεRI) and CD69 are strongly expressed in acne lesions (42). MC number and CD69 expression peaked in the closed comedone stage, indicating that activated MCs are involved in early acne lesions. The increase in the number of MCs depends on keratinocyte-produced stem cell factor (SCF). Lipoteichoic acid (LTA), a gram-positive cell wall component, stimulates an increase in the production of SCF in keratinocytes, indirectly influencing the recruitment and maturation of MCs (88). A colocalization experiment showed that most $IL\text{-}17A^+$ cells are positive for tryptase (a MC marker) and negative for CD3 and CD4, markers of T cells. Thus, MCs are possibly the cellular source of IL-17A rather than $CD4^+$ T cells in closed comedone (42). Activated Th cells drive IL-17A production in MCs via cell-cell contact. Neither classical MC stimuli nor Th cell cytokines induce IL-17 production in MCs, which means the mechanism underlying IL-17 production by MCs is tightly regulated (42, 89). IL-17A, a proinflammatory cytokine, increases CXC ligand (CXCL)8 production in epithelial cells and activates fibroblasts to recruit neutrophils (90), while neutrophils generate reactive oxygen species (ROS) that irritate and destroy follicular integrity, causing inflammatory progression of acne lesions, which are then classified as pustules (91, 92). Moreover, IL-17A synergizes with other inflammatory cytokines, leading to increased production of IL-6 and IL-8 (93). IL-17 is not a typical mast cell cytokine, but it is increasingly appreciated that innate immune cells can produce IL-17 during an inflammatory response (94). However, the underlying mechanisms by which mast cells secrete IL-17 are not clear. To understand the complex pathophysiology of acne vulgaris, it is imperative to define the mechanisms mediating IL-17 release.

4.4 Innate lymphoid cells

Innate lymphoid cells (ILCs) exhibit a lymphoid morphology; they do not express rearranged antigen-specific receptors but do have important functions in innate immunity and tissue remodeling. ILCs are subdivided into 3 subsets, ILC1s, ILC2s and ILC3s. ILC2s are the predominant tissue-resident skin ILC subset under steady state and during inflammation (95, 96). Lack of ILCs causes sebaceous hyperplasia and alters the equilibrium of skin commensal bacteria by modulating the production of palmitoleic acid, a component of sebum with antimicrobial properties, and inhibiting the growth of several species of gram-positive cocci (97). Sebaceous hyperplasia and dyshomeostasis of skin commensal bacteria induce inflammation in the pathogenesis of acne vulgaris,

which means that ILCs may be involved in the early stage of inflammation in acne. A large number of ILC3s are present in the non-lesional skin in hidradenitis suppurativa (HS) (98). Both IL-1β and IL-23 can activate ILC3s to produce IL-22 and IL-17 (99, 100). With expression of multiple Th17- and Th1-derived cytokines, ILCs are subsequently replaced by adaptive Th mediated response. It remains to be seen whether ILCs operate in the same way in humans as they do in experimental animal models. Future study is needed to investigate ILC subsets in skin of patients with acne and characterize the functional capacity of ILC to contribute to immune responses.

5 Skin cells involved in acne inflammation

5.1 Keratinocytes

As the major cell type in the epidermis, keratinocytes not only form a physical barrier but also secrete cytokines to modulate the immune response and inflammation (101). Keratinocytes express different types of pattern recognition receptors (PRRs), recognizing various pathogens and secreting cytokines, chemokines, and AMPs (102). Keratinocytes constitutively synthesize IL-1α and IL-1β (103). Excessive skin colonization of *C. acnes* can activate TLR-2 and TLR-4 on keratinocytes, resulting in the production of a panel of inflammatory mediators, including IL-8, IL-6, IL-1α, TNF-α, granulocyte–macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinase (MMP)-9 and hBD-2 (74, 104–107). These mediators activate tissue-resident immune cells to induce and perpetuate an inflammatory response. *C. acnes* is also recognized by CD36, a scavenger receptor expressed on keratinocytes, inducing a rapid production of ROS by keratinocytes, consequently leading to inhibition of bacterial growth and production of inflammation (108). Moreover, keratinocytes in hair follicles express squalene epoxidase, which converts squalene to squalene epoxide (79). Lipid peroxides, in particular squalene peroxides, have been shown to activate lipoxygenases and increase the production of IL-6 in keratinocytes in a dose-dependent manner (109). In addition, hypoxia due to increasing intraductal pressure may induce hypoxia inducible factor (HIF)-1 production, stimulating keratinocytes to produce proinflammatory cytokines (24, 110). Thus, keratinocytes can contribute at least in part to the inflammation in acne vulgaris.

5.2 Sebocytes

Sebocytes form the sebaceous gland acini belonging to the upper PSU (111, 112). Matured sebocytes secrete their contents in a holocrine manner, leading to DNase2-mediated programmed cell death (113), which affects skin barrier function (114). Human sebum is a lipid mixture, and wax esters and squalene are characteristic of sebocytes (115, 116). Sebocytes may act as immune-active cells, recognizing microorganisms and then producing AMPs and cytokines. Sebocytes are not only a target of inflammation, but also modulate of immunity (117, 118). Increased activity of androgen hormones and insulin-like growth factor 1 (IGF-1) stimulates the proliferation and differentiation of sebocytes, resulting in hyperseborrhea (119). Clinical research has demonstrated a positive correlation between serum IGF-1 levels and disease severity, especially in female acne patients (120). IGF-1 induces the expression of proinflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNF-α, in sebocytes via the NF-κB signaling pathway (121). Sebocytes express PRRs, such as TLR2, TLR4, TLR6 and CD14, to recognize *C. acnes* and produce IL-1β, IL-6 and TGF-β *in vitro*, which drives a Th17 immune response (37, 122–125). GATA6 expressed in differentiating sebocytes can induce the expression of IL-10 and negatively regulates acne-driven IL-8 and IL-17 cytokines. Expression levels of GATA6 are reduced in early acne lesions, resulting in increased acne-driven cytokines (126).

Bacterial lipases hydrolyze some of the triglycerides in the sebum to free fatty acids (FFAs), which have a proinflammatory effect and antibacterial activity (127, 128). Proteases produced by *C. acnes* activate protease-activated receptor-2 (PAR-2) on sebocytes can also induce the production of inflammatory cytokines and antimicrobial peptides (129). FFAs and *C. acnes* upregulate the expression of hBD-2 in human sebocytes to enhance innate immune defense (47, 130). The development of more anaerobic conditions in hair follicles can lead to outgrowth of *C. acnes* and buildup of short-chain fatty acids (SCFAs) (131, 132). SCFAs have been shown to amplify TLR-driven cytokine responses from sebocytes through inhibition of histone deacetylase activity and the activation of fatty acid receptors (132).

Moreover, sebocytes secrete biologically active lipids to regulate inflammation. Sebum from acne patients contains lower levels of linoleic acid and higher levels of squalene, lipoperoxides, and monounsaturated fatty acids (MUFAs), particularly palmitoleic acid (C16:1) and oleic acid (C18:1) (83, 133–135). Stearoyl-CoA desaturase (SCD) and fatty acid desaturase (FADS)-2, two enzymes responsible for the biosynthesis of MUFAs in sebocytes, are upregulated by the TLR-2 ligand macrophage-activating lipopeptide-2 (MALP2) (122, 136). Excessive generation of squalene and MUFAs increases the rate of lipid peroxidation, and their oxidation products create a proinflammatory environment and induce comedogenesis (135, 137). Palmitic acid activates the NLRP3 inflammasome to induce release of IL-1β (138) and inflammatory response in sebocytes via TLR2 and TLR4 signaling (128). Epidermal growth factor together with palmitic acid may augment the inflammatory properties of sebocytes (139). In contrast, linoleic acid has an anti-inflammatory effect via inhibition of IL-1β production in *C. acnes*-activated macrophages (81). It is qualitative changes, not quantitative changes, in sebum composition that play a central role in the development of acne (26). Finally, sebocytes can release leptin after being triggered by TLR-2 and TLR-4 or mTORC1 pathway (118, 140). Sebocyte-derived leptin induces the expression of proinflammatory lipids, such as cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LOX), and augments the expression of IL-6 and IL-8 (141, 142). Leptin also plays a pivotal role in Th17 cell differentiation (143). Sebocytes expressing leptin receptor (LEPR) may perpetuate inflammation in an autocrine manner (144). Collectively, sebocytes can provoke inflammation in acne via multiple mechanisms.

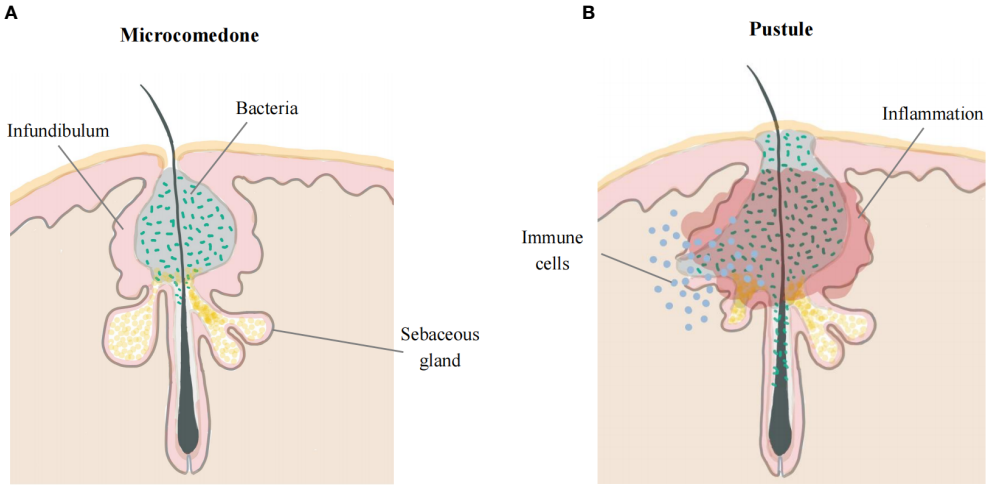

FIGURE 1
Early acne lesions and late acne lesions. **(A)** The early stage of acne occurs in the hair follicle infundibulum. Microcomedone is mostly composed of lipids with clusters of bacteria, and the outer shell is made up of corneocyte layers. **(B)** The walls of the follicles rupture, leading to extrusion of the content and causing a rapid inflammatory response.

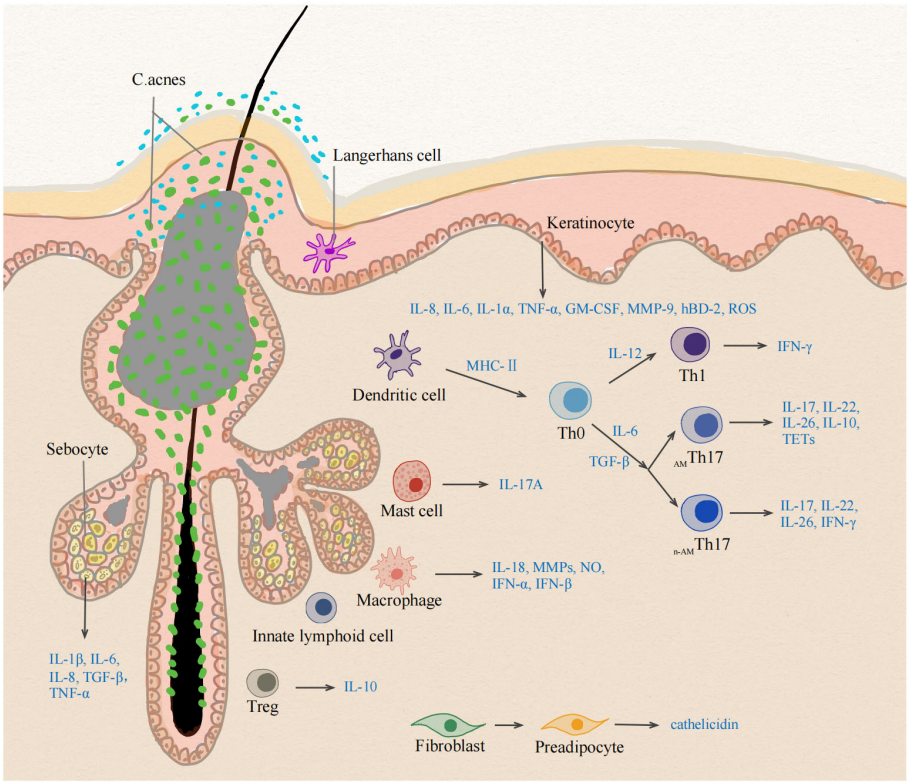

FIGURE 2
Different cell types at the early stage of inflammation in acne vulgaris. The early stage of acne vulgaris manifests microcomedones and small papules, which has no disruption of the follicle wall. The change of follicle microenvironment in acne initiate the immune activation of skin cells. Activated sebocytes, keratinocytes and skin-resident APCs upregulate the production of pro-inflammatory mediators, such as IL-1β, IL-6, IL-12 and TGF-β. IL-6 and TGF-β induce the differentiation into Th17 cells, whereas IL-12 drives a Th1 differentiation program. Healthy-related *C. acnes* induce IL-10-producing $_{AM}$Th17 cells, whereas acne-associated strains promote the development of n-$_{AM}$Th17 cells. $_{AM}$Th17 cells release IL-17, IL-22, IL-26, IL-10 and TETs, n-$_{AM}$Th17 cells induce IFN-γ. Treg lose their suppressive function for deviations of the Th17/Treg balance. MCs are the cellular source of IL-17A in early acne. Lack of ILCs leads to sebaceous hyperplasia and alters the equilibrium of skin commensal bacteria. Accumulation of intracellular lipids and lipid metabolic products induce the production of proinflammatory cytokines in macrophages. *C. acnes* triggers dermal fibroblast differentiation and enhances cathelicidin expression. APC, Antigen presenting cell; TGF-β, Transforming growth factor- Beta; IFN-γ, Interferon gamma; MC, mast cell; TETs, T-cell extracellular traps; ILCs, innate lymphoid cells. $_{AM}$Th17 cells, antimicrobial Th17 cells; n-$_{AM}$Th17 cells, non-antimicrobial Th17 cells.

5.3 Fibroblasts

Dermal fibroblasts are essential cells that support the structural integrity of tissues. Dermal white adipose tissue (dWAT) is a unique tissue layer made up of adipocytes mainly concentrated around the PSUs (145). Intradermal infection with *Staphylococcus aureus* induces proliferation and differentiation of fibroblasts into the preadipocyte lineage, leading to rapid expansion of the dWAT layer and triggering the production of antimicrobial peptides, a process dubbed reactive adipogenesis (146). Recent studies have shown that reactive adipogenesis occurs in the perifollicular stroma of acne. *C. acnes* triggers dermal fibroblast differentiation and enhances cathelicidin expression, which is partially dependent on TLR2 activity (147). Hence, dermal perifollicular fibroblasts are involved in the pathogenesis of acne and represent a potential target for acne therapy.

6 Conclusions

Acne lesions begin with the formation of microcomedones. Follicular epidermal hyperproliferation, increased sebum production and the growth of *C. acnes* in PSUs contribute to microcomedone formation.

The alteration of the follicle microenvironment stimulates skin-resident antigen presenting cells (APCs), sebocytes, and keratinocytes to produce proinflammatory cytokines, such as IL-1β, IL-6, and TGF-β. Macrophages phagocytose oxidized lipids and produce proinflammatory cytokines. MCs appeared as pioneer cells to produce IL-17, followed by the appearance of ILCs and Th cells. With the expression of multiple Th17- and Th1-derived cytokines, adaptive Th-mediated response plays a pivotal role in the early stage of acne. Deviations of the Th17/Treg balance may contribute to the initiation of inflammatory processes and negatively affect PSU homeostasis destabilizing the hair follicle infundibulum (Figure 2). The follicle walls eventually rupture, and neutrophils take over, increasing the latter stage of IL-17 production and triggering a rapid inflammatory response. The crosstalk of different skin cells in the early stage of acne remains to be revealed. Understanding these skin immune cells in the pathogenesis of early acne can facilitate the identification of biomarkers as well as the development of targeted therapies for acne vulgaris. Because of immune overactivation in acne, anti-inflammatory treatments should be employed in the management of acne.

Author contributions

LH: Writing – original draft, Writing – review & editing. SY: Writing – original draft, Writing – review & editing. XY: Writing – review & editing. FF: Writing – review & editing. LZ: Writing – review & editing. LW: Writing – review & editing. XZ: Writing – review & editing. CY: Writing – review & editing. QQ: Writing – review & editing. TZ: Writing – original draft, Writing – review & editing.

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Conflict of interest

The 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.

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Interferon type I signature associated with skin disease in juvenile dermatomyositis

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Background: Interferon type I (IFN-I) signaling system hyperactivation plays an important role in the pathogenesis of juvenile dermatomyositis (JDM).

Aim of the study: To analyze IFN-I score with disease activity in patients with JDM.

Materials and methods: Clinical manifestations laboratory data, and treatment options were analyzed in 15 children with JDM. Disease activity was assessed by CMAS (childhood myositis assessment tool) and CAT (cutaneous assessment tool) scores. IFN I-score was assessed by RT-PCR quantitation of 5 IFN I-regulated transcripts (IFI44L, IFI44, IFIT3, LY6E, MXA1).

Results: All patients had skin and muscle involvement, some had a fever (n = 8), swallowing disorders (n = 4), arthritis (n = 5), calcinosis (n = 3), lipodystrophy (n = 2), and interstitial lung disease (n = 5). Twelve patients had elevated IFN I-score and it was correlated with skin disease activity. Ten patients had clinically active disease and the level of IFN I-score and its components were higher than in patients with inactive disease (8.8 vs. 4.2, p = 0.011). IFN I-score was evaluated in nine patients during follow-up. The simultaneous reduction of IFN I-score and its components, CMAS and CAT scores was observed.

Conclusion: Skin involvement in refractory JDM is a challenging problem requiring the use of additional medications. Serum IFN I-score might be suggested as the promising biomarker of skin disease activity in JDM patients. Further investigations on patients with JDM and recurrent disease activity are needed, especially concerning biomarkers that determine the response to JAK inhibitors and treatment options for patients who don't respond to them.

KEYWORDS

inflammatory myopathy, juvenile dermatomyositis, IFN-I signaling pathway, interferon score, interferon type I signature

Introduction

Juvenile dermatomyositis (JDM) belongs to a group of idiopathic inflammatory myopathies (IIM), affecting children until 16 years and characterized by muscle involvement with skin vasculopathy (1–3). The etiology of JDM is still unclear. Hyperactivation of the interferon-I signaling system is one of the key moments of

pathogenesis, as well as the production of auto-antibodies, however, 30–40% of patients do not have auto-antibodies, which indicates other mechanisms of disease development (4, 5).

There are several sets of JDM classification criteria proposed by Bohan И Peter (1976), Tanimoto (1995), and ACR/EULAR (2017), but Tanimoto criteria predominantly uses for JDM diagnostics (6–8). Children have a higher prevalence of dermatomyositis, calcinosis, and lipodystrophy while adults are characterized by the prevalence of different subtypes of IIM, higher rate of lung and myocardial involvement, and antisynthetase autoantibodies (9).

Several special scores—CMAS (childhood myositis assessment tool) and MMT-8 (manual muscle testing) are used for the assessment of muscle involvement (10, 11) as well as CAT (cutaneous assessment tool), and CDASI (cutaneous disease area and severity index) are used for skin disease (12, 13).

There are no validated biomarkers for the assessment of JDM activity. Nowadays neopterin, CXCL11, and galectin-9 are considered as the most perspective biomarkers for JDM (14).

The ultrasound is a promising tool for assessment of skin disease in patients with connective tissue diseases (15–17).

Interferon (IFN) signature is a surrogate biomarker of IFN signaling cascade hyperactivation. It might be assessed in different tissues, e.g., blood, skin, and muscles. IFN type I and chemokine profile depend on the subtype of inflammatory myopathies (18, 19).

Patients with monocyclic JDM are good responders for traditional treatment with corticosteroids and methotrexate. Otherwise, the management of patients with polycyclic course or recurrent skin disease is still challenging for pediatric rheumatologists. Different treatment options have been used in such cases including JAK inhibitors blocking activation of IFN type I signaling system (17). In several trials and studies of agents inhibiting the IFN-I signaling pathway in IIM, the IFN signature was used as the biomarker of efficacy (20–22).

This study aimed to analyze the association between serum IFN-I score and signs of disease activity in children with JDM.

Methods

Patients

In the cohort study, 15 children (10 girls and 5 boys) with JDM from different parts of Russia have been included (nine patients are from St Petersburg, five patients are from the central area of Russia, and one patient is from the southern part of Russia).

Abbreviations: ACR/EULAR, American College of Rheumatology/European Alliance of Associations for Rheumatology; ALT, alanine aminotransferase; ANA, antinuclear antibodies; AST, aspartate aminotransferase; CANDLE, chronic atypical Neutrophilic dermatosis with Lipodystrophy and elevated temperature syndrome; CAT, cutaneous assessment tool; CDASI, cutaneous disease area and severity index; CK, creatine kinase; CMAS, childhood myositis assessment tool; CRP, C-reactive protein; DAS, disease activity score; dCAT, cutaneous assessment tool damage score; ESR, erythrocyte sedimentation rate; IIM, idiopathic inflammatory myopathies; IFN-I, interferon type I; JAK-inhibitors, Janus kynase inhibitors; JDM, juvenile dermatomyositis; LDH, lactate dehydrogenase; MMT, manual muscle testing; Me, median; PGA, physician global activity; SAVI, STING-associated early onset vasculopathy.

JDM diagnosis and disease activity assessment

The diagnosis was made by Tanimoto criteria (7). Clinical and laboratory parameters and treatment options were evaluated. Disease activity was assessed by CMAS (childhood myositis assessment tool) and CAT (Cutaneous Assessment Tool) scores (11, 12). Muscle and skin disease activity parameters (CMAS, CAT) have not been evaluated in the disease onset.

IFN signature assessment

Whole blood was collected in Tempus^{TM} Blood RNA tubes. Total RNA was extracted from blood leukocytes using Tempus RNA Isolation Kit according to the manufacturer's instruction. cDNA was subjected to reverse transcription (RT). RT reaction (final volume of 20 uL) contained 5X reverse transcriptase reaction buffer, 200 U of RevertAid Reverse Transcriptase (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania), 20 U of RiboCare RNase Inhibitor (Evrogen, Moscow, Russia), dNTP mix (20 nM each), random hexamers (0.25 μmol). The mixture of RNA, dNTPs, and primers was consecutively incubated for 5 min at 70, 65, and 60°C to achieve primer annealing, and then cooled at 0°C for 2 min; after the adding of the enzymes the reaction mix was incubated at 20°C for 5 min, 38°C for 30 min and 95°C for 5 min. A total of 40 uL of sterile water was added; 1 uL of cDNA solution was used for qPCR. PCR reaction contained 1X GeneAmp PCR Buffer I (Applied Biosystems, USA), 250 mkM of each dNTP, 200 nM of each primer and probe, 2.5 mM MgCl2 and 1U of TaqM-polymerase (AlkorBio, Russia) in a final volume of 20 uL. The following forward (f) and reverse (r) primer and probe (p) sequences were used for quantitative real-time PCR:

ifi44l_f1 ACTGTGCATGGATGACATTCC, ifi44l-r CAGGTG TAATTGGTTTACGGGAA, ifi44l_p FAM-TAAACTGATATC TGTCTGGCATACAACCTT-BHQ1, ifi44_f GAAAGAAAGAT AAAAGGGGTCATTG, ifi44_r CCATATGGTTCATAAGTTCTC AAGG, ifi44_p FAM-TCAGGAAGAGCTTACTGTCTGCCTTGA-BHQ1, ifit3_f GAACAAATCAGCCTGGTCAC, ifit3_r GAA GGATTTTCTCCAGGGAATTC, ifit3_p FAM-AACAGCAGAGA CACAGAGGGCAGTCAT-BHQ1, ly6e-f CTGCTGGTACCTG CGTCC, ly6e-r CATTCTGGAGAGGATGGCCG, ly6e_p FAM-TCACAAACCAAAGCAGCCTGTCCT-BHQ1, mx1_f CTGAATGGAGATGCTACTGTGG, mx1_r CACCTTCTCC TCATACTGGCTG, mx1_p FAM-TTGTTCTCAGCCACCGAG CCT-BHQ1, sdha_f CCACTCGCTATTGCACACC, sdha_r ATCCAAGGCAAAATACTCCAC, sdha_p R6G-CTGGTATC ATATCGCAGAGACC-BHQ2.

PCR reaction was performed using Bio-Rad CFX96 machine; conditions included enzyme activation step (10 min at 95°Ñ) followed by 50 cycles of amplification (15 s at 95°Ñ, 20 s at 58°Ñ, 30 s at 72°Ñ). Relative expression was analyzed using Bio-Rad Gene Expression software. The samples were normalized against the expression of the household SDHA gene. Fold change values were determined using the $2^{-\Delta\Delta CT}$ method.

Interferon type signature was measured by quantitation of 5 IFN I-regulated transcripts (*IFI44*, *IFI44L*, *IFIT3*, *LY6E*, *MX1*). To determine the normal range of IFN-I score values we previously

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TABLE 1 Characteristics of patients with JDM at the study inclusion.

Parameter	Results
The median age of inclusion, years, Me (25%; 75%)	8.8 (5.7; 10.8)
The median age of JDM onset, years, Me (25%; 75%)	6.2 (3.6; 7.6)
Trigger, *n* (%) patients	
Insolation	5 (33)
Acute infections	3 (20)
Unknown	7 (47)
Clinical manifestations in the onset and disease development, *n* (%) patients	
Muscle involvement	15 (100)
Gotton's papules	15 (100)
Face erythema	15 (100)
Heliotrope rash	14 (93)
Body erythema	9 (60)
Livedo reticularis	9 (60)
Skin ulceration	3 (20)
Fever	8 (53)
Cheilitis	6 (40)
Stomatitis	2 (13)
Difficulty in swallowing	4 (27)
Arthritis	5 (33)
Calcinosis	3 (20)
Lipodystrophy	2 (13)
Hepatomegaly	7 (47)
Splenomegaly	3 (20)
Interstitial lung disease	5 (33)
Myositis confirmation, *n* (%) patients	
US or MRI	9 (60)
Electroneuromyography	4 (27)
Muscle biopsy	1 (7)
Laboratory in the disease onset, *n* (%) patients	
Leucopenia	2 (13)
Elevated CRP/ESR	9 (60)
High ALT	9 (60)
High AST	13 (87)
High LDH	15 (100)
High CK	10 (67)
ANA positivity	10 (67)
Autoantibodies:	2 (13)
SRP	4 (27)
SAA	2 (13)
PM-Scl	1 (7)
Jo-1	2 (13)
CENP-B	1 (7)
Ku	
Treatment, n (%) patients	
- Pulse methylprednisolone	14 (93)
- Intravenous immunoglobulin	14 (93)
- Prednisone	15 (100)
- Methotrexate	15 (100)
- Mycophenolate mofetil	3 (20)
- Cyclosporine A	2 (13)
- Cyclophosphamide	1 (7)
- Azathioprine	1 (7)
- Tofacitinib	3 (20)
- Baricitinib	1 (7)

ANA, antinuclear antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CK, creatine kinase; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; Me, median; MRI, magnetic resonance imaging; US, ultrasound.

analyzed RNA samples from 30 clinically healthy individuals who do not have a history of a recent infectious disease. The IFN-I score in this group ranged from 0.5 to 1.9 (median 1.2); the value of ≥ 2 was considered a diagnostic threshold indicative of IFN I-pathway hyperactivation. Samples from patients with a genetically confirmed diagnosis of interferonopathy (DADA2 and SAVI syndromes) and known IFN-I scores measured in another lab were used as positive controls.

In nine patients IFN-signature was evaluated repeatedly. The analysis was done in the following subgroups: (i) active ($n = 10$) and non-active ($n = 5$) JDM patients; and (ii) JDM patients with elevated ($n = 12$) and normal ($n = 3$) IFN I-score.

The patients were divided into 2 groups according to IFN-I score (high and normal) and disease activity (active and non-active).

Statistics

The sample size was not calculated. The software Statistica (release 10.0, StatSoft Corporation, Tulsa, OK, USA), Biostat, and MedCalc were used for the data analyses.

All continuous variables were checked by the Kolmogorov-Smirnov test: no normal distribution was identified. The descriptive statistics were reported in medians and interquartile ranges (IQRs) for continuous variables and absolute frequencies and percentages for categorical variables.

We used the Mann-Whitney *U*-test to compare two independent quantitative variables and the chi-square test for the comparison of two categorical variables, or the Fisher's exact test in case of expected frequencies <5.

A comparison of two dependent quantitative variables was carried out using Wilcoxon's matched paired test and the Mac-Nemar test was applied for dependent categorical variables.

Spearmen correlation analysis between categorical and quantitative variables was performed.

A *p*-value of less than 0.05 was considered statistically significant.

TABLE 2 Dynamics of the disease activity scores in patients depending on IFN-I scores during the study.

Assessed parameters (baseline)	Whole group (n = 15)	IFN-I score, high (n = 12)	IFN-I score, normal (n = 3)	p-value
CMAS, units, Me (25%; 75%)	38 (28; 46)	35 (23; 42)	46 (40; 48)	0.07
CAT-activity, units, Me (25%; 75%)	3 (1; 5)	3 (2; 6)	0 (0;0)	0.004
CAT-damage, units, Me (25%; 75%)	0 (0; 1)	0 (0; 1)	1 (1; 1)	0.101
JDM activity changes (2nd assessment, changes since baseline)	Decreasing in IFN score	p-value	Δ IFN score	p-value
Δ CMAS, points	$r = -0.116$	0.767	$r = 0.339$	0.372
The improvement of CMAS, yes	$r = 0.189$	0.626	$r = 0.300$	0.433
Δ aCAT, points	$r = 0.105$	0.788	$r = 0.688$	0.041
The improvement of aCAT, yes	$r = 0.286$	0.456	$r = 0.821$	0.007
The improvement of dCAT, yes	$r = 0.357$	0.345	$r = 0.444$	0.231

CAT, cutaneous assessment tool; aCAT, CAT activity score; CAT, CAT damage score; CMAS, childhood myositis assessment tool; Δ, delta, difference between two measurements; IFN, interferon.

Results

Patients' demography

The median age of inclusion in the study was 8.8 (5.7; 10.8) years while the median age of the disease onset was 6.2 (3.6; 7.6) years. All patients had skin (Gottron's papules, face erythema) and muscle involvement at the onset of the disease (Table 1). Active disease status was in 10 patients and 5 patients were inactive at the time of inclusion in the study. Insolation triggered JDM in 5/15 patients and two of these five patients had sun exposure during seaside vacations with a duration of about 1–2 weeks and acute infection in 3/15 (20%). In the remaining patients, the trigger was not identified.

At the disease onset, all patients (100%) had muscle involvement, Gotton's papules, and face erythema. Other skin involvement included heliotrope rash (93%), body erythema (60%), livedo reticularis (60%), skin ulceration (20%), calcinosis (20%), and lipodystrophy (13%). Interstitial lung disease had five (33%) patients. All patients initially received the standard of care treatment with corticosteroids (intravenous, followed oral) and methotrexate. Intravenous immunoglobuline received 93% of patients. Three patients (20%) with refractive skin disease, failed standard of care treatment and IVIG received JAK-inhibitors.

Interferon I score assessment and its association with disease activity

Muscle and skin disease activity parameters (CMAS, CAT) have not been evaluated in the disease onset. The elevated IFN I-score was in 12 (80%) patients. There was no difference in laboratory parameters between patients with normal and elevated IFN I-score. Median CMAS was 35 (23; 42) units in patients with elevated and 46 (40; 48) units with normal IFN I-score ($p = 0.07$).

Active skin disease was only in patients with elevated IFN I-score: CAT score was 3 (2; 6) points in patients with elevated IFN I-score compared with patients with normal IFN I-score–0 (0; 0) points ($p = 0.004$). The levels of CMAS and CAT depending on the IFN-I score level are in Table 2. Ten patients had active disease. Comparison between patients with active and non-active diseases revealed differences in IFN I-score and its components, in laboratory parameters and activity scores (Table 3).

Follow-up interferon type I assessment

Interferon type I-score has been measured in nine patients during follow-up. During the study IFN I-score decreased in 7/9 (78%) patients, CAT score in 7/9 (78%) patients and CMAS increased in 8/9 (89%) patients. In all patients with skin disease activity CAT score and IFN I-score reduction were observed ($r = 0.687$; $p = 0.041$) (Supplementary Figure 1 and Table 2). Positive correlations between IFN I-score, its components, and

TABLE 3 IFN I-score in patients depending on the JDM disease activity.

Parameters	Active disease (n = 10)	Non-active disease (n = 5)	p-value
IFN I-score, total, Me (25%; 75%)	13.6 (8.9; 24.7)	1.4 (1.4; 2.0)	0.006
IFI44, Me (25%; 75%)	30.5 (18.3; 42.1)	2.6 (1.4; 2.9)	0.006
IFI44L, Me (25%; 75%)	48.6 (27.2; 87.5)	2.3 (2.2; 3.1)	0.009
IFIT3, Me (25%; 75%)	11.7 (5.1; 15.9)	1.0 (0.9; 1.5)	0.006
LY6E, Me (25%; 75%)	10.2 (4.7; 23.6)	1.3 (1.0; 1.3)	0.012
MX1, Me (25%; 75%)	12.2 (6.1; 20.3)	1.5 (1.4; 2.0)	0.006
ESR, mm/h, Me (25%; 75%)	12 (6; 15)	2 (2; 2)	0.004
ALT, UE/ml, Me (25%; 75%)	35 (23; 124)	15 (12; 16)	0.037
LDH, U/l, Me (25%; 75%)	364 (272; 461)	(163; 233)	0.024
CMAS, Me (25%; 75%)	34 (18; 38)	46 (40; 48)	0.019
CAT activity, Me (25%; 75%)	4 (3; 6)	0 (0; 1)	0.001

CAT, cutaneous assessment tool; aCAT, CAT activity score; CMAS, childhood myositis assessment tool; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase.

TABLE 4 Correlation between IFN-I score (r) and its components with activity and symptoms of JDM.

Presented parameter	IFN-score at baseline	IFN-score follow-up	IFI44	IFI44L	IFIT3	LY6E	MX1
Disease activity	0.577*	0.707*	0.637*	0.643*	0.54*	0.541*	0.570*
aCAT	0.498	0.691*	0.548*	0.457*	0.525*	0.449	0.551*
dCAT	−0.194	−0.522*	−0.352	−0.199	−0.312	−0.114	−0.287
Cheilitis	0.458	0.408	0.352	0.488*	0.440	0.524*	0.361
Arthritis	0.999*	0.500	0.999*	0.999*	0.999*	0.999*	0.999*

aCAT, cutaneous assessment tool activity score; dCAT, cutaneous assessment tool damage score; IFN, interferon. *$p < 0.05$.

disease activity, CAT activity, cheilitis, and arthritis were observed (Table 4).

Treatment with JAK-inhibitors

Three patients with refractory skin disease were treated with tofacitinib. All patients had an increased IFN-I score. One patient achieved complete remission (no skin and muscle disease activity) with normalization of IFN-I score and the remaining two patients had a partial response to tofacitinib. They had an initial improvement of skin disease, followed by a flare when prednisone tapered less than 0.2 mg/kg. In both of these patients have decreased IFN-I score, but its normalization could not be achieved.

Discussion

We performed indirect measurements of type I interferon activity using relative expression levels of five IFN I-stimulated genes previously used in JDM patients (23, 24). The exact functions of molecules encoded by the corresponding genes and their role in JDM pathogenesis are unclear; the expression of these IFN I–I-stimulated transcripts is used as a surrogate marker of IFN I signature.

Our study supports previous results that the IFN I-score is associated with skin activity and could be used as a skin disease biomarker. In our group of patients, a decrease in IFN I-score corresponded with a decrease in disease activity. A correlation between IFN I-score and arthritis was found.

Interferon type I hyper-activation in JDM pathogenesis

The role of interferons in the development of dermatomyositis has been studied for the last 10–15 years. The majority of these studies demonstrated hyperactivation of the IFN I signaling pathway in blood, muscle, and skin tissue in patients with dermatomyositis (4).

Baechler et colleagues analyzed IFN-inducible gene and IFN chemokine scores in adult and pediatric patients with dermatomyositis. Both groups of patients had higher scores than healthy controls. The correlation of IFN gene score with disease activity has been demonstrated only in adult patients, while IFN chemokine score correlated with muscle disease activity and global VAS in adults and children (25). The positive correlation of IFN type I signature in blood with disease activity in the majority of patients with dermatomyositis and polymyositis was observed, except in the patients with inclusion body myositis (26).

The assessment of IFN signature might help distinguish between subtypes of IIM in adults. IFN I-signature in muscles was associated with dermatomyositis, whereas IFN- γ signature inclusion body myositis and antisynthetase myositis (18).

The changes in interferon chemokine score (IP-10, MCP-1) corresponded with changes in extra muscular disease activity score during two subsequent visits in 20 children with JDM (27).

Interferon type I score based on measuring 28 transcript expression profiles was compared between patients with JDM and monogenic type I interferonopathies (CANDLE, SAVI). Patients with JDM had higher IFN I scores than healthy controls, but lower than patients with interferonopathies. High IFITI expression led to the elevation of IFN I-score in JDM than in SAVI and CANDLE. IFN I-score moderately correlated with JDM disease activity scores (physician global activity (PGA), manual muscle testing (MMT), extra-muscular global and skeletal activity, and Disease Activity Score–DAS) (28).

Interferon type I blockade with JAK-inhibitors

Several studies demonstrated the efficacy of JAK inhibitors in patients with pediatric and adult dermatomyositis. IFN I-score could be considered as a biomarker of treatment efficacy. Four adult patients with refractory dermatomyositis (remained active disease after initiating of two different immunosuppressive drugs with corticosteroids with or without immunoglobulin) and elevated IFN I-score received ruxolitinib up to 40 mg per day for 3 months. Clinical remission (no skin and muscle disease activity) and IFN I-score reduction were reported in all patients (29).

Facial skin rash and CDASI score improved in all four patients and muscle strength improved in patients with clear muscle weakness and creatine kinase levels also decreased significantly in one of them. All patients reported an improvement in their quality of life score. Ruxolitinib decreased IFN levels and interferon-stimulated genes score in PBMCs (29).

Tofacitinib treatment allowed for achieving clinical improvement (increased CMAS from 18 to 40 points) and reduction of IFN I-score in JDM patients with persistent disease activity and high IFN I-score (30). During treatment with

tofacitinib 11 mg/day of adults and children with refractory dermatomyositis, 50% of the patients experienced moderate improvement and 50% had minimal improvement and the mean change in the CDASI activity score over 12 weeks was statistically significant (since 28 ± 15.4 at baseline vs. 9.5 ± 8.5 at 12 weeks) (p = 0.0005) with the decreasing of serum chemokine levels of CXCL9/CXCL10 from baseline was demonstrated (31).

Our previous experience demonstrated the high efficacy of tofacitinib in one of two patients with refractory JDM. Tofacitinib controlled skin disease and allowed discontinued corticosteroids (32).

Study limitations

The study limitations are related to the small number of patients, heterogeneity of the studied population according to disease activity and disease duration, and absence of testing the most frequent autoantibodies for JDM (anti-TIF1 and anti-NXP2). The absence of the clinical and interferon type I score assessment (CMAS, CAT) in the disease onset influences study results. Previous treatment (pre-assessment of interferon type I score) may misrepresent study results.

Conclusion

Skin involvement is refractory JDM is a challenging problem requiring using additional medications. Hyperactivation of the IFN I-signaling system in JDM patients was observed. Serum IFN I-score might be suggested as the promising biomarker of skin disease activity in JDM patients. Further investigations on patients with JDM and recurrent disease activity are needed, especially concerning biomarkers that determine the response to JAK inhibitors and treatment options for patients who don't respond to them.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Saint-Petersburg State Pediatric Medical University approved the study (protocol # 1/3 îò 11.01.2021). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and approved the final version to be published.

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Conflict of interest

The 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/fmed.2024.1214920/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The dynamics of IFN I-score and JDM activity scores measurement during the study.

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Spatial transcriptomics reveals altered lipid metabolism and inflammation-related gene expression of sebaceous glands in psoriasis and atopic dermatitis

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Sebaceous glands drive acne, however, their role in other inflammatory skin diseases remains unclear. To shed light on their potential contribution to disease development, we investigated the spatial transcriptome of sebaceous glands in psoriasis and atopic dermatitis patients across lesional and non-lesional human skin samples. Both atopic dermatitis and psoriasis sebaceous glands expressed genes encoding key proteins for lipid metabolism and transport such as *ALOX15B, APOC1, FABP7, FADS1/2, FASN, PPARG*, and *RARRES1*. Also, inflammation-related *SAA1* was identified as a common spatially variable gene. In atopic dermatitis, genes mainly related to lipid metabolism (e.g. *ACAD8, FADS6*, or *EBP)* as well as disease-specific genes, i.e., Th2 inflammation-related lipid-regulating *HSD3B1* were differentially expressed. On the contrary, in psoriasis, more inflammation-related spatially variable genes (e.g. *SERPINF1, FKBP5, IFIT1/3, DDX58*) were identified. Other psoriasis-specific enriched pathways included lipid metabolism (e.g. *ACOT4, S1PR3)*, keratinization (e.g. *LCE5A, KRT5/7/16*), neutrophil degranulation, and antimicrobial peptides (e.g. *LTF, DEFB4A, S100A7-9*). In conclusion, our results show that sebaceous glands contribute to skin homeostasis with a cell type-specific lipid metabolism,

which is influenced by the inflammatory microenvironment. These findings further support that sebaceous glands are not bystanders in inflammatory skin diseases, but can actively and differentially modulate inflammation in a disease-specific manner.

KEYWORDS

sebaceous glands, psoriasis, atopic dermatitis (AD), spatial transcriptomics, lipid metabolism, inflammatory skin diseases

Introduction

Acne, one of the most prevalent diseases in adolescents, provides evidence that sebocytes may be disease drivers by increasing lipid production (1–4). Gene expression analyses of whole tissue acne samples and sebocyte cell lines showed that sebocytes are able to respond to a wide repertoire of both local and systemic stimuli, such as hormones, growth factors and neuroendocrine mediators, with an increased expression of inflammatory cytokines, cholesterol biosynthesis, cyclooxygenase and lipoxygenase (5, 6). This suggests that sebocytes may contribute to the pathogenesis of acne and have a complex impact on skin metabolism and inflammation. Advances in sebaceous gland (SG) research including the detection of Toll-like receptors (TLRs) on the surface of SGs (7), changes in gene expression patterns in response to their activation (8, 9), and the production of antimicrobial peptides (10–13) have led to the introduction of "sebaceous-immunobiology" (14), suggesting that the active role of SGs in disease pathogenesis may extend far beyond acne.

Results from immunostainings and whole tissue gene expression data suggest that seborrhoeic dermatitis is centered around dysfunctional SGs, in which metabolized sebum lipids may induce inflammation (15, 16). The presence of enlarged SGs in rosacea also suggests a central role in the pathology of this disease (17, 18). Therefore, SG-rich areas, enlarged SGs and seborrhoea are thought to contribute to inflammatory skin diseases. However, our increasing knowledge of the immune-competence of sebocytes allowed further intriguing speculations as to whether SGs could indeed independently drive disease pathologies in two of the major inflammatory skin diseases such as atopic dermatitis (AD) and psoriasis (PSO).

AD is characterized by dry skin and inflammation, starting in SG poor areas, and later involving SG-rich parts, such as the face (19). Lipid analysis of the epidermis showed that the characteristic lipid barrier disruption in AD is a result of keratinocyte dysfunction and reduced levels of sebum lipids (20, 21). In contrast, PSO often starts on the scalp, especially in the early-onset form, and subsequently prefers sites with low sebum production, i.e. elbows and knees. However, in the distinct entity known as "sebopsoriasis" or "seborrhiasis" (seborrhoeic dermatitis + psoriasis), PSO lesions occur at the same sites as seborrhoeic dermatitis (22). This topographical coexistence, as well as other findings such as SG atrophy observed in the chronic phase of both diseases (23, 24), provide excellent starting points to further investigate the functional sebaceous (immuno)biology in PSO and AD (25, 26).

In this work, we aim to clarify the role of SGs in the development and disease homeostasis of AD and PSO. Therefore, we investigated and compared the spatial transcriptomic changes in SGs of lesional (L) and non-lesional (NL) human skin samples.

Results

SGs are characterized by their active lipid metabolism, lipid-related gene expression and protein abundance. Recently, sebocytes have been implicated in immunoregulatory functions (14). However, comprehensive analyses of their *in vivo* gene expression profile are lacking. Therefore, we aimed to identify differentially expressed (DEGs) and spatially variable genes (SVGs) in SGs of human NL, AD and PSO skin by spatial transcriptomics (Methods). Briefly, we manually annotated sebaceous glands in PSO, AD and NL skin samples (Figures 1A, B), visualized the data (Figure 1C), analyzed spatial patterns of SG-specific SVGs (Figure 1D), DEGs (Figure 1E) and pathway enrichments (Figure 1F).

Sebaceous glands exert a specific pattern of gene expression in the skin

First, we identified the gene expression profile of SGs in NL skin samples. Our results showed that SGs have a specific gene expression signature that clearly distinguishes them from other structures within the skin (Figure 1C). Our analyses of SGs in NL skin compared to the rest of NL skin delivered a large set of 5,449 differentially expressed genes highlighting the unique characteristics of SGs (Supplementary Table S2, Supplementary Figure S3).

To further dissect the spatial expression profile of SGs in NL skin, we identified SVGs and distinct spatial expression patterns (Figures 2A–J; Methods) (27). Four of the expression patterns were significantly enriched in SGs (Figure 2K): pattern 1 (1,178 genes, padj value: 9.20e-23), pattern 7 (1,071 genes, padj value: 1.92e-07), pattern 8 (495 genes, padj value: 6.77e-18), and pattern 9 (393

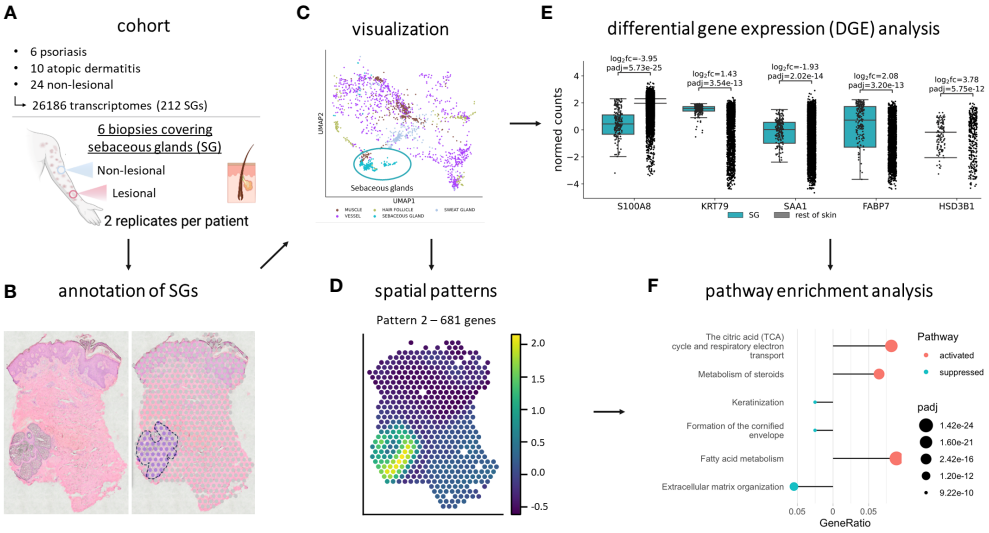

FIGURE 1
Study cohort and workflow. **(A)** The spatial transcriptomics dataset contains 6 lesional and non-lesional skin samples from psoriasis and atopic dermatitis patients. 6 psoriasis, 10 atopic dermatitis, and 24 non-lesional spots, containing 26,186 transcriptomes, of which 212 were of sebaceous glands, were analyzed. After **(B)** manual annotation for sebaceous glands and **(C)** visualization, the dataset was subject to **(D)** SpatialDE, **(E)** differential gene expression, and **(F)** pathway enrichment analysis. Created with BioRender.com.

genes, padj value: 5.02e-29). Pathway enrichment analysis provided further insight into the SG-related patterns (Supplementary Table S3). Genes from pattern 9 revealed SG-typical pathways related to lipid, fatty acid, steroid, and cholesterol metabolism, and energy production (Figure 2L). Genes from pattern 1 were associated with mitochondrial function, the citric acid cycle and energy production (Figure 2M). Pattern 7 genes were linked to intracellular transport and cell cycle (Figure 2N).

Sebaceous gland transcriptome is different in atopic dermatitis and psoriasis

Extending our studies to L samples of AD and PSO, distinct gene expression profiles of NL and L SGs were revealed (Figure 3A). We identified genes with significantly altered expression levels in SGs compared to the rest of the skin in each of the above conditions and applied pathway enrichment analysis (Figure 3B). The top 20 pathways enriched in NL SGs compared to the rest of NL skin showed SG-typical functions related to lipid, cholesterol, or steroid metabolism, among others, and were used as a reference for the analysis of changes in DEGs in L SGs. Comparing the enriched pathways of DEGs in SGs in NL and AD skin, we found that SGs altered their specific gene expression signature related to synthesis of very long chain fatty acyl-CoAs, SREBP-regulated cholesterol biosynthesis, glycerophospholipid biosynthesis, and biotin transport in AD SGs. When assessing DEGs in SGs of PSO samples, pathways such as the citric cycle, electron transport and ATP synthesis, vitamin metabolism and branched-chain amino acid catabolism, which were enriched in NL and AD SGs, could not be identified. Importantly, gene clusters determining key SG functions such as peroxisomal lipid, steroid, fatty acid, cholesterol, and linoleic acid metabolism, as well as the activity of SREBP, were detectable in both AD and PSO.

To better understand the biology of SGs at a finer spatial scale, SVGs were identified using spatialDE (see also Materials & Methods). In both AD and PSO, SGs continued to express genes encoding key proteins for lipid metabolism and transport such as *ALOX15B* (Figures 3C, D), *APOC1, FABP7* (Figures 3E, F), *FADS1, FADS2, FASN, PPARG*, or *RARRES1* among others at high levels (Supplementary Table S4). Inflammation-related *SAA1* was also identified as a common AD/PSO SVG (Figures 3G, H). AD SG-specific SVGs included lipid metabolism-related genes such as *ACAD8, FADS6*, or *EBP* (Figure 3I), but also revealed inflammation-related *CCL17* and *HSD3B1* (Figure 3K). In PSO SGs, *SERPINF1* (Figure 3J) and immune function-related *FKBP5* (Figure 3L) were identified as SVGs. Other PSO-specific SVGs were the typical lipid metabolism-related gene *ACOT4, and S1PR3*, which is involved in proliferation and inflammation in PSO (28) (Supplementary Table S4). SVG expression was shown on previously annotated lesional atopic dermatitis (Figure 3M) and psoriasis (Figure 3N) slides.

Sebaceous glands show profound changes in their lipid production-related gene expression profile in atopic dermatitis

Having identified the genetic programs specific to SGs in the context of the whole skin, we aimed to define further disease-specific gene expression changes. Therefore, we compared the gene expression profiles of SGs in L AD skin with those of SGs in NL samples. The top 3 enriched pathways were cholesterol biosynthesis, fatty acid metabolism and steroid metabolism

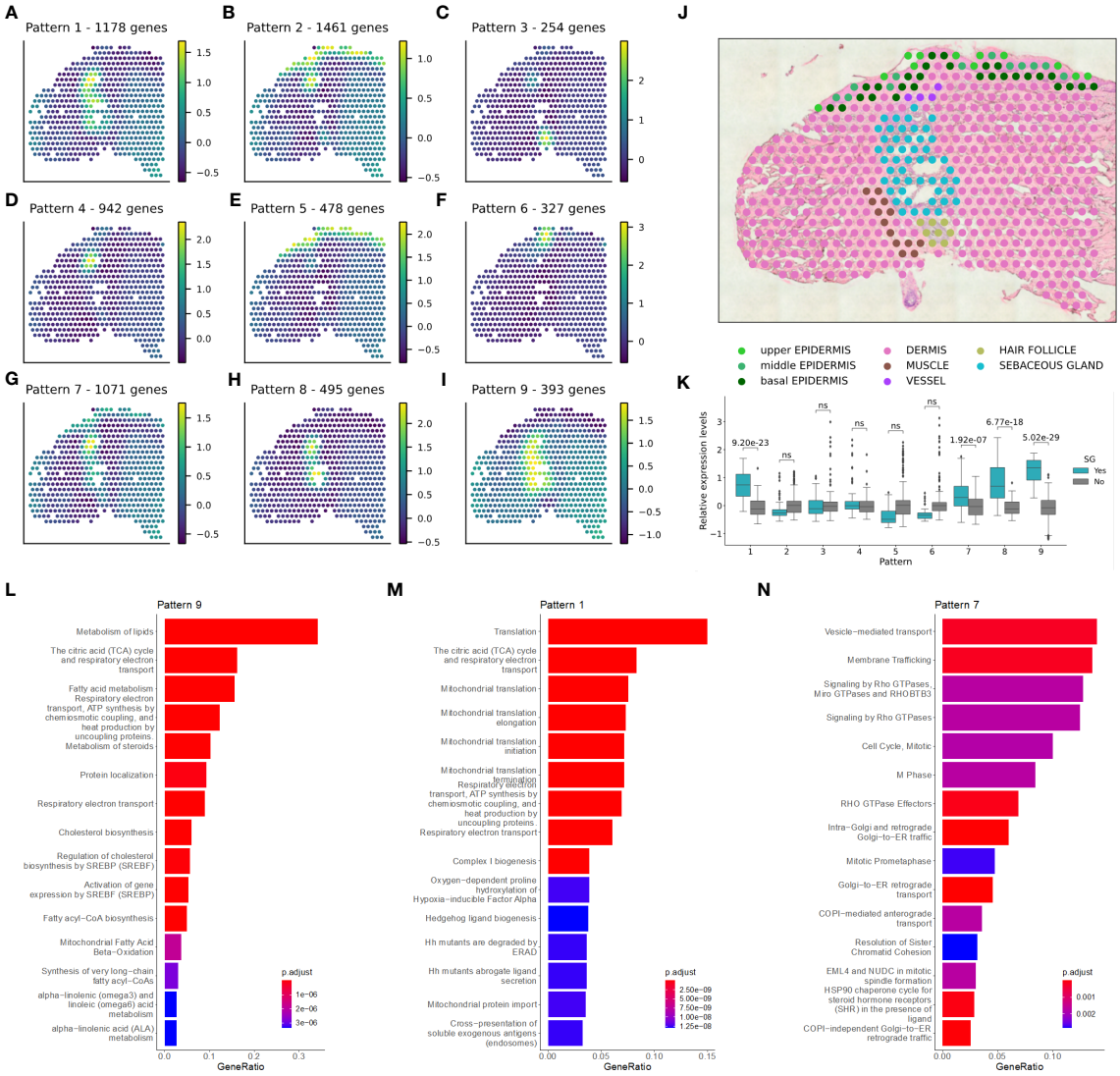

FIGURE 2
Sebaceous glands have a pivotal role in lipid metabolism-related tasks in non-lesional skin. Spatially variable genes and distinct spatial expression patterns were identified in non-lesional skin samples. **(A–I)** Enriched patterns for one replicate of a **(J)** non-lesional skin sample is shown. **(K)** Significant enrichment of sebaceous gland spots in the pattern intensities was calculated using Mann-Whitney U one-sided (greater) test. Pathway enrichment analysis in patterns **(L)** 9, **(M)** 1, and **(N)** 7.

(Figure 4A). These results provide further evidence that SGs in AD actively modify their lipid profile already at the level of gene expression. Clusters such as ATP synthesis and electron transport further reveal an altered metabolic activity for SGs in AD skin.

Gene signature encoding type 3/Th17-related immune functions distinguishes sebaceous glands in psoriasis and atopic dermatitis

By comparing the gene expression profile of SGs from L PSO and NL samples, we identified PSO-typical pathways related to differentiation (keratinization, cornified envelope formation) and inflammation (neutrophil degranulation, antimicrobial peptides; Figure 4B). In further analyses, we compared the gene expression profiles of L PSO vs. L AD SGs. In PSO, SGs gained immunocompetence. Besides immune features such as interferon signaling (e.g. *IFIT1/3, DDX58*) and production of antimicrobial peptides (e.g. *LTF, DEFB4A, S100A7-9*), significant differences were found in the expression of genes related to keratinization (e.g. *LCE5A, KRT5/7/16*) and SUMOylation in PSO (Figure 4C).

Discussion

In this manuscript, we present an *in vivo* human spatial transcriptome signature analysis of SGs. Compared to the limitations of whole tissue analysis or *in vitro* data, spatial transcriptomics allowed us to define the transcriptome of sebocytes within small groups of cells *in vivo*. Using SpatialDE, a spatial gene clustering approach that enables expression-based

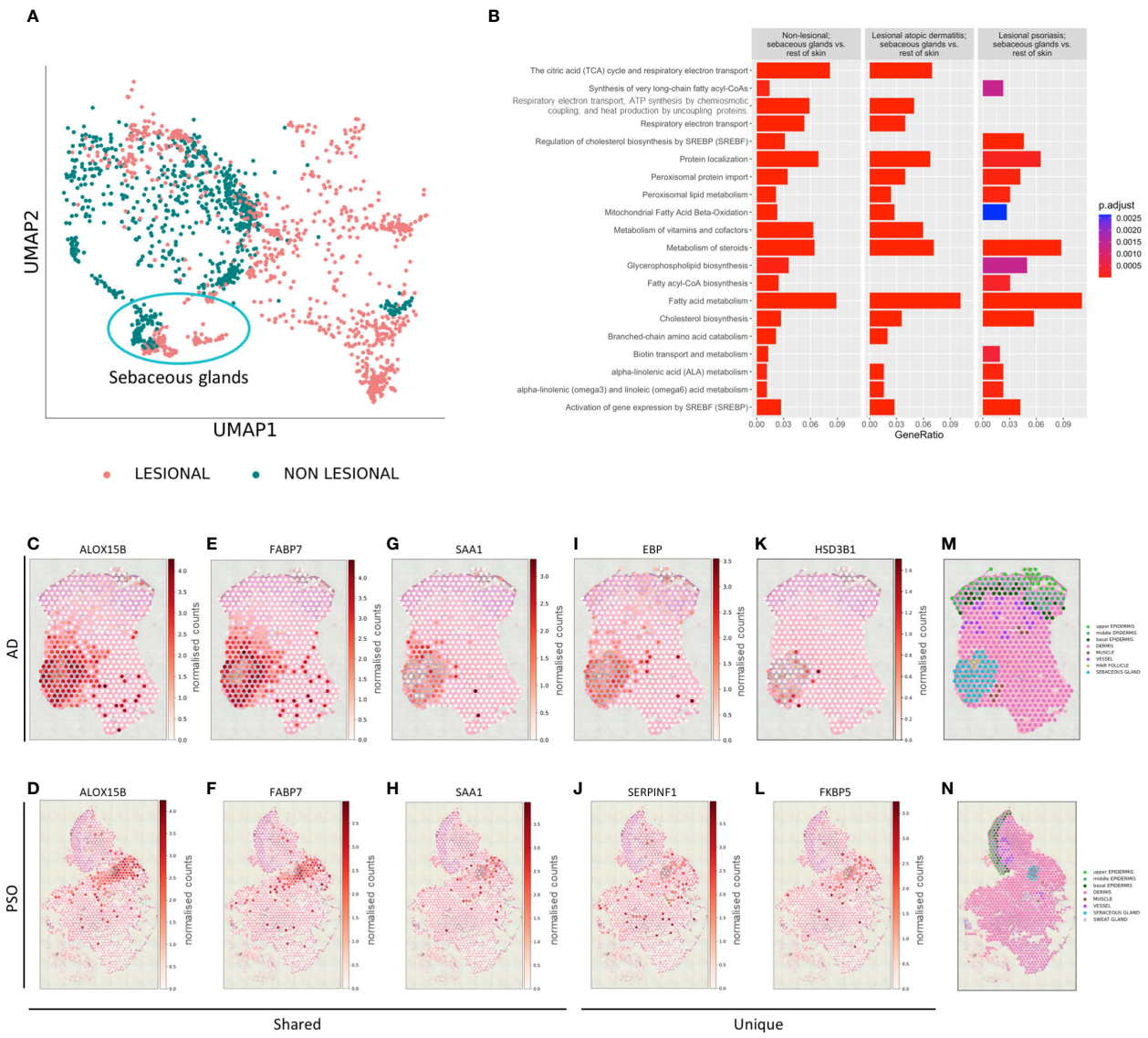

FIGURE 3
Sebaceous glands' transcriptome changes in inflammatory microenvironment. **(A)** UMAP plot of gene expression of non-lesional and lesional SGs. **(B)** Top 20 Reactome pathways of enrichment analysis comparing non-lesional sebaceous glands vs. the rest of non-lesional skin and corresponding enrichment in lesional atopic dermatitis sebaceous glands vs. the rest of lesional atopic dermatitis skin, and lesional psoriasis sebaceous glands vs. the rest of lesional psoriasis skin. Selected spatial variable genes enriched in sebaceous glands **(C–H)** shared across atopic dermatitis and psoriasis, **(I, K)** unique to lesional atopic dermatitis and **(J, L)** unique to lesional psoriasis. Annotated lesional **(M)** atopic dermatitis and **(N)** psoriasis slides.

tissue histology (27), we were able to study the biology of SGs at an even more granular scale.

SGs are well-defined, easily identifiable structures within the skin, composed predominantly of sebocytes. Although this minimizes annotation or contamination errors, a methodological limitation of our work is that the 55 µm spot size of the Visium Spatial Gene Expression slide (10x Genomics) used to analyze the samples does not allow conclusions to be drawn at the level of individual cells. This is more pronounced in acne samples, where the inflammatory cell infiltrate is also localized in the partially damaged pilosebaceous unit; therefore, we stuck to the two most common inflammatory skin conditions, PSO and AD, where the pilosebaceous unit is not the target of inflammation. A comparison of our data to the SG-specific transcriptome of acne lesions would have been desirable. Nevertheless, aside from the above mentioned limitations, published whole tissue analyses do not provide sebocyte-specific gene expression data (29), while available single cell RNA results on acne samples lack sebocyte-specific data (30). Future spatial transcriptomics studies focusing on SGs in acne lesions will allow further conclusions on the specific role and comparison of SGs in acne and other inflammatory skin diseases. Other limitations are that SGs are rare in lesional PSO and AD samples, and the size of the cohort analyzed in our study is also small, although the total of more than 26,000 transcriptomes analyzed allowed us to delve deep into the SG transcriptome.

While confirming the overexpression of lipid metabolism-related genes in SGs, our spatial transcriptomics analysis shed light on previously unstudied pathways. The highly active cell

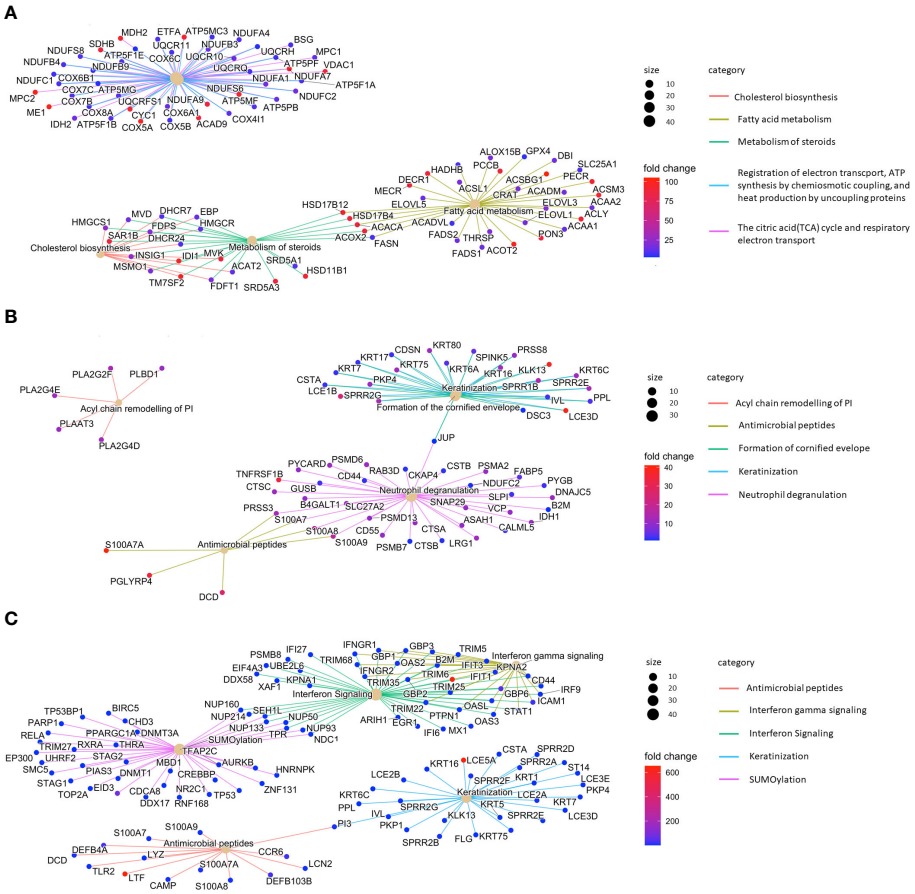

FIGURE 4
Sebaceous glands contribute to type 3 inflammation/Th17 immunity. Top 5 enriched pathways of **(A)** lesional atopic dermatitis sebaceous glands vs. non-lesional sebaceous glands, **(B)** lesional psoriasis sebaceous glands vs. non-lesional sebaceous glands, and **(C)** lesional psoriasis sebaceous glands vs. lesional atopic dermatitis sebaceous glands.

type-specific lipid metabolism of sebocytes has been progressively revealed over the last two decades of sebocyte research (14). Here, we confirm the *in vivo* relevance of widely studied enzymes and signaling pathways like delta-6 desaturase/FADS2 or stearoyl-coenzyme A desaturase (31, 32). Furthermore, the previously reported central role of nuclear receptors such as PPARs or retinoic acid (33–35), and the characterization of other transcription factors such as SREBP-1 or FoxO1 (36) in the regulation of SG proliferation and lipid metabolism (37) are supported by our findings. Based on our data, linoleic acid, a known activator of PPAR-γ and also the source of arachidonic acid, could be a potent natural stimulus behind the unique features of sebocytes (38, 39). We also confirmed the central role of genes involved in lipid synthesis (*FASN, THRSP*, and *ELOVL5*), metabolism (*FADS2* and *ACSBG1*) and transport (*APOC1*), and keratinization (*KRT79*), which were found to be expressed in a combined subpopulation of healthy, L and NL AD inner root sheath and SG cells (40). In the present study, we identified each one of these genes and many more as SVGs in L AD and PSO SGs. In addition, our transcriptome analyses revealed enzymes and pathways for further studies, such as the role of SUMOylation and the HSP90 chaperone cycle for steroid hormone receptors in sebocytes.

The results of our study support the postulated inflammatory capacity of sebocytes in AD. AD is characterized by dry skin and inflammation, which is primarily associated with an impaired skin barrier. The findings that AD skin has low levels of sebocyte-specific lipids (20, 41, 42), and a recent publication showing that the amount of sebum secreted by SGs was decreased in AD patients and was negatively correlated with barrier function and disease severity (43), further support that SGs may play an active role in the pathogenesis of AD. Importantly, a recent study has also linked the cytokine milieu of AD to sebocyte functions by showing that IL-4 upregulates the expression of 3β-hydroxysteroid dehydrogenase 1 (HSD3B1), a key enzyme in the conversion of cholesterol to sebum lipids (44). Here, we support these findings by identifying *HSD3B1* as an AD SG-specific SVG.

SGs appear to be involved in type 2/Th2 inflammation. *ALOX15B*, a common AD/PSO SVG, is a key player in fatty acid metabolism, and cholesterol homeostasis. In our previous studies investigating the eicosanoid/docosanoid signaling in the skin of human AD patients, we found that the sum of 15-LOX metabolites

was significantly increased (45). Furthermore, studies have shown that in activated human macrophages, *ALOX15B* is induced by the Th2 cytokines IL-4 and IL-13 and has an effect on IL-4-induced *CCL17* in an *SREBP-2*-dependent manner (46). This further supports a potential involvement of SGs in type 2/Th2-inflammation. However, the identification of *ALOX15B* as an SVG in PSO SGs requires further validation to define its role in type 3/Th17-inflammation.

We found further evidence for the active contribution of SGs in inflammation. *CCL17* plays a potential role in the pathogenesis of AD (47), which was also identified as an AD-specific SVG in the present study. While *SAA1* encoding serum amyloid A1, previously described as a marker of TLR 1/2- and 4-activated SGs (8), was also found to be a common SVG of AD/PSO SGs in the present work, highlighting the importance of further investigating the inflammatory capacity of SGs.

An alteration of the retinoic acid signaling at the level of the SGs may be pathologically relevant, as *RARRES1* expression levels were also altered in SGs of AD and PSO samples. Notably, *RARRES1* is one of the key genes found to be upregulated in skin samples from acne patients treated with the potent skin drying agent isotretinoin, as well as in both the SEB1 (48) and SZ95 sebocyte cell lines (49) in response to isotretinoin.

Overall, the SG transcriptome signature in AD revealed numerous genes involved in the formation of the lipid skin barrier. The clusters of mitochondrial functions, ATP synthesis and respiratory electron transport that were altered in AD SGs provide further important starting points for studies on how changes in lipid production might be linked to an altered energy expenditure (50, 51).

Our data confirmed that PSO SGs not only maintained their active lipid metabolism, but also acquired immune-competence via their gene expression profile. PSO is characterized by atrophy and sometimes absence of SGs in the affected skin samples, raising the questions of whether this plays a role in the development and progression of the disease and whether the alterations in the expression of lipid metabolism-related genes (*AWAT2, DHCR7, ELOVL5* or *FAR2*) identified in this study are specific to PSO. The involvement of PSO SGs in skin inflammation was confirmed by comparing SGs from PSO samples with SGs from NL and AD samples. The detected transcripts encoding keratins and differentially down-regulated genes related to cell cycle and proliferation suggest that the driving mechanism behind SG atrophy may share similarities, such as the involvement of NOTCH signaling, but is generally different in the two diseases. Immune-related clusters, such as interferon signaling, neutrophil activation and the induction of genes encoding antimicrobial peptides, clearly dissected the two diseases also at the level of SGs, suggesting an active contribution of SGs to type 3/Th17 inflammation.

Notably, *S1PR3* was identified as a PSO SG-specific SVG in our study, suggesting an involvement of SGs in the pathogenesis of PSO. The lncRNA H19/miR-766-3p/S1PR3 axis has previously been shown to contribute to keratinocyte hyperproliferation and skin inflammation in PSO via the AKT/mTOR pathway (28). The PSO-specific SVG *SERPINF1* may also play a role in the immune regulation of PSO (52).

FKBP5 was identified as another PSO-specific SVG. Recently, the immunoregulatory *FKBP5* has been shown to contribute to NF-κB-driven inflammation and cardiovascular risk (53), and is also associated with depression susceptibility (54, 55). Both cardiovascular risk and depression are known and common comorbidities of psoriasis (56, 57). Further studies are needed to investigate a potential role of *FKBP5* in the link between systemic inflammation, cardiovascular risk and depression susceptibility in psoriasis patients.

In conclusion, this study provides human *in vivo* data which confirmed that beyond altering their lipid metabolism in a disease-specific manner in an inflammatory microenvironment, SGs can be considered as an active and immunocompetent structure in L skin with possible pathological and therapeutic relevance. Moreover, our data serve as a starting point for further studies at protein level to better understand the role of SGs in inflammatory skin diseases in the future.

Materials & methods

Study cohort and spatial transcriptomics

The study cohort leverages patients from the Schäbitz et al. study (58). L and NL skin from each patient was collected and subsequently processed using the software SpaceRanger-1.0.0 from 10x Genomics. L skin was defined by clinical presence of typical hallmarks of AD or PSO inflammation, such as involvement of predilection sites, erythematous papules and plaques, or scaling. After taking the biopsies, the diagnosis was confirmed by 2 independent dermatopathologists, considering typical histological hallmarks of AD or PSO, including presence of immune cells, spongiosis, acanthosis, papillomatosis, and hyperkeratosis, amongst others. NL skin was defined as skin clinically and histologically absent of the mentioned AD and PSO (or any other dermatosis) hallmarks. The study was approved by the local ethics committee (Klinikum Rechts der Isar, 44/16 S). Each patient gave written informed consent for sample collection for research purposes.

Spatial transcriptomics data preprocessing

Leveraging the cohort from Schäbitz et al. (58), we performed the preprocessing using `scanpy` (59). First, we conducted quality control on spot and gene level. Spots having a mitochondrial fraction above 25%, less than 30 genes, and less than 500 UMI-counts or more than 500,000 UMI-counts were filtered out. Genes were required to be measured in at least 20 spots. The R-package `scran` (60) was used to normalize the data using size factors. We added a pseudo count of 1 to the normed counts and transformed them into log counts per million (logCPM). Next, we identified highly variable genes for each specimen using the flavor cell_ranger. We corrected for technical artifacts caused by the project co-variate using `scanorama` (61). In order to embed the data in 2D, we calculated principal components (PCs) and selected n_pcs = 15 explaining the most variance. PCs were leveraged to create a nearest

neighbor graph using the default parameters. Using the graph, the data was embedded in 2D using UMAP (62). For the downstream analysis we selected only those specimen having SG annotations. In total we got 1 PSO, 1 AD, and 1 non-lesional sample with 2 replicates each (6 slides in total) (Supplementary Table S1).

Differential gene expression and pathway enrichment analysis of spatial transcriptomics

To identify significantly up- and down-regulated genes in SGs at a spatial resolution, we compared spots annotated as SG with the remaining spots using the R-package 'glmGamPoi' (63). Raw counts and size factors which have been calculated during the preprocessing step were used as input for the differential gene expression (DGE) analysis. In addition, we also considered biological variances, i.e., cellular detection rate (cdr), patient heterogeneity, and tissue layers. Variables of the differential gene expression (DGE) analysis were NL skin, AD, PSO, and a pool of PSO and AD. The following designs were used.

$$Y_{s,g} \sim cdr + patient + annotation + condition$$

and

$$Y_{s,g} \sim cdr + annotation + condition$$

Here, $Y_{s,g}$ is the raw count of gene g in a spot s. The later design was used to compare L, PSO vs. AD in Figure 4C, as the design matrix needed to be of full rank. P-values were corrected using the multiple testing method of Benjamini-Hochberg (BH) (64). In addition, DEx genes had to have a $adj.\ p-value \leq 0.1$ and $|log_2FC| > 1$.

Pathway enrichment analysis was performed using the Bioconductor packages 'ReactomePA' (65) and 'org.Hs.eg.db' (66). Pathways were considered enriched at a false discovery rate (FDR) of 10%, corrected with BH.

Discovering spatial patterns and variable genes

We used spatialDE (27), which allowed us to determine spatial patterns and their associated genes per sample. Following the spatialDE workflow, we assumed normal distributed data, corrected for library size and ran spatialDE with default settings to obtain spatial variable genes (SVGs). Automatic expression histology (AEH) was used to identify spatial patterns using the previously observed and prefiltered SVGs requiring a q-value < 0.05. We set the number of expected patterns C to nine and used the mean length scale as optimal characteristic length scale parameter l as recommended by spatialDE. In order to determine whether a pattern is enriched in a SG, we used the alternative hypothesis that pattern intensity in SG is greater than in other spots. The tests for all patterns on a specimen were conducted using the one-sided Mann-Whitney U test (67) in the python package `statannotations` (68). P-values were corrected with the multiple test correction method Bonferroni (69). We called the null hypothesis rejected if the $adj.\ p-value \leq 0.05$. Default parameters of Bioconductor's R package "ReactomePA" were used for p-value and q-value cut-offs, and a minimal gene set size of five was required.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, GSE206391. Source code is available at github: https://github.com/MendenLab/ST_SebaceousGlands.

Ethics statement

The studies involving humans were approved by Klinikum Rechts der Isar, Munich, Germany, 44/16 S. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were primarily isolated as part of our previous study (58) for which ethical approval had been obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

PS: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. CH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. ASc: Data curation, Investigation, Writing – review & editing. MJ: Data curation, Investigation, Writing – review & editing. AP: Investigation, Writing – review & editing. SE: Conceptualization, Methodology, Resources, Writing – review & editing. ASz: Formal analysis, Writing – review & editing. MS: Formal analysis, Writing – review & editing. FG: Formal analysis, Writing – review & editing. CZ: Formal analysis, Writing – review & editing. TB: Resources, Supervision, Writing – review & editing. MM: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing. KE: Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. DT: Conceptualization, Data curation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The 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.2024.1334844/full#supplementary-material

SUPPLEMENTARY TABLE 1
Study cohort.

SUPPLEMENTARY TABLE 2
Differentially expressed genes of non lesional sebaceous glands vs. the rest of non-lesional skin.

SUPPLEMENTARY TABLE 3
Pathway enrichment analysis of patterns where sebaceous glands were significantly enriched in non-lesional skin (patterns 1, 7, 8, and 9).

SUPPLEMENTARY TABLE 4
Unique and shared spatially variable genes of atopic dermatitis and psoriasis sebaceous glands.

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Identification of immunological patterns characterizing immune-related psoriasis reactions in oncological patients in therapy with anti-PD-1 checkpoint inhibitors

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Introduction: Immunotherapy with biologics targeting programmed cell death protein-1 (PD-1) is highly effective in the treatment of various malignancies. Nevertheless, it is frequently responsible for unexpected cutaneous manifestations, including psoriasis-like dermatitis. The pathogenesis of anti-PD-1-induced psoriasis has yet to be clarified, even though it is plausible that some innate and adaptive immunity processes are in common with canonical psoriasis. The genetic predisposition to psoriasis of patients could also be a contributing factor. Here, we investigated the immunological and genetic profiles of two patients with metastatic melanoma and one patient affected by lung cancer, who developed severe psoriasis after receiving anti-PD-1 nivolumab therapy.

Methods: The immune patterns of the three patients were compared with those detectable in classical, chronic plaque-type psoriasis or paradoxical psoriasis induced by anti-TNF-α therapy, mostly sustained by adaptive and innate immunity processes, respectively. Therefore, immunohistochemistry and mRNA analyses of innate and adaptive immunity molecules were conducted on skin biopsy of patients. Genetic analysis of polymorphisms predisposing to psoriasis was carried out by NGS technology.

Results: We found that anti-PD-1-induced psoriasis showed immunological features similar to chronic psoriasis, characterized by the presence of cellular players of adaptive immunity, with abundant $CD3^+$, $CD8^+$ T cells and $CD11c^+$ dendritic cells infiltrating skin lesions, and producing IL-23, IL-6, TNF-α, IFN-γ and IL-17. On the contrary, a lower number of innate immunity cells ($BDCA2^+$ plasmacytoid dendritic cells, $CD15^+$ neutrophils, $CD117^+$ mast cells) and reduced IFN-α/β, lymphotoxin (LT)-α/β, were observed in anti-PD-1-induced psoriasis

lesions, as compared with anti-TNF-α-induced paradoxical psoriasis. Importantly, the disintegrin and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5) psoriasis autoantigen was significantly upregulated in psoriasis lesions of anti-PD-1-treated patients, at levels comparable with chronic plaque-type psoriasis. Finally, NGS analysis revealed that all patients carried several allelic variants in psoriasis susceptibility genes, such as *HLA-C*, *ERAP1* and other genes of the major psoriasis susceptibility *PSORS1* locus.

Discussion: Our study showed that adaptive immunity predominates over innate immunity in anti-PD-1-induced psoriasis lesions, consistently with the local ADAMTSL5 overexpression. The presence of numerous SNPs in psoriasis susceptibility genes of the three patients also suggested their strong predisposition to the disease.

KEYWORDS

psoriasis, melanoma, anti-PD-1 therapy, immune-related cutaneous adverse event (ircAE), paradoxical skin reactions, adaptive immunity, innate immunity

1 Introduction

Immune checkpoint inhibitors (ICIs) are increasingly used as first-line therapy in various malignancies, including melanoma and lung cancer (1, 2). In these conditions, treatment with monoclonal antibodies against immune checkpoint molecules, such as programmed cell death protein-1 receptor (PD-1) and/or cytotoxic T lymphocyte-associated protein 4 (CTLA-4), can result in enhanced cytotoxic activation of tumor antigen-specific T cells and in eradication of tumor lesions. However, immune activation by ICIs may lead to immune-mediated adverse events, among which cutaneous reactions are the most common (approximately 40%) (3, 4). Among immune-related cutaneous adverse events (ircAE), maculopapular rashes, lichenoid eruptions, and vitiligo, as well as less common inflammatory and autoimmune manifestations, such as hidradenitis suppurativa (HS) and *de novo* or worsening of pre-existing psoriasis, have been described (3–5). Most ircAE are of low-grade and are treated with corticosteroids, a therapy that seems not to interfere with the anti-tumor immune responses activated by ICIs. Severe ircAEs have been observed in the 1-2% of patients and may require additional topical or systemic agents, including antihistamine compounds and biologics. In the case of ircAEs, dose changes were made in up to 21% of patients, whereas therapy discontinuation was necessary only in about 8% of patients (4).

Pathogenically, ircAEs induced by anti-PD-1 therapies result from breaking of peripheral T cell tolerance and unleashing of immune and inflammatory responses, likely dependent on $CD8^+$ T cells and IL-6 enhanced production (6–8). For instance, PD-1 signal blockade-induced psoriasis-like dermatitis is characterized by a prominent epidermal infiltration of $CD8^+$ T cells and overexpression of IL-6, IL-23 and IL-17A cytokines, as demonstrated in a murine model of psoriasis induced by imiquimod (IMQ), carried out with genetically modified mice lacking PD-1 in $CD8^+$ T cells (8).

An aberrant migration and accumulation of $CD8^+$ T lymphocytes into the epidermis has also been shown in classical psoriasis, especially during the establishment of adaptive immune responses (9). In stable plaques, intraepidermal $CD8^+$ T cells display highly pathogenic features, as they abundantly produce cytokines, such as IL-17A and IFN-γ, which in turn dictate specific and inflammatory gene signatures in keratinocytes and in other resident skin cells (10). The dialogue between $CD8^+$ T lymphocytes and keratinocytes, as well as with skin cells of adaptive immunity, such myeloid dendritic cells (mDC), is strictly depends on the recognition of peptide (auto)antigens presented by MHC class I molecules, such as *HLA-Cw6*, which is the strongest psoriasis susceptibility allele (11). Among them, the disintegrin and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5), a protein modulating microfibril functions, has been identified as autoantigen presented by melanocytes and keratinocytes in an HLA-Cw6-restricted fashion (12), and localized throughout the psoriatic epidermis (13, 14).

Systemic administration of anti-TNF-α biologics can be responsible for unexpected paradoxical psoriasiform reactions in patients treated for immune-mediated inflammatory conditions, such as HS, rheumatoid arthritis, and inflammatory bowel disease (15, 16). Our previous studies on the characterization of anti-TNF-α-induced paradoxical psoriasis reactions revealed an overactivation of innate immunity in the skin lesions of HS patients, also due to a strong predisposition of HS patients to develop immune responses against innate stimuli, and the presence

of an immunological infiltrate mainly represented by BDCA2$^+$ plasmacytoid dendritic cells (pDCs), CD15$^+$ neutrophils, c-kit/CD117$^+$ mast cells, CD68$^+$ macrophages and monocytes (17). A local overproduction of the type I IFNs, IFN-β and IFN-α2a, concomitantly to other innate immunity molecules, such as lymphotoxin (LT)-α and LT-β, was also detected in paradoxical psoriatic skin of patients treated with anti-TNF-α biologics (17).

To date, some of the pathogenic mechanisms underlying immune-related psoriasis reactions in patients treated with anti-PD-1 have been described, even though the specific patterns of innate and adaptive immunity prevailing in the skin lesions are still to be elucidated. The influence of genetic predisposition of patients to psoriasis is also unproven.

In this study, we investigated skin immunological patterns of two patients with metastatic melanoma and one patient affected by lung cancer, who developed severe psoriasis after receiving anti-PD-1 nivolumab therapy. The immune patterns characterizing psoriasis lesions of patients were compared with those detectable in classical, stable plaque-type psoriasis or paradoxical psoriasis induced by anti-TNF-α therapy, mostly sustained by adaptive and innate immunity processes, respectively. The expression of the psoriasis autoantigen ADAMTSL5 and the genetic susceptibility to psoriasis of the three patients were also studied.

2 Materials and methods

2.1 Patients and samples

Two patients with metastatic melanoma, stage IV (AJCC, version 8) (18), and one patient affected by metastatic lung cancer, stage IVB (19), and developing psoriasis after receiving nivolumab (240 mg every 2 weeks) were included in the study.

Six patients affected by classical plaque-type psoriasis (Psoriasis area and severity index, PASI: 8-21 range), and three patients with severe HS (Hurley III, Sartorius score: 41.5-61.5 range) showing paradoxical psoriasis after treatment with adalimumab (40 mg, weekly) were also enrolled for the study. Clinical data, as well as skin biopsies and blood, were collected from patients with the permission of the IDI-IRCCS Local Ethics Committee (Prot. CE 475/2016). The participants provided their written informed consent to participate in this study.

For oncological patients, the investigator-determined objective response was assessed radiologically with computed tomography scans approximately every 12 weeks after treatment initiation. Tumor response was classified according to the immune response evaluation criteria in solid tumors (iRECIST 1.1) (20), and therapy efficacy evaluation was based on best overall response determined as best time-point response according to iRECIST. Eight-mm skin biopsies were taken from psoriasiform lesions arising in enrolled patients. For patient 2, biopsy specimens from primary and melanoma skin metastasis, as well as from vitiligo lesions were collected from the archives of the Anatomical Pathology Unit of IDI-IRCCS. Skin biopsies were also taken from normal-appearing, non-lesional skin of psoriatic patients. Biopsies were divided into two parts for immunohistochemistry and RNA isolation. A 2-ml sample of peripheral blood was used to extract DNA.

2.2 Immunohistochemistry

Skin samples from healthy donors, immune-related psoriasis induced by anti-PD-1, chronic plaque-type psoriasis and anti-TNF-α-induced paradoxical psoriasis. were fixed in 10% formalin and embedded in paraffin. Five-µm sections were dewaxed and rehydrated and stained with hematoxylin and eosin (H&E) or processed for immunohistochemistry. In this case, endogenous peroxidase was quenched by 3% H_2O_2 treatment and then antigen retrieval was achieved by treating sections with citrate buffer pH 6.0 or Tris-EDTA buffer pH 7.8 (both from UCS Diagnostic, Rome, Italy), depending on the primary antibodies (Abs). After blocking nonspecific binding sites with a blocking solution (Dako, Glostruk, Denmark), sections were incubated with the primary Abs. The latter were as follows: anti-CD3 (#A0452, Dako, 1:100 dilution), anti-CD8 and anti-IFN-α2A (#AB217344, 1:75 dilution and, #AB198914, 1:75 dilution, respectively; both were from Abcam, Cambridge, UK), anti-CD11c and anti-CD117 (#MON3371, 1:50 dilution and #MONX10234, 1:100 dilution, respectively; both Abs were purchased from Monosan, Uden, Netherlands), anti-BDCA2 (DDX0043-TDS, Dendritics, Lyon, France, 1:30 dilution), anti-CD15 (#347420, BD Biosciences, Milan, Italy, 1:30 dilution), anti-IL-17A (#AF-317-NA, R&D Systems, Abingdon, UK, 1:30 dilution) and anti-ADAMTSL5 (#NBP1-93438, Novus Biologicals, Centennial, USA, 1:50 dilution). Immunoreactivity was visualized with peroxidase reaction using 3-amino-9-ethylcarbazole (AEC) or 3,3'-diaminobenzidine (DAB) in H_2O_2, and specimen counterstained with hematoxylin. As a negative control, the primary Abs were omitted or replaced with an irrelevant isotype-matched Ab. Positivity was evaluated in five adjacent fields at a 200X magnification. Cells infiltrating dermis and epidermis were also counted in five adjacent fields for each skin specimen.

2.3 Real-time PCR analysis

Total RNA was extracted from skin biopsies using RecoverAll Total Nucleic Acid Isolation (Life Technologies). mRNA was reverse transcribed into cDNA by using SuperScript IV VILO master mix (Invitrogen) and analyzed by QuantStudio5 real-time PCR System (Thermo-Fisher Scientific, Waltham, MA, USA) using SYBRGreen or Taqman PCR reagents. The primer sets were as follows: IFN-β, 5'CAGCAATTTTCAGTGTCAGAAGC3'/5'TCATCCTGTCCTTGAGGCAGT3'; LT-α, 5'CTACCGCCCAGCAGTGTC3'/5'GGTGGTGTCATGGGGAGA3'; LT-β, 5'GGCGGTGCCTATCACTGT3'/5'GAAACCCCAGTCCTTGCTG3'; TNF-α, 5'CTCTTCTGCCTGCTGCACTTTG3'/5'ATGGGCTACAGGCTTGTCACTC3'; IL-6, 5'GGCACTGGCAGAAAACAACC3'/5'CACCAGGCAAGTCTCCTCAT3'; IL-23, 5'GACAACAGTCAGTTCTGCTTGC3'/5'GAGAAGGCTCCCCTGTGAAA3'; β2M, 5'GATGAGTATGCCTGCCGTGTG3'/

5'CAATCCAAATGCGGCATCT3'. IL-17A, IL-22 and IFN-γ genes were analyzed by the TaqMan gene expression assay (assay ID: Hs00174383_m1, Hs00220924_m1 and Hs00174143_m1, respectively). mRNA levels were normalized to β2M mRNA expression. The values obtained from triplicate experiments were averaged, and data presented as mean 2^−ΔΔCT ± SD.

2.4 SNP analysis

DNA was extracted from blood using the QIAcube®system (Qiagen, Hilden, Germany), and 10 ng were used for high-throughput sequencing by NGS technology. SNPs were selected based on an extensive review of articles on the association between psoriasis and SNPs or response to biological therapeutics (21–27). The customized designed SNP panel permitted to identify 417 genetic variants together with additional SNPs located in proximity of the investigated genomic regions. The SNP panel was analyzed by targeted sequencing, using Ion AmpliSeq™ Library kit Plus (Thermo Fisher Scientific) and the Ion GeneStudio™ S5 Plus platform (Thermo Fisher Scientific, Massachusetts, USA). Sequencing data were processed with the Ion Torrent Suite software v.5.10. Positive calls were selected applying a read depth>30X and allelic frequency >0.3. Reads were aligned to human genome sequence (build GRCh37/human genome 19). Variants were collected using Variant Caller. Variants' annotations were finally verified using ANNOVAR.

2.5 Statistics

The significance of differences in the numbers of immunoreactive cells in skin biopsies was calculated using the unpaired Student's *t*-test and values are expressed as the median + interquartile range. Unpaired Student's *t*-test was also used to compare differences in mRNA content in skin biopsies of patients. All statistical analysis were conducted using Prism v.10.1.0 (GraphPad Software, Boston, MA, USA) and statistical significance was assumed at a p value of 0.05 or less.

3 Results

3.1 Clinical characterization of immune-related psoriasis reactions in patients undergone anti-PD-1 therapy

We studied two patients with metastatic melanoma and one patient affected by lung cancer, who developed cutaneous reactions after receiving anti-PD-1 immunotherapy.

Patient 1, a 70-year-old Caucasian man, was affected by metastatic lung cancer and, considering the best overall response, he responded positively to nivolumab therapy with an immune complete response (iCR, Table 1). He reported a personal history of psoriasis, and after 2-week treatment with anti-PD-1, showed a re-occurrence of the disease (PASI 21) (Figure 1A, panels i-iii). For psoriasis condition, patient 1 received therapy with systemic dexamethasone and topical clobetasol, which however resulted unsuccessful. Patient 1 also developed bullous pemphigoid, arising after 100-week treatment with nivolumab (Figure 1A, panels iv-v). Patient 2, a 67-year-old Caucasian man, was affected by metastatic melanoma and, after treatment with anti-PD-1 immunotherapy he achieved an iCR as the best response (Table 1). After 20 weeks of ICI therapy, he showed vitiligo, and after 52 weeks of treatment he concomitantly developed plaque-type psoriasis on the legs, elbows, and trunk (PASI 8) (Figure 1B). Patient 2 was not treated for psoriasis condition, neither systemically nor topically. Patient 2 reported previous psoriasis. Anti-PD-1 therapy was not discontinued in patient 1 and 2, even after ircAE manifestation (Table 1). Finally, a 62-year-old Caucasian woman, patient 3, with metastatic melanoma treated with anti-PD-1 and with an immune unconfirmed progressive disease (iUPD) as the best response, after 4 weeks of immunotherapy developed psoriasis (PASI 15) and discontinued ICI treatment for this ircAE. For psoriasis condition, she was successfully treated with acitretin and with topical corticosteroids. Patient 3 never restarted anti-PD-1 therapy for the re-occurrence of psoriasis condition. Interestingly, none of patients had a positive family history for psoriasis (Table 1), neither showed psoriasis reactions before ICI treatment. Histological examination of the psoriasis lesions of all the patients showed epidermal hyperplasia with parakeratosis, papillary vessel ectasia and perivascular infiltrate compatible with a psoriasiform dermatitis (Figure 1C). A neutrophilic infiltrate was present in corneal abscesses (Figures 1C).

TABLE 1 Characteristics and treatment outcomes of enrolled patients.

Characteristics	Patient 1	Patient 2	Patient 3
Sex	male	male	female
Age[a]	70	67	62
BMI[b]	31.96	24.76	22.86
Disease	lung cancer	melanoma	melanoma
Stage[c]	IVB	IV	IV
Checkpoint inhibitor[c]	nivo	nivo	nivo
Best Response[d]	iCR	iCR	iUPD
ircAEs (week)[e]	Psoriasis (2), Bullous Pemphigoid (100)	Psoriasis (52), Vitiligo (20)	Psoriasis (4)
PASI (ircAE)	21	8	15
ICI discontinuation for ircAEs	no	no	yes
Previous psoriasis	yes	yes	no
Psoriasis family history	no	no	no

[a]Age, years.
[b]BMI, (kg/m^2)
[c]Staging before starting immunotherapy: nivo, nivolumab.
[d]Best Response according to the iRECIST criteria, at the last observation: iCR, immune complete response.
iUPD, immune unconfirmed progressive disease.
[e]Weeks of treatment before the onset of immune-related cutaneous adverse events (ircAEs); PASI, psoriasis area severity index.

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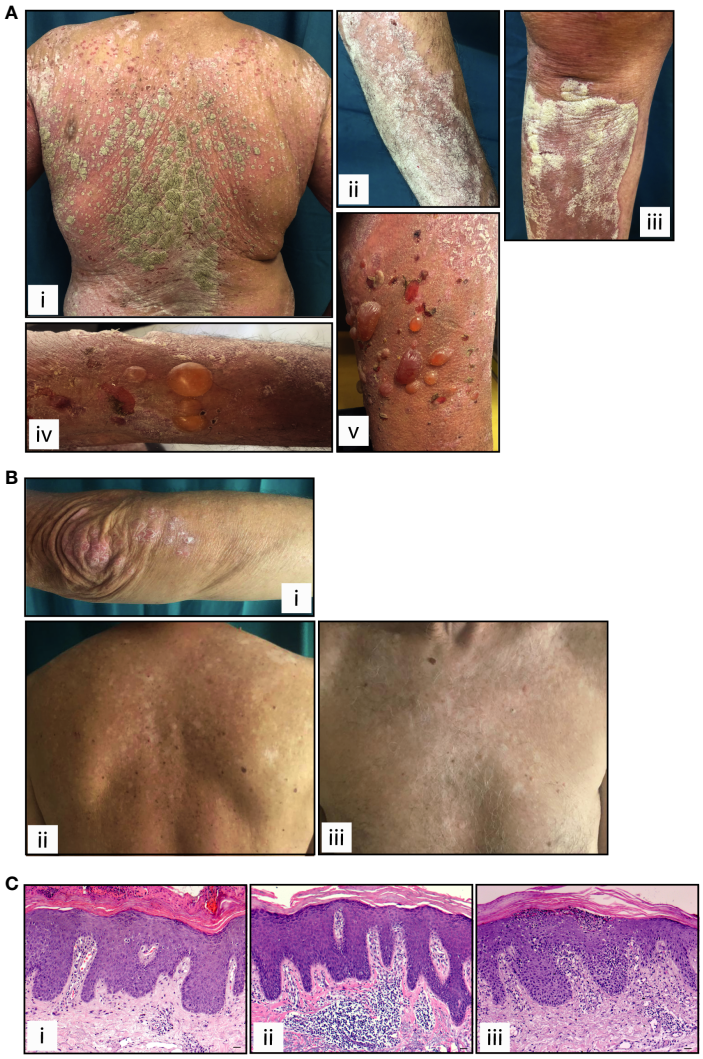

FIGURE 1
Clinical and histological presentation of psoriasis induced by anti-PD-1 therapy. Cutaneous manifestations of patients 1 and 2 affected by lung cancer and melanoma, respectively, presenting psoriasis reaction after receiving anti-PD-1 treatment. **(A)** Patient 1, panels i-iii show paradoxical erythemato-squamous plaques localized on the trunk, arms and lower limbs. Patient 1 also developed generalized bullous pemphigoid, arising after treatment with nivolumab (panels iv-v). **(B)** Patient 2 shows squamous plaques on the elbows and upper limbs (i) and on the trunk (ii), concomitantly with vitiligo (ii and iii). **(C)** H&E staining for the corresponding histology specimens of patients 1 (i), patient 2 (ii) and patient 3 (iii) was also performed. Scale bars, 20 μm.

3.2 Adaptive immunity predominates over innate immune responses in psoriasis lesions induced by anti-PD-1 therapy

Leukocyte subpopulations were characterized by immunohistochemistry in immune-related psoriasis induced by anti-PD-1 and compared to those present in chronic plaque-type psoriasis (n=6 patients) and in paradoxical skin lesions of HS patients undergone anti-TNF-α therapy (n=3) (Figures 2, 3). Immune-related psoriasis induced by PD-1 blockade exhibited immunological aspects of chronic inflammation, as skin lesions of all patients showed a prominent infiltrate of $CD3^+$, $CD8^+$ T cells and $CD11c^+$ DC, at levels and patterns of distribution similar to stable psoriasis (Figure 2). In fact, $CD8^+$ T lymphocytes and $CD11c^+$ DC massively localized in the epidermis and in the papillary dermis, respectively, together with $CD15^+$ neutrophils accumulating in the epidermis to form focal subcorneal aggregates (micro abscesses of Munro) (Figure 2). In contrast, anti-PD-1-induced psoriasis did not show the immunological signs of paradoxical psoriasis induced by TNF-α blockade or acute psoriasis, characterized by an overactivation of innate immunity pathways and prominent infiltration of innate immunity cells. $CD15^+$ neutrophils, $BDCA^+$ pDC, and c-kit/$CD117^+$ mast cells, poorly infiltrated the mid and interpapillary dermis of skin lesions of the three patients, differently to what observed in anti-TNF-α-induced psoriasis lesions (Figure 3).

The quantification of immunoreactivity for markers for different leukocyte subpopulations showed that chronic psoriasis and anti-PD-1-induced lesions were characterized by a higher number of epidermal $CD8^+$ T cells, dermal $CD11c^+$ DCs and IL-$17A^+$ cells than psoriasiform lesions induced by TNF-α blockade (~11.4-, 1.4- and 1.3-fold increase,

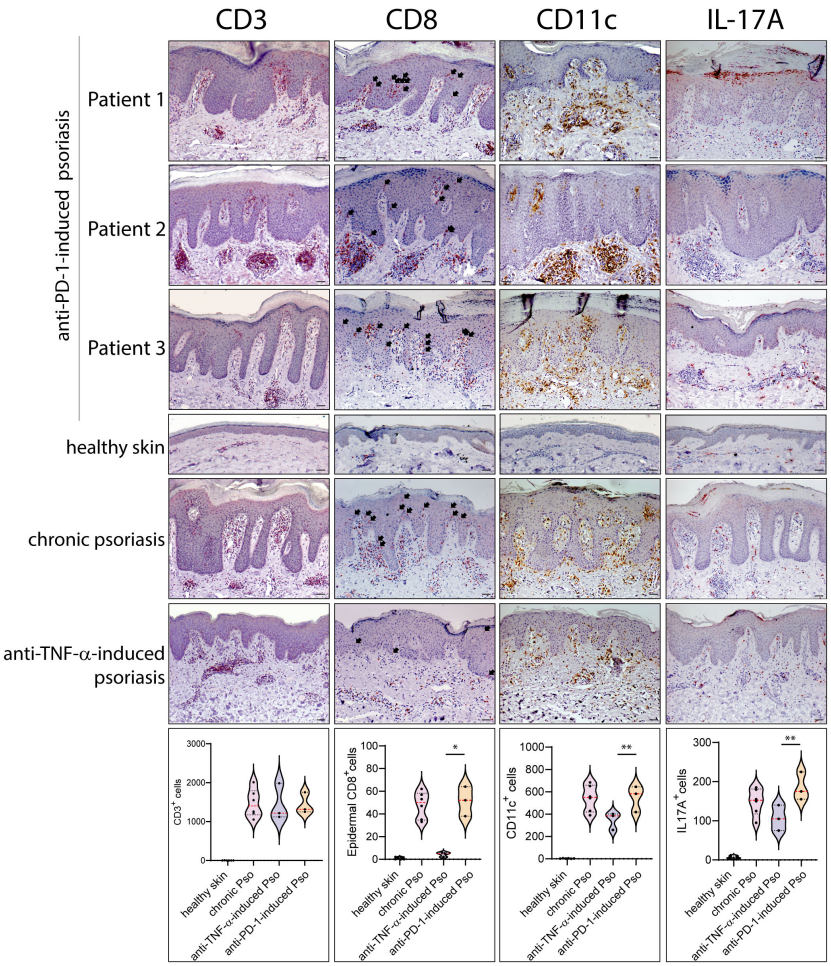

FIGURE 2
Paradoxical psoriasis induced by PD-1 blockade show a prominent infiltrate of $CD3^+$, $CD8^+$ T cells, $CD11c^+$ DC and IL-17^+ cells, at levels and patterns of distribution similar to chronic psoriasis. Leukocyte subpopulations were characterized by immunohistochemistry in paradoxical psoriasis lesions induced by anti-PD-1 (Patient 1, 2 and 3), and compared to those present in chronic plaque-type psoriasis (n=6 patients) and in paradoxical psoriasis induced by anti-TNF-α therapy (n=3). The distribution of numerical data relative to cell immunoreactivity for CD3 (red staining), CD8 (red), CD11C (brown), and IL-17A (red staining) in the three types of psoriasis reactions, are represented in the violin plots. Immunohistochemistry analysis of anti-PD-1 psoriasis skin reactions obtained from patient 1, patient 2 and 3 shows similar numbers of $CD3^+$ cells and higher number of epidermal $CD8^+$, dermal $CD11C^+$, and IL-$17A^+$ cells, when compared with paradoxical psoriasis induced by anti-TNF-α. Chronic psoriasis and anti-PD-1-induced psoriasis showed similar values in immunoreactive cells. No immunoreactivities were observed in skin samples from healthy donors (n=6). Arrows indicate $CD8^+$ T cells localized within epidermis. Slides were analyzed by two pathologists with experience in dermatology. Positive cells were counted in five adjacent fields at a total magnification of ×200. For chronic or anti-TNF-α-induced psoriasis, one representative set of staining is shown. For each patient, one out of three representative stainings is shown. *$p < 0.05$, **$p < 0.01$ versus anti-TNF-α-induced psoriasis. Scale bars, 40 µm.

respectively) (Figure 2). Conversely, the infiltrate of dermal $CD15^+$ neutrophils $BDCA^+$ pDC, and c-kit/$CD117^+$ mast cells, was less abundant (~3.8-, 6.3-, and 2.8-fold decrease, respectively) (Figure 3). Psoriasiform reactions of anti-PD-1-treated patients were weakly immunoreactive for the type I IFN-α2A, at lower levels than paradoxical psoriasis induced by anti-TNF-α (~1.6-fold decrease) (Figure 3).

Consistently with a prevalence of adaptive immunity over innate immune responses in psoriasis induced by anti-PD-1 therapy, we found that the mRNA expression levels of psoriasis-related cytokines, such as IL-17A, IL-23, IFN-γ and IL-22 were significantly higher in the skin of the three patients, as compared to psoriasis-like reactions to anti-TNF-α, and similar to mRNA levels detected in chronic psoriasis plaques (Figure 4A). In line with previous studies (6, 8), aberrant IL-6 and TNF-α mRNA amounts were detected in the immune-related reactions to anti-PD-1 (Figure 4A). Finally, the analysis of selected innate immunity molecules showed that the type I IFN-β and the lymphotoxins LT-α and LT-β (belonging to the TNF cytokine family) mRNA are poorly expressed in psoriasis lesions induced by anti-PD-1, as compared to anti-TNF-α-induced psoriasis (Figure 4B).

3.3 ADAMTSL5 psoriasis autoantigen is overexpressed in psoriasis lesions induced by nivolumab treatment

We next evaluated the expression of the melanocyte- and keratinocyte-derived autoantigen ADAMTSL5, whose expression

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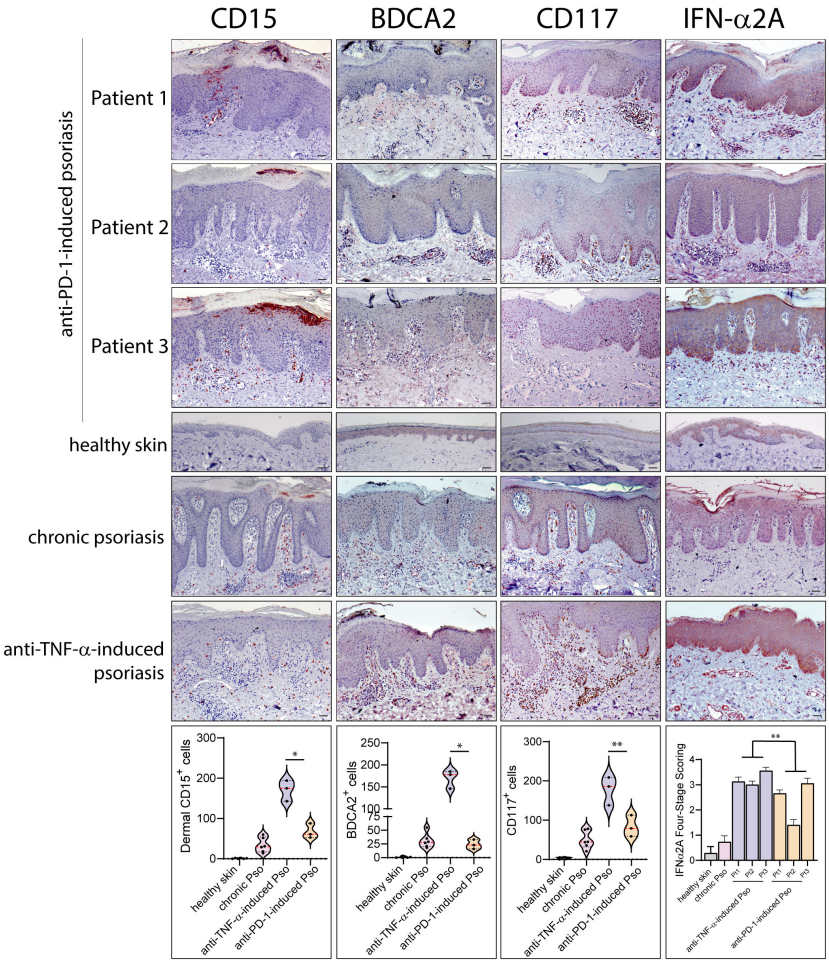

FIGURE 3
Innate immunity patterns are lacking in anti-PD-1-induced psoriasis. Innate immunity cell subpopulations were characterized by immunohistochemistry in paradoxical psoriasis lesions induced by anti-PD-1 (Patient 1, 2 and 3), and compared to those present in chronic plaque-type psoriasis (n=6 patients) and in paradoxical psoriasis induced by anti-TNF-α therapy (n=3). The distribution of numerical data relative to cell immunoreactivity for CD15 (red staining), BDCA2 (brown) and c-kit/CD117 (red staining) in the three types of psoriasis reactions are represented in the violin plots. Graph shows the mean ± SD of semiquantitative, four-stage scoring, ranging from negative immunoreactivity (0) to strong immunoreactivity (4+) of IFN-α2A staining (red). The infiltrate of dermal $CD15^+$ neutrophils $BDCA2^+$ pDC, and c-kit/$CD117^+$ mast cells, was less abundant in immune-related psoriasis induced by anti-PD-1 as compared to paradoxical psoriasis induced by anti-TNF-α. IFN-α2A also was less abundant in psoriasiform reactions of anti-PD-1-treated patients, as compared to paradoxical psoriasis induced by anti-TNF-α. Similar immunoreactivity values were observed in anti-PD-1-induced and chronic psoriasis. No significant immunoreactivities were observed in skin samples from healthy donors (n=6). Slides were analyzed by two pathologists with experience in dermatology. Positive cells were counted in five adjacent fields at a total magnification of ×200. For chronic or anti-TNF-α-induced psoriasis, one representative set of reactions is shown. For each patient, one out of three representative staining is shown. *$p < 0.05$, **$p < 0.01$ versus anti-TNF-α-induced psoriasis. Scale bars, 40 μm.

has been found to be dysregulated in psoriasis (13, 14) and in many cancer types, including melanoma (28). Immunohistochemistry analysis carried out on immune-related psoriasis induced by anti-PD-1 treatment revealed a strong positivity for ADAMTSL5 antigen in skin lesions of all three patients (Figure 5). Immunoreactivity was mainly present in keratinocytes throughout the epidermis and in scattered basal cells with the morphology of melanocytes (Figure 5A). ADAMTSL5 was also highly expressed in most dermal infiltrating cells, expectedly DCs and macrophages, localized in the papillary and mid dermis, as well in perivascular and endothelial cells (Figure 5A). Non-lesional areas adjacent to developed psoriasiform reactions showed ADAMTSL5 immunoreactivity exclusively confined to melanocytes (not shown). ADAMTSL5 expression pattern observed in immune-related psoriasis induced by anti-PD-1 was similar to that observed in plaque-type psoriasis (Figure 5A), even though ADAMTSL5 antigen was not always detectable in all psoriasis specimens (3 of 6 psoriasis patients were $ADAMTSL5^+$). None of the three paradoxical psoriasis to anti-TNF-α showed ADAMTSL5 positivity (Figure 5A).

Finally, for patient 2, we performed ADAMTSL5 immunohistochemistry on skin specimens obtained from lesional, perilesional and 3-cm distant areas of the primary melanoma, from melanoma skin metastasis and anti-PD-1-induced vitiligo. As shown in Figure 5B, ADAMTSL5 was expressed in most melanoma cells of primary tumor, with different intensity of staining. ADAMTSL5 immunoreactivity could be weak, moderate, or intense and localized in cytoplasmic foci/granules. An intense

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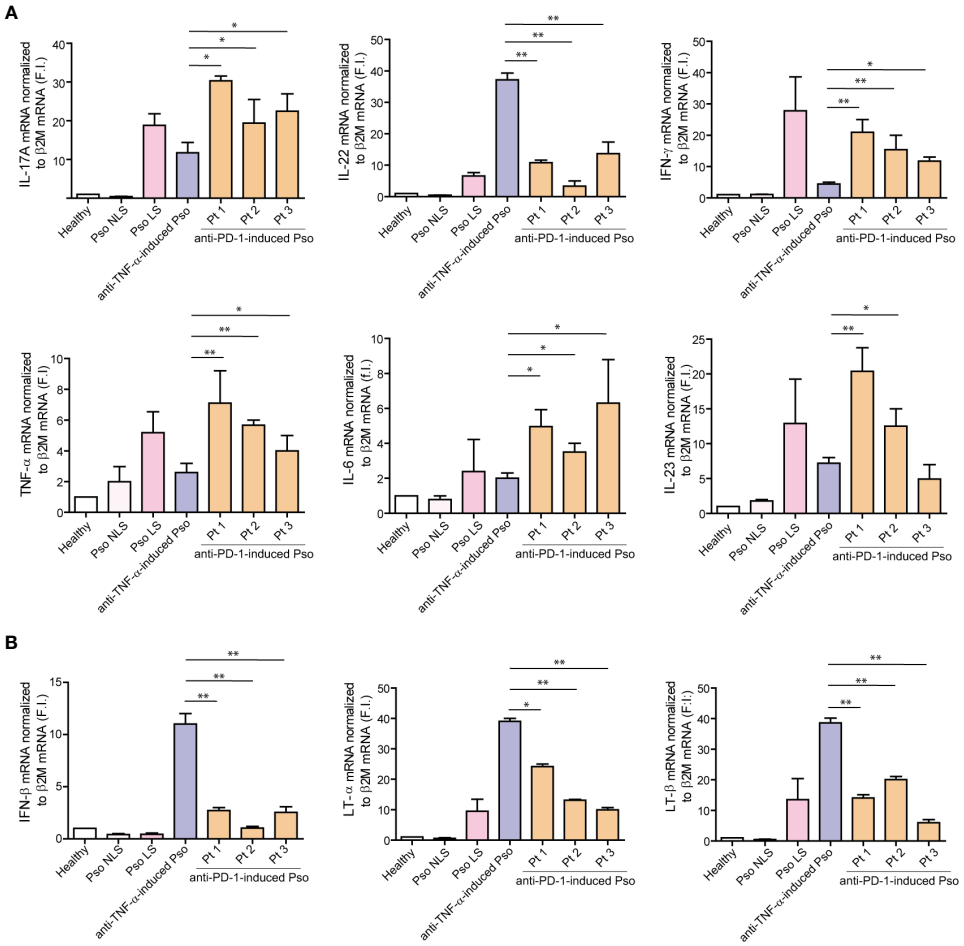

FIGURE 4
Inflammatory cytokines typical of chronic psoriasis are overexpressed in immune-related psoriasis reactions to anti-PD-1. **(A)** mRNA expression of IL-17A, IL-22, IFN-γ, TNF-α, IL-6 and IL-23 was analyzed by real-time PCR in psoriasis lesions induced by anti-PD-1 (Pt 1, Pt2 and Pt3), and compared to those present in skin biopsies from lesional (Pso LS) and non-lesional (Pso NLS) skin of psoriatic patients (n=6) and in paradoxical psoriasis induced by anti-TNF-α (n=3). Healthy skin from healthy donors (Healthy, n=6) was also analyzed. Levels of all mRNAs were significantly higher in the skin of the three patients, as compared to psoriasis-like reactions to anti-TNF-α, and similar to those detected in chronic psoriasis plaques. **(B)** Real-time PCR analysis of selected innate immunity molecules showed that IFN-β, LT-α and LT-β mRNAs are less expressed in psoriasis lesions induced by anti-PD-1, as compared to psoriasis reactions to anti-TNF-α. mRNA values were normalized to β2M mRNA. All data shown are the mean of three different experiments ± SD. Statistical significance was assessed by paired Student's t test, $*p \leq 0.05$, $**p \leq 0.01$.

staining was also observed in perilesional area neighboring primary melanoma, specifically in melanocytes undergoing transformation and in few infiltrating dermal cells (Figure 5B, panel ii). Conversely, in the area 3-cm distant from the margin of tumor, ADAMTSL5 positivity was only found in melanocytes (Figure 5B, panel i). ADAMTSL5 staining was also observed in melanoma skin metastasis of patient 2, with an intense and granular positivity in the cytoplasm (Figure 5B, panel vi). Of note, we could detect numerous ADAMTSL5$^+$ melanophages/macrophages infiltrating tumoral areas and characterized by a strong accumulation of melanosomes ingested from neighboring melanocytes (Figure 5A, panel iii-vi). These observations suggest that ADAMTSL5 protein is produced by tumor cells in significant amounts and undergoes subsequent uptake by melanophage/macrophage subpopulation. Finally, in vitiligo specimens of patient 2, ADAMTSL5 expression was absent in both perilesional and lesional skin, except for few cells infiltrating the dermis (Figure 5C).

3.4 SNP characterization in anti-PD-1-treated patients developing immune-mediated psoriasis reactions

In order to understand whether immune-related psoriasis development in patients undergone nivolumab treatment had a genetic basis, we analyzed by high-throughput NGS a panel of SNPs predisposing to psoriasis. Among them, we studied SNPs frequent in psoriatic population, such as polymorphisms in *HLA-C, HLA-B, ERAP1, PSORS1C3, MICA* and other genes of *PSORS1* locus. We also analyzed genetic variants of pathogenic cytokines, receptors, signal transducers and regulators of cytokine signaling (i.e., TNF-α, IL-17F, IL-17RA, IL-23R, IL-12B, TNFSF15, TNFRSF1B, TRAF3IP2, TNAIP3, NFKBIZ, SOCS1 and Tyk2), as well as SNPs in genes encoding skin-barrier proteins (i.e., *CDSN, CCHCR1*) and innate immunity molecules (i.e., IFIH1, DDX58 and LTA) (Supplementary Table 1). All patients showed variants of ERAP1

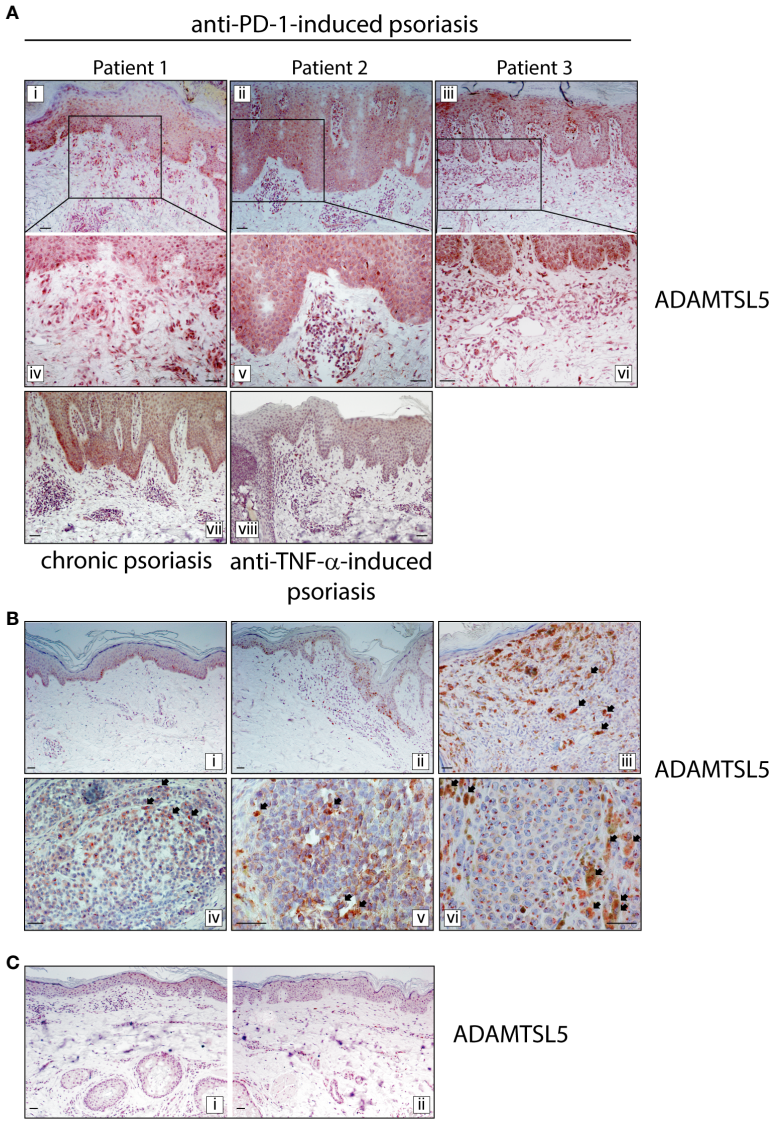

FIGURE 5
ADAMTSL5 psoriasis autoantigen is overexpressed in psoriasis lesions induced by nivolumab treatment, as well as in melanoma tissues. **(A)** Immunohistochemistry for ADAMTSL5 was conducted on psoriasis lesions induced by anti-PD-1 (Patient 1, 2 and 3), and compared to those present in chronic plaque-type psoriasis (n=6 patients) and in psoriasis induced by anti-TNF-α therapy (n=3). ADAMTSL5 immunoreactivity was mainly present in paradoxical psoriasis to anti-PD-1, in keratinocytes and in scattered basal cells with the morphology of melanocytes. It was also highly expressed in most dermal infiltrating cells, localized in the papillary and mid dermis, as well in perivascular and endothelial cells. Panels iv, v and vi represent the insets of panels i, ii, and iii, respectively, at a magnification of x200. ADAMTSL5 expression pattern observed in psoriasis to anti-PD-1 was similar to that observed in plaque-type psoriasis (vii). Anti-TNF-α -induced psoriasis lesions were negative (viii). **(B)** For patient 2, ADAMTSL5 was also detected in non-lesional (3-cm distant area from tumor lesion) (i), perilesional (ii) and lesional (iii-v) skin specimens obtained from the primary melanoma and melanoma skin metastasis (vi). Numerous ADAMTSL5^{+} melanophages/macrophages infiltrated melanoma tissues (arrows, iii-vi). **(C)** ADAMTSL5 expression was absent in both perilesional (i) and lesional (ii) skin of anti-PD-1-induced vitiligo developed by patient 2 after nivolumab treatment. For each specimen, one out of three representative staining is shown. Scale bars, 40 µm.

and HLA-C region, either in homozygosis or heterozygosis (Supplementary Table 1). SNP pattern in *ERAP1* were specific for each patient and affected both exons and intronic regions of the gene. Several variants in HLA-C promoter were found in patient 1, who showed concomitant SNPs in HLA-C upstream region (~35 kb) (rs12191877, rs17192519, rs198874, rs17198895, rs17192526, rs4406273, rs2524095, rs2853922) (Supplementary Table 1). Patient 2 showed only two SNPs in HLA-C upstream region (rs9264942, rs10484554), whereas patient 3 showed four critical SNPs in the 3'UTR of HLA-C (rs1130538, rs1130580, rs1130592, rs1094) and five SNPs in HLA-C upstream region (rs9348865, rs9264944, rs9264946, rs76703505, rs3094691). Interestingly, patient 2 showed three SNPs (rs2523473, rs2428476, rs28366116) in homozygosis in *MICA* (Supplementary Table 1). A specific SNP pattern was observed for each patient, even though all patients carried out numerous SNPs in genes involved in skin barrier function, namely in *LCE 3A-B*, *LCE1C*, *CDSN* and *CCHCR1* (Supplementary Table 1). Concerning variants of genes encoding cytokines, receptors, signal transducers and regulators of cytokine signaling, patient 1 showed SNPs in *TNFRSF1B* (five SNPs of which

four were in homozygosis), *RUNX3* (rs7536201), *SLC12A8* (rs651630), *TNIP1* (three SNPs in heterozygosis), *IL12B* (rs3213094 and rs2546890), *SOCS1* (rs431918 in homozygosis), *FBXL19* (rs10782001 in homozygosis) and *IL17RA* (rs4819553 and rs4819958) (Supplementary Table 1). Patient 1 and 2 showed SNPs in *TNFA* (rs3093662 in patient 1 and rs3093661 in patient 2) and in *TNFRSF1A* (rs767455 in both patients). Patient 2 and 3 shared SNPs in *NFKBIZ* encoding the IKB-ζ signal transducer of IL-17 (rs595788, rs9881690, rs7625614 in patient 2 and rs9818678 in patient 3), *TNFAIP3* (rs582757 in both patients and rs610604 in patient 3). Patient 1 and 3 showed variants of *IL-23R* (rs12567033 in patient 1 and rs2201841 in patient 3), *TRAF3IP2* (rs33980500 and rs76228616 in patient 1 and rs71562288 and rs240993 in patient 3) and of *TNFRSF15* (rs6478109 in both patients). Patient 3 only carried out two SNPs in *CTLA4* (rs231721 and rs3087243, both in homozygosis) and in *RUNX1* (rs2834760) (Supplementary Table 1). All patients shared SNPs in *TNFSF15*, a gene encoding a cytokine belonging to the TNF ligand family.

4 Discussion

Psoriasis pathogenesis involves both innate and adaptive immunity responses, which are overactive in different clinical phases of the disease and characterized by specific patterns of inflammation. Innate immunity processes predominate in the early phase of psoriasis development, with pDC, neutrophils and mast cells being abundant in skin lesions. Conversely, adaptive immune responses driven by mDCs and T lymphocytes, mostly IL-17- and IFN-γ-producing $CD8^+$ T cells, are typical of chronic, stable psoriasis (10, 29–31).

In this study, we found that immune-related psoriasis evoked by anti-PD-1 therapy in three patients affected by malignancies strongly resembles chronic psoriasis. In fact, by comparing skin lesions of immune-related psoriasis to anti-PD-1 with chronic plaque-type psoriasis and psoriasiform reactions to anti-TNF-α, we observed a prominent infiltrate of $CD8^+$ T cells and $CD11c^+$ DC, at levels and patterns of distribution of stable psoriasis, together with $CD15^+$ neutrophils accumulating in sub corneal aggregates. In parallel, the mRNA expression levels of psoriasis-related cytokines, such as IL-17A, IL-23, IFN-γ and IL-22 greatly increased in immune-related skin reactions to anti-PD-1. Conversely, the immunological patterns typical of paradoxical psoriasis by anti-TNF-α and acute psoriasis, including overexpression of innate immunity molecules and dermal infiltration of $BDCA^+$ pDC, $CD15^+$ neutrophils, and c-kit/$CD117^+$ mast cells could not be found.

Concerning mechanisms involved in anti-PD-1-induced psoriasis reactions, Tanaka R et al. described the contribution of $CD8^+$ T cells, whose pathogenicity has been related to enhanced IL-6, IL-23 and IL-17A production. These findings were obtained by blocking IL-6 receptor in IMQ-induced psoriasis reactions in mice genetically modified and lacking PD-1 expression specifically in $CD8^+$ T cells (8). We confirmed the observation that $CD8^+$ T cells strongly infiltrate psoriasis-like lesions of patients undergone anti-PD-1 therapy, with a preferential accumulation of these cells in the epidermal compartment. $CD8^+$ T cells were in close contact with keratinocytes and distributed as in chronic psoriasis, where an aberrant crosstalk *via* MHC class I molecules and (auto)antigen presentation by keratinocytes occurs (10). We also found that IL-6 and TNF-α are aberrantly expressed in psoriasis reactions induced by anti-PD-1, at levels comparable with chronic psoriasis. Increased IL-6 production in patients affected by psoriasis has been extensively described (32) and correlated to several pathological effects within affected tissues, including differentiation of type-17 lymphocytes and dampening of regulatory T (T_{reg}) cell function (33). In chronic psoriasis, IL-6, abundantly released by Th17 cells, sustains deleterious loops leading to Th17/T_{reg} unbalance (34, 35). Of note, IL-6-induced effects, which are deleterious in patients affected by psoriasis, could be instead protective in the cancer context and limit tumor growth and expansion, by promoting expansion of cytotoxic IL-17-producing T cells and preventing immune suppression by T_{reg}. However, although melanoma microenvironment can provide an optimal cytokine milieu for Th17 recruitment/expansion by expressing high IL-6 (36), and IFN-γ-releasing Th17 cells show antitumor effects through recruitment of cytotoxic $CD8^+$ T cells (37), IL-17A-expressing cells were generally few around the primary melanoma lesions (38). It would be of interest to investigate whether Th17 lymphocytes are present in melanoma lesions in patients developing cutaneous ircAEs.

Here, we also show that ADAMTSL5 antigen was strongly expressed in psoriasis lesions of the three oncological patients, at similar expression levels and localization of chronic plaque-type psoriasis. However, ADAMTSL5 was not detectable in all patients affected by plaque psoriasis, confirming that different (auto) antigens can elicit adaptive immune responses in the disease (39, 40). In addition, ADAMTSL5 was not expressed in psoriasis reactions to anti-TNF-α, in line with previous findings showing an overactivation of innate immunity, and not adaptive responses, in these conditions (17, 41). Importantly, in patient 2, ADAMTSL5 expression was not only found in psoriasis skin lesions but also in most melanoma cells of the primary tumor and in perilesional areas, specifically in melanocytes undergoing transformation. ADAMTSL5 also accumulated in melanophages/macrophages infiltrating tumoral and peritumoral areas, indicating that ADAMTSL5 protein is produced by melanoma cells in significant amounts and undergoes subsequent uptake by melanophages. These latter cells are macrophage-tumor hybrids, which have been related to the tumorigenicity and metastatic potential of melanoma (42). In fact, melanophages have a strong migratory capacity *in vitro* and can spread to skin-draining lymph nodes (43), where cross-presentation of melanoma antigens by DCs or other antigen presenting cells to naïve T cells typically occurs (44). The presence of ADAMTSL5 antigen in primary melanoma, as well as in cutaneous and lymph node metastasis (data not shown) suggest that ADAMTSL5-specific T-cell responses can be driven in melanoma patients, with the possibility of induction of immune responses triggering/exacerbating psoriasis in permissive conditions (anti-PD-1 treatment). Besides melanoma, ADAMTSL5-specific immune responses could also be induced in other cancer types, being ADAMTSL5 dysregulated in a wide variety of malignant

tumors, including lung cancer and hepatocarcinoma (28, 45). In hepatocarcinoma condition, ADAMTSL5 overexpression derives from hypermethylation of *ADAMTSL5* gene body and localizes in hepatocarcinoma cells and in macrophages of necrotic areas of tumors. Importantly, ADAMTSL5 confers tumorigenicity by upregulating oncogenic inputs (i.e., MET, EGFR, PDGFRβ, IGF1Rβ, FGFR4), and its abrogation increases sensitivity of tumor cells to clinically relevant drugs (45).

In psoriasis patients, specific immune responses induced by ADAMTSL5 autoantigen are represented primarily by IL-17A-producing $CD8^+$ T cells and directed against melanocytes and surrounding keratinocytes (12). ADAMTSL5 recognition occurs through HLA-Cw6 class I molecule, which presents peptide ligands by recognizing Vα3S1/Vβ13S1 TCR on $CD8^+$ T cells (12). It would be interesting to examine whether ADAMTSL5 might represent a potential antigen also for melanoma and other malignancies, recognized by $CD8^+$ T cells with specific TCR repertoire. There is the possibility that anti-PD-1-induced immune responses specific for ADAMTSL5 could be responsible for both anti-PD-1-induced psoriasis, and for an effective immune response against melanoma cells. The favorable prognosis of patients 1 and 2 after nivolumab treatment is in line with this hypothesis since both of them showed high expression of the ADAMTSL5 autoantigens. Instead, the poor prognosis of patient 3 could depend on the short-term treatment with nivolumab and concomitant immunosuppressive therapies for limiting psoriasis and immune-related responses.

Psoriasis disease manifestation occurs only in a portion of subjects undergone anti-PD-1 therapies for advanced solid tumors, with an incidence rate of ~ 4.3% in patients developing ircAEs (46). Therefore, it is reasonable to speculate the influence of genetic factors predisposing to immune-related psoriasis to anti-PD-1, and specifically being involved in driving and amplifying type-17 and type-1 adaptive immune responses. Indeed, a genetic predisposition to other types of psoriasis reactions induced by therapies with immunomodulators have been described (17, 47). To date, no evidence correlating the presence of SNPs and the development of psoriasis in patients in treatment with anti-PD-1 exist. In our study, we found that all patients carried numerous allelic variants in *HLA-C*. None of the patients showed the *HLA-Cw6* susceptibility allele, even though other SNPs possibly involved in the regulation of HLA-C expression levels (i.e., *HLA-C* promoter, *HLA-C* 3'UTR and *HLA-C* upstream region) were found. These findings are important since specific HLA-C haplotypes have been correlated with the clinical course of psoriasis disease, and over one-hundred SNPs of *HLA-C* genic and intergenic region have been described in patients (48). In addition, evidence has emerged for the presence of susceptibility alleles of other MHC class I genes and regulatory regions, potentially influencing HLA expression in the psoriatic population. They include polymorphic regions in proximity to *MICA*, which encodes MHC class I-related proteins with potential immunological functions on IL-17A-producing and $CD8^+$ T cells (49). These latter polymorphisms were only found in patient 2, who showed the most favorable response to anti-PD-1 treatment. Concomitantly, oncological patients carried allelic variants in the *ERAP1* gene, consistently with the presence $CD8^+$ T-cell responses in psoriasis reactions to anti-PD-1. Interestingly, different ERAP1 haplotypes controlling the likelihood and strength of the immune response have been identified (50). Among them, the haplotype 2 (rs26653) can control the autoimmune response against melanocytes in psoriasis by generating ADAMTSL5 antigen epitopes (50). All patients show specific ERAP1 haplotypes, which may determine the generation and different amounts of certain autoantigens for HLA-class I presentation with the subsequent risk of autoimmune $CD8^+$ T-cell activation (50). Other than having a role in MHC class I antigen presentation, ERAP1 is involved in the activation of inflammasome pathways and production of cytokines and chemokines involved in psoriasis development (i.e., IL-6, TNF-α, and CCL2) (51).

Although all patients carried numerous SNPs in genes involved in skin barrier function, a specific SNP pattern in *LCE 3A-B* and *LCE1C*, as well as in *CDSN* and *CCHCR1*, was observed for each patient. In particular, several SNPs were identified in *LCE* gene cluster, located in the epidermal differentiation complex of *PSORS4* locus and encoding structural proteins with a role in epithelial barrier formation, as well as peptides with antimicrobial activity (52). All LCE allelic variants are likely involved in the pathogenic responses induced by IL-17 in psoriatic keratinocytes, in terms of terminal differentiation and proliferation, two processes contributing to epidermal acanthosis typical of psoriatic lesions (10). Previous studies identified several conserved, noncoding elements within *LCE* intergenic region exhibiting dynamic regulatory activity and coordinating LCE expression in differentiating or proliferating cells (53). In all patients, we found SNPs located between *LCE3B* and *LCE3A*, in an intergenic region potentially involved in the regulation of expression levels of LCE3 genes. This genomic sequence could have regulatory functions like the entire *LCE3B/C* region, whose deletion leads to increased LCE3A mRNA expression in psoriatic skin under the influence of Th1 and Th17 cytokines (54).

We found few SNPs in exon 2 of *CDSN* overlapping with *PSORS1C1*, which can give rise to missense variants strongly impacting on corneocyte adhesion and skin desquamation, as well as associating with increased risk of psoriasis severity (55). The potential effects of SNPs on PSORS1C1 expression and function in patients is unpredictable.

Concerning *CCHCR1*, we found several SNPs in the three patients, with different distribution in introns and exons of the gene. In patient 1, we detected SNPs potentially leading to amino acid substitution in exon 4 (rs130065, rs130066, rs130076), exon 14 (rs130079) and in exon 18 (rs1576) and two SNPs in intron 10 (rs746647, rs2240065). Patient 2 and 3 showed two SNPs in homozygosis in *CCHCR1*, both present in intron 13 (rs3094226, rs2073719). Although these latter two SNPs are irrelevant for amino acid composition of CCHCR1 protein, their potential regulatory function of CCHCR1 mRNA expression cannot be excluded. The consequence of the SNP presence in CCHCR1 in patients could be multiple, depending on SNP presence and haplotypes, which give raise different CCHCR1 mRNA and protein variants (56). CCHCR1 influences keratinocyte proliferation by regulating cytoskeleton as well as other processes including RNA surveillance and transport (57). The function of CCHCR1 isoforms in psoriasis together with the IL-17-dependent mechanisms regulating their expression pattern in psoriatic skin remains to be elucidated. NGS analysis of variants of genes encoding cytokines, receptors, signal transducers and regulators of cytokine signaling showed that all

patients carried SNPs in *TNFSF15*, a gene encoding TL1A. TL1A is a TNF-like protein overexpressed in psoriasis, that competitively binds to death receptor 3, providing stimulatory signal for proliferation, activation, apoptosis and cytokine in effector immune cells (58). Interestingly, patient 1 had a peculiar genetic pattern, and differently from the other two patients carried several SNPs predisposing to psoriasis. These SNPs were present in *TNFRSF1B, RUNX3, SLC12A8, TNIP1, IL12B, SOCS1, FBXL19* and *IL17RA*, and were associated with the severity and early onset of the disease (59). Indeed, patient 1 developed the most severe form of psoriasis among the three patients and had a personal history for the disease. On the other hand, patient 2 and 3 shared SNPs in *NFKBIZ* and in *TNFAIP3*, encoding the IL-17/TRAF6 signaling regulators IKB-ζ and A20, respectively, whose genetic variability could contribute to immune dysregulation and chronic inflammation in psoriasis lesions (60). Patient 3 only showed polymorphisms in a region downstream of *CTLA4*, which have been previously associated to paradoxical psoriasis to anti-TNF-α (61). In the future, it will be necessary to extend the analysis of psoriasis-related SNPs to a largest cohort of oncological patients developing psoriasiform reactions to anti-PD-1, but also in a population successfully responding to the treatment, to identify differences in the genetic background of the patients. The identification of genetic biomarkers correlating with an adverse response to anti-PD-1 therapy will be useful to predict the risk of developing immune-related psoriasis.

In conclusion, our study shows that immune-related psoriasis induced by anti-PD-1 therapy in three oncological patients has immunological features common to chronic phase psoriasis, mainly characterized by cellular and molecular players of adaptive immunity. Among them, $CD8^+$ T cells were found in the epidermis of skin lesions and in close contact with keratinocytes, consistently with the local overexpression and exposure of ADAMTSL5 psoriasis autoantigen. The latter has also been found in melanoma tissues, suggesting a possible role of ADAMTSL5 in evoking T-cell responses in common with psoriasis. The genetic susceptibility of patients to develop immune responses typical of psoriasis would confirm this possibility. It will be important to evaluate the presence and features of ADAMTSL5-specific T-cell responses in oncological patients developing chronic psoriasis following anti-PD-1 therapies, and to assess whether these responses may concomitantly trigger the onset of psoriasis and the protection against tumor.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra/PRJNA107495558, PRJNA1074955.

Ethics statement

The studies involving humans were approved by the Ethics committee of Istituto Dermopatico dell'Immacolata Hospital, Rome, Italy (Prot. CE 475/2016). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. MC: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. GS: Formal analysis, Software, Writing – review & editing. CS: Formal analysis, Methodology, Validation, Writing – review & editing. VF: Methodology, Writing – review & editing. SP: Resources, Writing – review & editing. FG: Resources, Writing – review & editing. SR: Formal analysis, Writing – review & editing. SM: Conceptualization, Supervision, Writing – review & editing. CF: Conceptualization, Supervision, Writing – review & editing. CA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The 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.2024.1346687/full#supplementary-material

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Heterogeneity and plasticity of tissue-resident memory T cells in skin diseases and homeostasis: a review

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Skin tissue-resident memory T (Trm) cells are produced by antigenic stimulation and remain in the skin for a long time without entering the peripheral circulation. In the healthy state Trm cells can play a patrolling and surveillance role, but in the disease state Trm cells differentiate into various phenotypes associated with different diseases, exhibit different localizations, and consequently have local protective or pathogenic roles, such as disease recurrence in vitiligo and maintenance of immune homeostasis in melanoma. The most common surface marker of Trm cells is CD69/CD103. However, the plasticity of tissue-resident memory T cells after colonization remains somewhat uncertain. This ambiguity is largely due to the variation in the functionality and ultimate destination of Trm cells produced from memory cells differentiated from diverse precursors. Notably, the presence of Trm cells is not stationary across numerous non-lymphoid tissues, most notably in the skin. These cells may reenter the blood and distant tissue sites during the recall response, revealing the recycling and migration potential of the Trm cell progeny. This review focuses on the origin and function of skin Trm cells, and provides new insights into the role of skin Trm cells in the treatment of autoimmune skin diseases, infectious skin diseases, and tumors.

KEYWORDS

heterogeneity, plasticity, tissue-resident memory T cells, psoriasis, vitiligo, melanoma

Highlights

- Tissue-resident memory T (Trm) cells are a group that remain in the tissue for long periods of time and are able to stay out of the peripheral circulation for months or even years.
- Skin Trm cells can be expanded from the original Trm source or developed from Trm precursors (Tcm cells, Tem cells, Tmm cells, etc.).

- $CD4^+$ Trm or $CD8^+$ Trm cells play a harmful role in autoimmune related skin diseases such as psoriasis and vitiligo, while $CD8^+$Trm cells play a protective role in tumor diseases such as melanoma.
- Skin Trm cells have the possibility of redifferentiation when they are encountered with pathogens or in a stable state. Mainly $CD4^+$ Trm cells can migrate to the remote skin or enter the circulation.
- The redifferentiation and migration of skin Trm cells have considerable prospects in the treatment of various skin diseases.

Introduction

The main components of skin are generally divided into the epidermis, dermis, and subcutaneous fat area. The basement membrane is the boundary between the epidermis and dermis. The skin is the first barrier to provide robust immune protection against invading pathogens in humans and many other organisms. It relies on an immunomodulatory network of innate and adaptive immune cells and many resident populations (1). The number of T cells infiltrating the skin is much higher than that in peripheral blood; memory T cells account for most of these cells including effector T (TEFF) cells, naive T cells, memory T cells and exhausted T cells (2). In the context of encountering pathogens in barrier tissues, dendritic cells (DCs) initiate the presentation of antigens to naive T cells through lymph node drainage, resulting in the activation, proliferation, and differentiation of naive T cells into effector T cells. Previous research has indicated that following the resolution of inflammation or infection, a small proportion of effector T cells differentiate into memory T cells and probably inhabit the infiltrated tissue, subsequently undergoing local exit or apoptosis (3). However, investigations by Gebhardt et al. utilizing herpes simples virus (HSV)-infected mouse models have identified a specific subset of effector memory T cells in peripheral tissues that persist within the same tissue for an extended period without recirculation after the infection has been eradicated, which subsequently developed into tissue-resident memory T (Trm) cells. They cannot be recycled after local or systemic viral infection and persist after transplantation of infected skin to the recipient (4–6). This results in the emergence of a population of Trm cells. In recent years, Trm cells have been found to reside in lymphoid and non-lymphoid organs for a long time. Traditionally, Trm cells in the skin exhibit prolonged survival for months or even years and reside at the site of the initial antigen encounter. The population of these cells can increase with host age. In the absence of antigenic stimulation, Trm cells can persist in peripheral tissues such as the skin and the female reproductive tract. During this time, they fulfill the role of tissue surveillance, extending dendritic projections within the local tissue to actively search for antigens (7). Upon reactivation by a specific antigen, Trm cells cease their motility and undergo rapid proliferation within the epithelial barrier tissue. Guided by the local tissue microenvironment, Trm cells promptly initiate a secondary immune response and differentiate into effector T cells, providing swift and effective protection against a potential secondary antigenic assault on the tissue (8). Notably, among the diverse array of memory T cells present in the skin, Trm cells occupy a distinct niche with a unique transcriptional profile and phenotype compared to those of other memory cells (9). Multiple studies have identified the expression of CD69 or CD103 as a notable characteristic of these cells, facilitating their tethering to the tissue and inhibiting their egress from the tissue. However, recent studies have suggested that the tissue residence of Trm cells is reversible (10). Trm cells exposed to secondary antigenic stimulation can differentiate into other types of memory cells. For example, the knockdown of CD69 in $CD4^+$ Trm cells promotes cell efflux from the skin, and these Trm cells not only reenter the blood circulation but also retain tissue propensity and recolonize secondary skin sites. In a mouse model, $CD8^+$ Trm cells were found to proliferate in draining lymph nodes and become circulating memory cells after antigen encounters (11). Thus, Trm cells are found in various non-lymphoid tissues, including the skin, and may reenter blood and distant tissue sites in response to recall, revealing the recycling and migratory potential of Trm cell generation (12).

This review examined a range of memory T cells found in the skin, focusing on Trm cells. Upon entering the skin, multiple subpopulations of memory T cells can differentiate into Trm cells, which exhibit diverse phenotypes in both healthy and diseased states. Furthermore, we will review the delicate balance between Trm cell settlement and recycling, summarize the crucial role of Trm cell plasticity in immune responses, and propose novel hypotheses regarding the involvement of this cell type in various diseases.

Different types of memory T cells exist in the skin

There are approximately 20 billion T cells in healthy human skin, most of which are $CD45RO^+$ memory T cells (up to more than 80%) (13). Memory T cells are usually divided into central memory T (Tcm) cells, Trm cells, effective memory T (Tem) cells and migrating memory T (Tmm) cells. Tcm cells can circulate between the bloodstream and secondary lymphoid organs (SLOs). CD62L and CCR7 are highly expressed in this group of cells, which allows them to maintain their circulatory capacity, similar to the characteristics of naive T cells. Upon encountering pathogen stimulation again, Tcm cells can be mobilized to lymphoid tissues or inflammatory sites beyond lymphoid tissues. These cells undergo rapid proliferation and differentiation into secondary effector T cells, which effectively combat pathogen invasion (7, 14). On the other hand, Tem cells can migrate between the bloodstream and non-lymphoid tissues (NLTs). These cells exhibit a strong inclination toward peripheral tissues and are characterized by low expression of L-selectin and CCR7 (15). Tem cells have primary effector functions and can quickly reach infection sites to form highly protective effector cells after encountering pathogens; thus, they are important for the formation of Trm cells and for the maintenance of Trm cells (9). Moreover, Tem cells are thought to be

capable of patrolling the interior of NLTs, but their specific function remains to be elucidated (16). In recent years, the concept of Trm cells, a group of cells that settle in the local tissue and are difficult to migrate, has emerged. Trm cells play a central protective role against NLTs; thus, most memory T cells in NLTs are Trm cells (17). They provide rapid and effective protection against pathogen invasion in barrier tissues. Healthy human skin contains $CD4^+$ and $CD8^+$ Trm cell populations that are more enriched in the epidermis than in the dermis. These groups of cells play essential roles in integrated memory responses and cutaneous immune responses (18). They present in small numbers in the circulatory system and in SLOs. Tmm cells are the most common CLA^+ memory T cell population in human blood. These cells exhibit high expression of CCR7 but low expression of L-selectin (19). The characteristics of these cells are between those of Tcm cells and Tem cells, and they have effector functions. However, Tmm cells appear to be excluded from lymph nodes that drain nonskin tissues. They can migrate from the skin to lymphatic vessels and into the skin-draining lymph nodes, a phenomenon not observed in other tissues (20).

The majority of memory T cells in healthy skin are phenotypic $CD62L^-CCR7^-$Tem cells. Tcm cells are present in small amounts in resting skin T cells, and Trm cell proportions can range from 20 to 60%, indicating that the percentage of Trm cells is highly variable in healthy individuals (21). Tissue-specific $CD4^+/CCR7^+$ Tcm cells and $CD4^+/CCR7^-$ Trm cells protected healthy human skin on most occasions, with the latter being significantly more protective. A recent study allergic contact dermatitis showed that TCR complementary-determining region 3 (CDR3) sequences are present in both cutaneous Trm cells and Tcm cells and that the generation of these two cell types in the skin may be related to specific priming signals from dendritic cells (22). A clinical experiment of alemtuzumab in treating leukemic cutaneous T-cell lymphoma (L-CTCL) revealed that Tcm cells and Tmm cells accounted for approximately one-third of the total cutaneous T cells. They circulate between the skin and peripheral blood and are closely linked to the development of the clinical symptoms of the disease. However, skin-resident $CCR7^-/CD69^+$ T cells are unaffected by alemtuzumab treatment, and this population can exert effector functions during disease development (23). Therefore, a variety of memory cells mediate the immune response of the skin, but Trm cells play a significant role in this process.

The origin of Trm cells in the skin

During the immune process of T-cell response, the fate of each T-cell is determined by the reception and release of different signals. They can undergo apoptosis or differentiation into memory T cells of different phenotypes after an immune response (24). When naive T cells encounter homologous antigens, they activate and proliferate in draining lymph nodes. This results in a corresponding population of effector T cells being able to specifically clear infected cells (25).

A small proportion of effector cells do not undergo apoptosis after pathogen clearance and instead differentiate into memory T cells (9). Skin antigen contact plays a vital role in the developmental trajectory of skin Trm cells. In the secondary immune response, the reactivation of Trm cells can recruit antigen-nonspecific memory T cells to the site of antigen encounter, thereby resulting in effector functions (26, 27). Recent studies have shown that the propensity of various T cells to produce Trm cells is uneven in the effector pool (28). Some studies have used lineage tracking tools to label naive T cells, and labeled TEFF cells, circulating memory T (TCIRCM) cells, and Trm cells have been obtained through mouse skin vaccines *in vivo* (29). The majority of naive T cells migrated into inflamed tissues and underwent differentiation into TCIRCM and Trm cell lineages (84.8%). Researchers have shown a specific subset of circulating TEFF cell clones has a high potential to develop into Trm cells. This suggests that the inclination toward Trm cell formation is acquired prior to tissue entry and that genes associated with Trm cell fate are abundantly expressed. Upon encountering an antigen again, these cells establish themselves as Trm cells (30). Nevertheless, there is also evidence indicating that Trm cells in tissue-draining lymph nodes are biased toward the skin due to their dependence on chemokines, and they do not necessitate antigen encounters. Moreover, $CD8^+$ T cells recruited by this route can be present in the skin for more than two months (31). However, little is known about the origin of Trm cells in the skin, that is, the origin of Trm cell precursors. The development of Trm cells is tissue-specific, but the underlying mechanism remains. Multiple subsets of memory T cells have been reported to initiate their differentiation program to become Trm cells, upon encountering homologous antigens in the skin (32, 33).. Among all CD45RO+ memory T-cell subsets, T cells are specifically recognized during maturation by high expression of skin lymphocyte-associated antigen (CLA) and migrate to the skin to form Trm cells (34, 35).. In the case of viral skin infections, $CD69^+KLRG1^-$ effector T cells are highly enriched in the early epidermis after the onset of T-cell infiltration, and the proportion of KLRG1 effector T cells is significantly higher in the spleen and blood than in the skin (36). Further support for circulating Trm cell precursors was provided by a study on Trm cell formation, in which CD127, which is present in humans and is the α chain of IL-7R, was identified. The interaction between IL-7 and CD127 plays an important role in the differentiation and maintenance of T-cell homeostasis (37). It is highly expressed in various autoimmune and inflammatory skin diseases. CD127 is a well-known marker of memory precursor cells. The generation of long lived T-cells is closely related to IL-7 and $KLRG1^-/CD127^+$ T cells are more likely to produce Trm cells in the skin (11). $CD8^+$ Tcm cells have been shown to persist as a significant tissue-resident population after pathogen clearance in the skin and to differentiate into functional $CD69^+CD103^-$Trm cells. An elegant study revealed that Tcm cells persist longer in circulation and enter the skin in more significant numbers as Trm cell precursors; these cells are the most dermatophilic and simultaneously produce high numbers of Trm cells and can complement other memory T-cell subsets (20, 38). $CCR7^+$ L-$selectin^+$ Tcm cells are the most potent precursors of Trm cells in human skin, and this population of cells has high potential to develop into Trm cells. However, the most efficient cell population for Trm cell transformation was Tem cells, which exhibited increased CXCR3 levels and produce interferon-γ (IFN-γ)$^+$ Trm

cells. In addition, Trm cells produced by migrating memory T cells preferentially express IL-17A (20, 39), and in psoriasis, $CD8^+$ Trm cells that produce IL-17A may constitute a pathogenic group in the skin (38).

Phenotype and localization of cutaneous Trm cells

The composition of Trm cells in the entire skin layer differs under stimulation by different antigens in the skin. Trm cells share a variety of common surface tissue markers and transcriptional signatures, which play crucial roles in Trm cell function (40). Two important surface markers of cutaneous Trm cells are CD69 and CD103. CD69 and CD103 were found on T cells but at different levels and with different dependent factors. $CD69^+CD103^{-/+}$ T cells constitute more than 70% of human skin T cells (41). CD69 is generally considered a marker of TCR-mediated activation, and skin Trm cells constitutively express CD69. CD69 downregulated S1P1 and decreased the expression of 'egress' sphingosine-1-phosphate receptor 1 (S1P) (42) to restrict S1PR1-mediated tissue export. Moreover, the expression of the transcription factor KLF2 can be transiently downregulated in response to antigens, thereby reducing the expression of its target gene, S1PR1. These mechanisms limit the efflux of memory cells from tissues, suggesting that S1P1 knockdown can lead to the long-term residency of Trm cells (43). CD103, the alpha chain of the $\alpha_E\beta7$ integrin, is induced by transforming growth factor-β (TGF-β) and binds to E-cadherin on epidermal cells in peripheral tissues, where it specifically restricts T-cell retention. Many researchers consider that the expression of CD103 is not a critical factor for skin Trm cell residency, and memory T cells with $CD103^-$ have also been found to have residual abilities. *Yersinia pseudotuberculosis* infection in the oral cavity induces the generation of $CD103^+$ or $CD103^-$Trm cells in the lamina propria (44). CD49a is the α-subunit of the α1β1 integrin receptor, also known as very late antigen 1 (VLA-1), and binds collagen IV enriched in the basement membrane separating the epidermis and dermis. CD49a expression is limited to only 15% of human skin-derived T cells, which may determine the cytotoxic function of Trm cells. Trm cell differentiation often depends on functional changes in this population of cells. T-cell phenotypes include the production of IL-17A and the upregulation of FOXP3 in $CD4^+$ T cells, and the upregulation of CD49a, CXCR3, and CXCR6 and the production of IFN-γ in CD8+ T cells (45). Under appropriate stimulation, $CD8^+$Trm cells with high CD49a expression had a higher ability to produce IFN-γ, perforin, and granzyme B (46, 47). The chemokine receptors CCR4, CCR8, CCR10, CXCR3, and CXCR6 are considered critical chemokine receptors for skin Trm cells. CCR8 is expressed in approximately 50% of Trm cells and is rarely expressed in Tcm cells (Figure 1).

The transcriptional profiles of Trm cells differ from those of Tcm cells and Tem cells, including the those of transcription factors

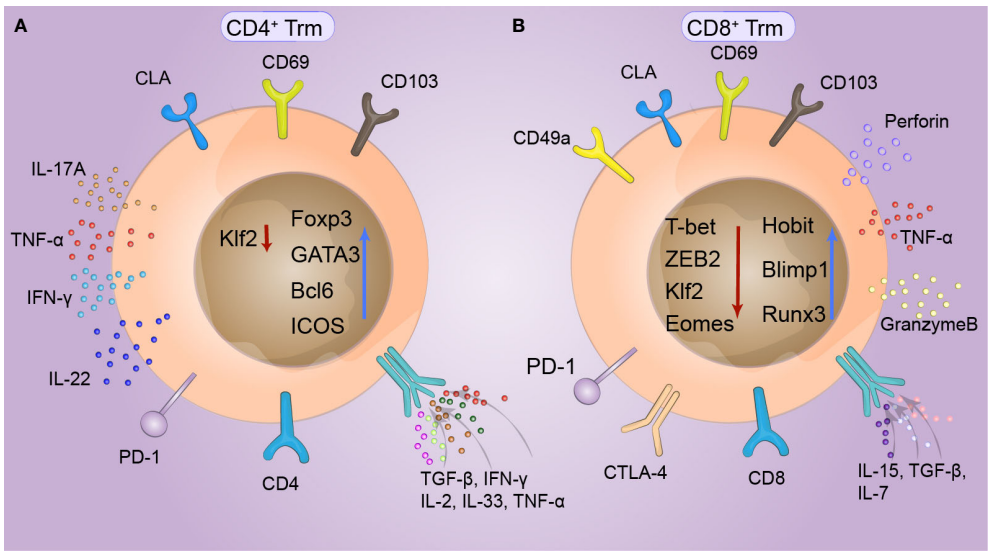

FIGURE 1
Phenotype of cutaneous tissue-resident memory T (Trm) cells. CD69 and CD103, which are expressed to varying degrees in both $CD4^+$ and $CD8^+$ Trm cells, are specific markers of skin Trm cells and can specifically limit the retention of T cells. Skin lymphocyte-associated antigen (CLA) is highly expressed in T-cell subsets with a special propensity for binding to skin tissue and is thus recognized by the skin. At the same time, the expression of KLF2 can be temporarily downregulated in response to antigens. **(A)** The survival of $CD4^+$Trm cells requires the intrinsic expression of Bcl6 by T cells and continuous signaling through ICOS, which is specifically upregulated in $CD4^+$Trm cells. FOXP3 and GATA3 are expressed in T helper cells. IL-17A, IL-22, and IFN-γ are produced by $CD4^+$Trm cells in healthy human skin, while TGF-β upregulates CD103 expression in $CD4^+$Trm cells, and IL-2, IL-33, IFN-γ, and TNF-α upregulate CD69 expression in $CD4^+$ Trm cells. **(B)** The transcription profiles of CD8+Trm cells, including Blimp1, Runx3, and Hobit cells, differed from those of Tcm and Tem cells. Hobit is specifically upregulated together with Blimp1 in $CD8^+$Trm cells. The key factors of the transcriptional regulation pathway, T-bet and ZEB2 are synergically downregulated under stimulation by local TGF-β signaling, promoting tissue retention. $CD8^+$ Trm cells also exhibit phenotypes similar to those of depleted T cells, including upregulation of a series of immune checkpoints, such as PD-1, LAG-3, TIM-3, and T-cell immune receptors (TIGIT). CD49a is upregulated in $CD8^+$ Trm cells, which can produce interferon (IFN-γ), perforin, and granulocase B under appropriate stimulation conditions.

Blimp1, Runx3 and Hobit (48). Blimp1 and Hobit are jointly and explicitly upregulated in Trm cells, promoting their retention in epithelial barrier tissues, and these two transcription factors have a synergistic effect on Trm cell development (49). In addition, the depletion of S1PR5 enhances the formation of Trm cells. S1PR5 plays a crucial role in T-cell infiltration and migration from peripheral organs and is downregulated in Trm cells. However, T-bet and ZEB2, which are critical factors in the S1PR5 transcriptional regulation pathway, are synergistically downregulated in Trm cells treated with S1PR5 under the stimulation of local TGF-β signaling (50). At the same time, a variety of tumor-associated Trm cells also exhibit phenotypes similar to those of depleted T cells, including upregulation of a series of immune checkpoints, such as TIM-3, LAG-3, PD-1 and T-cell immune receptor (TIGIT) (51). The survival of $CD4^+$Trm cells requires T cells to express Bcl6 internally and continuously transmit signals through ICOS and P2X7R. Thus, these two transcription factors are specifically upregulated in $CD4^+$Trm cells (Figure 1).

$CD4^+$ Trm cells

$CD4^+$ T lymphocytes are vital components of adaptive immunity. They can play an important auxiliary role in the function of innate cells, $CD8^+$T cells and B cells in the presence of various pathogens (43), especially Candida infection and Leishmania infection. In peripheral CD4 T cells, B-cell interactions lead to upregulation of the transcription factor Bcl6, which also inversely supports the differentiation of memory T cells (52). Similar to those of circulating $CD4^+$ memory T cells, the mechanism underlying the formation and diversity of memory $CD4^+$T cells are still unknown; therefore, extensive research on tissue-resident lymphocytes has focused on $CD8^+$ Trm cells. $CD4^+$ Trm cells typically gather in lymphoid tissue, and they interact with antigen-presenting cells upon re-encounter with pathogens. However, recent investigations have revealed a significant disparity between the numbers of $CD4^+$ and $CD8^+$ T cells within the T-cell population residing in the skin (53). $CD4^+$ T cells predominantly populate the dermal and epidermal compartments in humans, whereas in mouse skin, $CD4^+$ T cells are primarily concentrated in the dermis (54). In healthy human skin, the proportion of $CD4^+$ T cells in the two major layers of skin is approximately 75%, with $CD69^+CD103^+$Trm cells accounting for 50% and 25% of the T cells in the two layers, respectively. Among these, $CD4^+$ $CD69^+$ $CD103^+$ T cells are the most crucial resident population in the dermis. These cells exhibit specificity toward Candida albicans and Staphylococcus aureus and exhibit high expression levels of tumor necrosis factor-α (TNF-α), IL-22 and IFN-γ (55).Due to their limited proliferative capacity, $CD4^+$ $CD69^+$ $CD103^+$ T cells undergo rapid apoptosis when isolated from the skin (56, 57).

Conversely, $CD69^+CD103^-$ Trm cells are present in entire skin layers, constituting 20% and 40% of the T cells in the epidermis and dermis, respectively (54). These cells have a more robust proliferative capacity than double-positive cells; however, their effector function is relatively poor (58). In the dermal compartment, helper T (TH) cells were predominant compared to CD8+T cells, and the expression of CD103 was also relatively low compared to that in the epidermis. Consequently, $CD4^+$Trm cells exhibit enhanced migratory capacity but relatively diminished resident ability. In the skin of healthy mice, $CD4^+$T cells establish a dynamic equilibrium between migration and circulation with the circulating T-cell pool (59). Nonetheless, heightened skin inflammation or infection leads to augmented recruitment of $CD4^+$T cells to the skin and their retention in the dermis. This results in the development of a $CD69^+CD103^+$ $CD4^+$ T-cell phenotype embedded within the skin, which possesses potent effector functions (15). The adhesion of $CD4^+$T cells to keratinocytes and the upregulation of CD103 expression are facilitated by TGF-β.*In vitro*, TGF-β upregulated the expression of CD103, and TNF-α, IL-33 and IFN-γ upregulated the expression of CD69 (Figure 1). These cytokines and surface markers affect cutaneous $CD4^+$ Trm cell tissue retention more than antigen recognition (53, 60). $CD4^+$ Trm cells can supersede innate immunity upon re-exposure to antigens and exert a direct effector effect. However, the phenotypic and resident characteristics of $CD4^+$ Trm cells during infection remain unclear.

$CD8^+$ Trm cells

$CD8^+$ Trm cells are located mainly in the epidermal layer of the skin and the basement membrane between the dermis and epidermis. Skin $CD8^+$ Trm cells replace the original niche occupied by γδ T cells in the epidermis, enabling their stable existence for several years (61). These cells typically express surface markers such as CD69 and CD103 and have a core transcriptional signature distinct from that of circulating T cells. In the epidermis, $CD8^+$ $CD69^+$ $CD103^+$ Trm cells are a well-characterized subtype; however, at present, CD103 and CD69 are no longer considered the only identified markers of Trm cells. For example, after skin infection with herpes simplex virus 1 (HSV1), $CD8^+$ Trm cells in the epidermis are predominantly $CD69^+$ $CD103^+$ $VLA1^+$ $CD62l^-$ $CD122^-$ and exhibit strong effector function. In contrast, dermal Trm cells are $CD103^-$ but have a high proliferative capacity. Therefore, $CD8^+$ $CD103^+$ Trm cells do not proliferate in large quantities, but $CD103^+$ Trm cells are functionally superior to $CD103^-$ Trm cells (7). In $CD8^+$ Trm cells from mice, the responsiveness to TGF-β signaling is dependent on the suppression of T-box transcription factors, namely T-bet and Eomes, resulting in the upregulation of CD103 expression (62). Thus, the activation of TGF-β is mediated by the integrins avb6 or avb8 on keratinocytes and is regulated by T-box transcription factors. IL-15 plays a crucial role in maintaining the homeostatic proliferation and longevity of $CD8^+$ memory T cells. However, only low levels of T-bet expression can sustain the expression of IL-15R (63). Hence, T-bet and Eomes, which belong to the T-box family, regulate the responsiveness to both TGF-β and IL-15 signals, consequently influencing the normal development and function of $CD8^+$ Trm cells (64) (Figure 1). The requirements for establishing Trm cells were described in a study of skin infections by Jiang et al., who specifically identified ovalbumin (OT-I) and vaccinia virus expressing ovalbumin (VACV) using an adoptive transfer assay (65). The entry of CD8 T cells into the skin is not dependent on the helper function of $CD4^+$T cells or the expression

of IFN-γ, which is different from what occurs in other epithelial tissues. The expression of CD49a is limited to $CD8^+$ T cells and is present only in epidermal $CD69^+CD103^+$ T cells (65).

Trm cell phenotypes in skin diseases

Memory T cells in different tissues exhibit tissue specificity in pathological processes, whereas Trm cells in the skin can serve as a "double-edged sword". On the one hand, they can contribute to disease persistence and recurrence, acting as a "demon". On the other hand, in certain tumors such as melanoma, Trm cells can also act as "angel", where their activation in response to tumor proliferation leads to the production of effector functions (66). Thus, Trm cells play dual roles with both beneficial and detrimental effects. Therefore, Trm cells play a vital role in various diseases. The Trm cell phenotypes of 11 types of diseases are briefly summarized in Table 1. Here, we discuss vitiligo, psoriasis, and melanoma in the following section (Figure 2).

Psoriasis

Psoriasis is an autoimmune inflammatory skin disease, the pathogenesis of which involves the disruption of the immune balance of T cells, followed by excessive keratinocyte proliferation (78). Trm cells are highly enriched in active psoriasis lesions and are also found in stable psoriasis lesions (79). $CD3^+$ T cells infiltrated extensively throughout the entire skin layers of the lesion and included the $CD4^+$ and $CD8^+$ subgroups. $CD8^+$ Trm cells in psoriasis have recently been found to express CD69, CD103 and CLA and to play a key pathogenic role (54). In psoriatic skin lesions, CD103 is expressed in most epidermal cells by $CD8^+$ T cells, which are usually coexpressed with CD69, while this group of cells is negative for CD103 expression in the dermis, and a small number of $CD8^+CD103^+$Trm cells are observed under the dermal papilla (80). A small amount of $CD4^+$ Trm cells can infiltrate the epidermis and dermis. Trm cells in the dermis express high levels of CD103, while Trm cells in the epidermis express low levels of CD103 (81). The specific pathogenicity of this group of Trm cells depends on the cytokines they produce, and the key cytokines involved in this process are IL-17 and IL-22. In addition, recruitment of Trm cells is dependent mainly on IL-15 and IL-7 mediated immune responses. $CD8^+CD69^+CD103^+$Trm cells in the epidermis can express CCR6 and IL-23R, and under stimulation *in vitro*, these cells produce IL-17A and promote the recurrence of skin lesions in the same area via the secretion of IL-17 (38). A subset of $CD8^+$ $CD103^+$ T cells and $CD4^+CD103^{+/-}$ T cells also express IL-17A and produce IFN-γ or IL-22. Additionally, $CD4^+CD103^+$ Trm cells are capable of colonizing the epidermis. Interestingly, the population of $CD8^+CD103^+IL\text{-}17A^+$ Trm cells tends to be higher in patients treated with biological agents or systemic therapy (67). In addition, the proportion of $CD4^+/CD8^+$ $CD103^+$ $IL\text{-}17A^+$ Trm cells in the advanced treatment group was significantly higher than that in the nonadvanced treatment group (82). Under inflammatory and homeostatic skin conditions, a population of $CD8^+CCR10^+$ T cells is present but not enriched in

TABLE 1 Trm cell phenotypes in various skin diseases.

Disease	Epidermis	Dermis	Correlation factor	Reference
Psoriasis	$CD4^+CD69^+CD103^-$ (+++), $CD4^+CD69^+CD103^+$ (+), $CD8 + 103^+CD49a^-$ (+++), CD8 + 103 + 69^+ (+)	$CD8^+CD69^+CD103^-$, $CD8^+CD69^+CD103^+$	IL-17A, IL-15, Il-22	(67)
Vitiligo	$CD8^+CD49a^+CD69^+CD103^-$ (+++) $CD8^+CD49a^+CD69^+CD103^+$ (+)		Il-15 、 IFN-γ	(1, 68)
Melanoma	$CD8^+CD69^+CD103^+CLA^+$		IFN-γ and TNF-α, CXCL10, IL-17	(69, 70)
Cutaneous lupus erythematosus (CLE)	$CD8^+CD103^+$	$CD8^+CD103^+$		(71)
Atopic dermatitis		$CD4^+CD69^+$, $CD3^+CXCR4^+CD69^+$ (+++), $CD8^+CD69^+$ (+)	IL-4, IL-13, IL-17 and IL-22 IFN-γ	(72)
Pemphigus	$CD4^+CD69^+CCR7$-		IFN-g, IL-4, IL-17A, and IL-21	(73)
Primary cutaneous T-cell lymphomas (CTCL)	$CD4^+CD103^+$ (+++), $CD8^+CD103^+$ (+)	$CD4^+CD69^+CD103^-$	IL-7 and IL-15	(74)
Pelada		$CD8^+CD69^+$, $CD103^+$	IFN-γ, CCL5, CXCL9, CXCL10, STAT1	(59)
Polymorphic light eruption	$CD4^+CD69^+CD103^+CD49a$- (+++), $CD4^+CD69^-$ $CD103^+CD49a^-$ (+)	$CD4^+CD69^+CD103^+CD49a^-$ (+++), $CD4^+CD69$-$CD103^+CD49a^-$ (+)	IFN-γ and GzmB, IL-15	(75)
Fixed drug eruption	$CD8^+CD45RA^+CD69^+$		IFN-γ	(76)
Allergic contact dermatitis	$CD8^+CD69^+$ $CD103^+$		PD-1, TIM-3, IFN-γ, GzmB	(77)

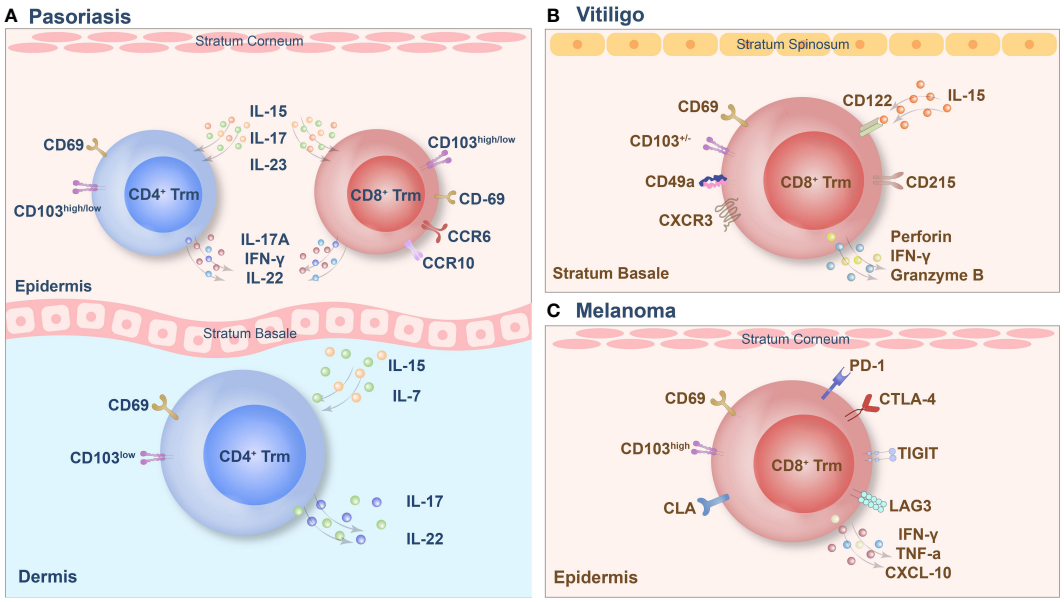

FIGURE 2
The tissue-resident memory T (Trm) cell phenotypes of psoriasis **(A)**, vitiligo **(B)** and melanoma **(C)**.

psoriatic lesions. These cells express GATA3, FOXP3, and many transcriptional features of Trm cells, including CD103 (79). In addition to T cells, the long-term role of Th22 and epidermal dendritic cells in psoriasis confirms that Trm cells persist in the epidermis of cured skin lesions and can function for several years (83). Therefore, targeting memory T cells in the psoriatic epidermis, particularly $CD8^+$ Trm cells, may be a promising approach for treating psoriasis. However, the presence of $CD4^+$ Trm cells should not be ignored because they may be an essential factor leading to disease recurrence and persistence.

Vitiligo

Vitiligo is a skin disease characterized by a high recurrence rate, and existing evidence supports the concept of local immune "memory." In addition, the recurrence of vitiligo suggests that autoimmune memory may develop within the lesion and contribute to disease relapse (84). The main factor in this immune memory is the formation of Trm cells, which play a vital role in the recurrence of vitiligo. Studies have shown that $CD8^+$ Trm cells are predominantly present in the epidermis and dermis of skin lesions in vitiligo patients. However, their numbers are significantly higher in the epidermal compartment than in the dermis (85). In mouse models, approximately 60–90% of melanocyte-specific $CD8^+$ T cells in vitiligo patients express the Trm cell markers CD69 and CD103, which are highly enriched in skin lesions. Trm cells can be present for more than a year, and their enrichment sites are mainly at the epidermal/dermal junction, which contains hair follicles with a marked reduction in melanocytes (86). $CD8^+$ Trm cell subpopulations expressing CD69, CD103, and CXCR3 were enriched in the epidermal compartments of patients with vitiligo, and they were significantly enriched in stable and active lesions. In lesions, two subsets of tissue-resident memory T (suTrm) cells are present: $CD69^+CD103^-$ Trm cells and $CD69^+CD103^+$ Trm cells. Moreover, $CD69^+CD103^+$ Trm cells are more abundant in the skin of patients with stable vitiligo than in that of patients with active disease (87). CD49a plays a crucial role in vitiligo lesions, and this group of cells exhibits strong cytotoxicity due to the constitutive expression of perforin and granzyme B in $CD8^+CD49a^+$ Trm cells. IL-15 is also integral in the development of vitiligo, as the CD122 subunit of the IL-15 receptor is expressed on Trm cells in both humans and mice (87). By targeting the IL-15 receptor, long-term blockade of CD122 depletes PMEL Trm cells, while short-term blockade of CD122 also achieves repigmentation by reducing its effector function. CD215, a subunit on the surface of Trm cells that is required for cell activation and cytokine expression, is highly expressed on keratinocytes (87). Recent studies have reported that the maintenance of decolorization of skin lesions in patients with vitiligo is simultaneously associated with autoreactive recirculating memory T (TRCM) cells in the blood, which bind to CXCR3 on the surface of TRCM cells via CXCL9 and CXCL10. Thus, TRCM cells are recruited to the lesion site. Under the synergistic effect of Trm cells and TRCM cells, decolorization is maintained for a long time by the production of IFN-γ and other cytokines. Therefore, simultaneously targeting Trm cells and TRCM cells to block the CXCL9/10-CXCR3 pathway in vitiligo patients may be an effective strategy for treatment (8).

Melanoma

Trm cells are a critical TIL subset that regulates the immune network in the melanoma microenvironment (88). Mice lacking Trm cell formation are more likely to develop tumors. Trm cells actively inhibit cancer progression, particularly $CD8^+$ Trm cells in

the epidermal layer. These compounds promote durable melanoma immune homeostasis. In particular, melanoma-specific Trm cells can exert profound inhibitory effects on tumor development independent of circulation before melanoma development (70). Melanoma-specific Trm cells reside in hair follicles primarily depleted of melanocytes. They do not require recirculating central memory T cells or lymph compartment recruitment for maintenance and persist in hair follicles for a long time. This population of cells expresses CD69, CD103, and CLA and can produce IFN-γ and TNF-α. Among these critical factors, the expression of CD103 is required for the formation of $CD8^+$ Trm cells in the skin. $CD103^+$ $CD8^+$ Trm cells play a solid protective role, and the long-term response of melanoma $CD8^+$T cells to immunotherapy for more than one year can effectively prevent the reattack of melanoma; thus, CD103-dependent Trm cells play a crucial role in antitumor immunity (89). Another TCR sequence analysis revealed the coexistence of Trm cells in the skin and Tem cells in the blood. This study also revealed the presence of identical clonotypes in both the skin and blood, as found in tumors (69). Furthermore, long-term survival of these T cells for up to nine years, along with high expression levels of IFN-γ and TNF-α in the tumor, skin, and peripheral blood clonotypes, has been demonstrated (69). High levels of these cytokines are sufficient to guarantee a good prognosis in patients with melanoma. It has also been demonstrated that the tumor-associated clonal repertoire is retained mainly in the skin. Trm cells have many similarities to tumors and viral skin infections. They have a protective effect on the skin and can be maintained for an extended period. However, it is worth mentioning that the expression of the immune checkpoints PDCD1, LAGLA3, and TIGIT is higher in tumor-specific Trm cells than in Tcm cells or Tem cells (37). $CD8^+CD69^+CD103^+$ Trm cells also showed higher PD-1 and lag3 immune checkpoint expression than $CD8^+CD69^-CD103^-$ cells. At the same time, they also express the first immune checkpoint molecule, CTLA-4, which inhibits the anticancer response of $CD8^+$ cells. Between T cells and dendritic cells, CTLA-4 interaction occurs mainly during antigen presentation in the draining lymph nodes. However, CTLA-4 inhibits T cell activation and proliferation by competing with CD28 to block the costimulatory signals required for T-cell activation. The constitutive expression of CTLA-4 suppresses T-cell-mediated immune responses in response to chronic TCR stimulation by infection or malignancy (90). However, the role of this critical checkpoint in Trm cell function remains unclear (37).

Plasticity of the skin Trm cell

By draining the lymph nodes, naive T cells initiate a primary immune response in SLOs, thereby draining the infected barrier tissue (Figure 3). Effector T cells proliferate to acquire effector capabilities before infiltrating local tissues to exert their functions. In the context of the secondary immune response, this process can be conceptualized as an "inside-out" model, where Tcm cells are promptly activated in lymph nodes and subsequently migrate to non-lymphoid tissues (91). In the skin, Trm cells possess the potential to govern local recall responses and exhibit effector functions (92). Interestingly, certain studies have indicated that the reactivation of Trm cells can trigger the recruitment of Tcm cells; however, the *in situ* differentiation of Trm cells can occur independently of antigen stimulation. The generation of secondary Trm cell populations relies on the proliferation of pre-existing Trm cells rather than their derivation from the circulating T-cell pool (93). Hence, Trm cells represent a relatively autonomous population capable of proliferating independently of the main memory or lymphoid tissue. They can even expand local immune surveillance autonomously without the involvement of antigen-presenting cells (10). However, the expansion ability of Trm cells is far lower than that of naive T cells or Tcm cells because of the inherent differences in the proliferative potential of T cells. For this reason, Trm cells are difficult to study *in vitro*, as these cells have difficulty surviving after being separated from the tissue microenvironment (94).

Recent studies have shown that cutaneous Trm cells have novel circulating characteristics in mice and humans, and Trm cells in the NLT, including the skin, exhibit significant levels of developmental plasticity. They can traverse tissue outlets to re-enter circulation and SLOs under both inflammatory and steady-state conditions, and their phenotypes can redifferentiated into various types of memory cells. Gebhardt identified two distinct subpopulations of antigen-specific memory T cells in the skin of patients with herpes simplex virus infection: a subset of $CD8^+$T cells that reside in the epidermis and a population of $CD4^+$T cells that can rush through the dermis, demonstrating that $CD4^+$T cells are more capable of migrating than $CD8^+$ T cells (6). Secondary antigen-stimulated Trm cells, particularly those with a $CD4^+$ phenotype, have been shown to migrate retrogradely, generate Tcm cells and Tem cells, and retain the biased homing and differentiation potential of Trm cells. In contrast, most skin $CD4^+$ T cells are in equilibrium with circulating T cells rather than stably residing in the tissue. In CD45.1 and CD45.2 congenic mice symbiosis experiments, almost half of the skin infiltrating T cells were $CD4^+$ Trm cells which express CD69 and CD103, and the cell subtypes exhibited by the co-organisms were very similar, demonstrating that these cells can flow through circulation (90). These tissue-derived Trm cells, called ex-Trm cells, retain dermatophagic and associated transcriptional characteristics. For example, the cutaneous extracellular matrix still expresses high levels of GATA3, cytoplasmic FABP4, and FABP5 but lacks the characteristics of other tissue-derived Trm cells, such as CCR9. According to a previous study (30), these cells can migrate back to the skin area under favorable conditions and opportunities. Klicznik et al. reported that $CD4^+CD69^+CD103^+$ Trm cells can exit human skin by downregulating CD69 expression. The authors also identified cell populations in the blood and lymph nodes with transcriptional profiles and clonotypes comparable to those of the human skin $CD4^+CD69^+CD103^+$ Trm cell population, confirming that part of the human skin $CD4^+CD103^+$ Trm cell population can reenter circulation (95). These cells may express an intermediate phenotype ($CCR7^{int/+}CD62L^{int}CD69^-CD103^{+/-}$E-selectin ligands$^+$) along the way and reenter distal lymph nodes, sites of nonspecific skin inflammation and even the circulatory system through draining lymph nodes (96). After this population of cells migrates to secondary human skin sites, the Trm cell phenotype reappears.

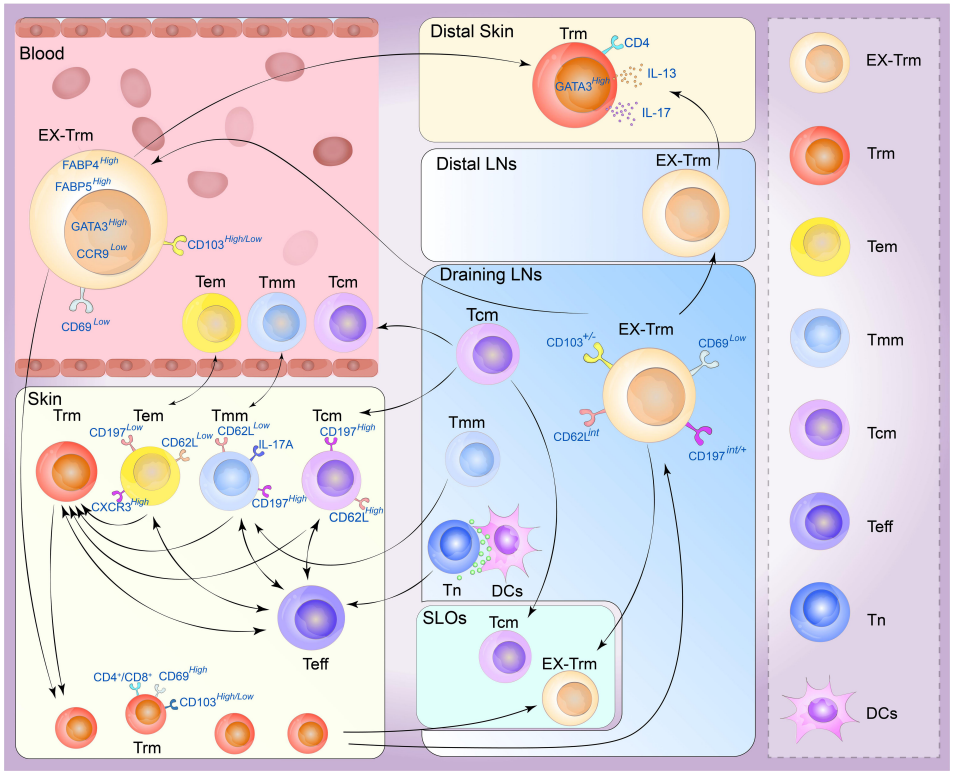

FIGURE 3
Plasticity of the skin tissue-resident memory T (Trm) cell. Memory T cells are generally divided into four types. Central memory T (Tcm) cells circulate between the blood and secondary lymphoid organs (SLOs) mostly through draining lymph nodes, and can be recruited into lymphoid tissue or to sites of inflammation beyond it. Effective memory T (Tem) cells can migrate between blood and non-lymphoid tissues (NLTs), showing a strong peripheral tissue bias. Tmm cells preferentially patrol peripheral tissues and migrate to lymph nodes and blood. They can migrate to draining lymph from the skin but are excluded from lymph nodes draining non-skin tissue. Trm cells are mainly derived from the differentiation of effector T-cell populations, and the generation of secondary Trm cell populations depends on the proliferation of preexisting Trm cells and a variety of Trm progenitor cells in the skin can also transform into Trm cells under certain conditions. Under stable and inflammatory conditions, skin Trm cells can cross the tissue outlet to reenter the circulation and SLOs, and these cells can form and redifferentiate to other types of memory cells. These Trm cells that flow out of tissues are commonly referred to as ex-Trm cells, which retain dermotropism and its associated transcriptional signatures. Migrating $CD4^+$ T cells may exhibit a transitional phenotype during migration and re-enter circulation, distal lymph nodes, and nonspecific skin inflammatory sites through draining lymph nodes. After this group of cells migrated to secondary human skin sites, Trm cells phenotypes could be represented. (Tn, navie T cell; Teff, effector T cell; DCs, dendritic cells; LNs, lymph nodes).

This provides new insights into the segmentation of human $CD4^+$ memory T cells (96). A recent allogeneic hematopoietic stem cell transplant (HSCT) study revealed host $CD4^+$ T cells with a skin-resident phenotype in patients who underwent immune cell reconstitution after HSCT. These cells showed Th2/Th17 characteristics with high GATA3, IL-13, and IL-17 expression. This population of cells is skin-derived and highly similar to cutaneous Trm cells (97). In a unique type of allogenic HSCT environment, host Trm cells form a symbiotic association with donor T cells, resulting in a chimera (98). These chimeric Trm cells can persist in human skin for decades without replenishing the circulation pool. In contrast, a subset of T-cell clones in the patient's skin and blood showed cross-sharing between tissues and time points. This suggests that tissue injury may stimulate Trm cell activation and retrograde migration and that Trm cell reseeding and inflammatory cell migration can occur in distant organs (88).

According to a lineage-tracing mouse model, $CD8^+$ Trm cells also have the potential to form circulatory effector cells and memory cells in the secondary immune response. $Hobit^+$ Trm cells demonstrate downregulation of Hobit expression upon encountering antigens, and $Hobit^+$ $CD8^+$ Trm cells can proliferate in draining lymph nodes. Consequently, they give rise to circulating memory cells following pathogen rechallenge. Moreover, pre-$hobit^+$ T cells undergo redifferentiation into $CD8^+$ Tem cells upon pathogen rechallenge, and these cells primarily acquire the phenotype of Tem cells (99). However, recent investigations have examined the gene profiles of $CD69^+$ $CD8^+$ T cells from various human tissues and revealed low to negligible levels of Hobit expression. As a result, further investigations are required to determine whether the differentiation mechanism of $CD8^+$ Trm cells in humans corresponds precisely to the findings observed in mice (64). However, another high-throughput sequencing analysis revealed that the methylation status of CpG regions in $CD8^+$ Trm cells indicated that $CD8^+$ Trm cells could redifferentiate in tissue regions and acquire the corresponding effector functions (100). These results suggest that $CD8^+$ Trm cells maintained at the site of local inflammation may also re-engage in systemic memory immune responses, supporting the inside-out characteristic of protective immunity (101). Memory responses can

be initiated *in situ* at sites of infection and within the tissue microenvironment in which skin Trm cells are located, and Trm cells have some proliferative capacity to redistribute and even support circulating memory T-cell pools (7).

Some T-cell populations, such as $CD4^+$ Trm cells or $CD8^+$ Trm cells, retain recycling programs in non-lymphoid tissues. This observation emphasizes mechanisms that enable T-cell migration to non-inflammatory tissues, and the extent of tissue specificity (and associated molecular mechanisms) of these recycling pathways remains a crucial area of research. However, the plasticity of $CD4^+$ Trm cells is more vital than that of $CD8^+$ Trm cells, and the recycling of $CD4^+$ Trm cells has been relatively well reported (102). Since S1P has been shown to promote the efflux of reactivated T cells from non-lymphoid tissues, we hypothesized that these recycled cells retain some of the functional and transcriptional properties of Trm cell precursors, such as Tcm cells or Tem cells (Figure 3). The direction of recycling may also be heterogeneous, and the T-bet axis may positively regulate this process. For example, the expression levels of CD62L and CD197 may be higher than those in Trm cells, and Trm cells may also express higher levels of CXCR3 or KLRG1. Further studies are required to determine the long-term migratory behavior of Trm cells, including their potential to reenter the circulation and migrate to distant tissue sites. These investigations may offer insights into the mechanisms of protective immunity in localized areas of the skin (103).

Discussion

Trm cells can develop from various memory cells, reside in lymphoid and non-lymphoid organs and are not traditionally recycled through the blood. The Trm cells in the skin are heterogeneous, and different cell subpopulations with different surface markers and functional expression levels of correlation factors may be involved in multiple diseases and anatomical conditions (104). $CD8^+$ Trm cells are essential for the development of diseases such as vitiligo, psoriasis, and melanoma. $CD8^+$ Trm cells can lead to recurrence or difficulty in curing disease through the local settlement of Trm cells. Therefore, Trm cell targeting is an attractive therapeutic strategy. Currently, the definition of Trm cells is constantly changing. Recent studies have shown that Trm cells reside in tissues for a long time. Various Trm cells, including $CD4^+$ and $CD8^+$, cells can exhibit a high degree of plasticity under steady-state and inflammatory conditions and thus migrate to draining LNs, circulation, distal LNs, and nonspecific skin inflammation sites. In both human and mouse models, $CD4^+$ Trm cells are traditionally considered a population with limited residency that is capable of exiting the skin, reentering the bloodstream, and potentially migrating to distant tissue sites (95). The characteristics of $CD4^+$ Trm cells display significant variability across tissues, and distinct cues contribute to their establishment and retention. Recent studies have also indicated that $CD8^+$ Trm cells can regain Tcm cell and Tem cell phenotypes in mouse models. These findings suggest that the current understanding of Trm cells may not be confined to their settlement solely within local tissues. Whether the residence of Trm cells in various diseases is time-limited remains uncertain, and further investigations are needed to explore whether Trm cells represent an intermediate stage rather than the final destination in the circulation of memory T cells across tissues, lymph nodes, and blood.

Some researchers believe this raises the possibility that the pathology can be distributed to distant tissue sites in the case of harmful effects mediated by Trm cells. For example, in autoimmune diseases involving Trm cells, the potential migration of Trm cells to distant sites could result in dissemination and metastasis to the lesion site, transforming a localized disease into a systemic disease. Alternatively, it progresses from a stable phase to an active phase. However, if the formation of Trm cells is reversible, then more possibilities for Trm cell-related treatment can be obtained. Therefore, to control the spread and recurrence of this disease, targeted Trm cell therapy will achieve improved efficacy. For example, treatment with Mart-1-specific Trm cells is very popular in the treatment of vitiligo. By inhibiting the generation of skin Trm cells, such as anti-IL-15 antibodies, at the lesion site, the recurrence of the disease can be reduced (84). Due to the positive effect of Trm cells on many tumors and infectious diseases, Trm cells can play a good role in disease resistance. The induction of redifferentiation and migration may become a new strategy for treating a variety of tumors using Trm cells and may also increase the targeting of various diseases. For example, Trm cells are a key group of $CD8^+$ T cells involved in immune checkpoint inhibitor therapy. The antitumor effect of anti-PD-1 depends on the localization of $CD8^+$ T cells at the tumor margin, proving that memory T cells are critical for mediating the anti-PD-1 response. Tumor-specific epidermal $CD69^+$ $CD103^+$ Trm cells have a sustained protective effect on melanoma patients, especially premelanoma melanoma-specific Trm cells, which can function independently of circulation and have a far-reaching inhibitory effect on tumor development (105). Melanoma-specific Trm cells can persist in the skin for a long time, and the $CD8^+$ T cells of melanoma patients exhibit a long-term response to immunotherapy for more than one year. In particular, $CD103^+$ $CD8^+$ Trm cells can play a key role in antitumor immunity and effectively prevent the reattack of melanoma cells (89). In other diseases, $CD8^+$ Tcm cells can migrate into the skin, where they are the first line of defense against subsequent infection after skin vaccinia virus infection has subsided (106). In addition, a colony of IFN-γ-producing Leishmania-specific memory $CD4^+$ Trm cells that formed in response to parasitic infection were able to remain in the skin when transplanted into juvenile mice. Their function is dependent on CXCR3, which recruits circulating T cells to the skin to provide optimal protective immunity against Leishmania (107). Therefore, an anti-Leishmania vaccine targeting the generation and amplification of Trm cells will be a hot new strategy in the future.

Conclusion and outlook

Overall, the resident and recycling functions of Trm cells should be viewed from a dialectical perspective. Skin Trm cells are two-sided. Although Trm cells are associated with the progression of many diseases, they still play a powerful role in the protection against many diseases in the local area. In recent years, the mechanisms of TRM cells in different skin diseases have begun to

be revealed. It is clear that TRM cells are strongly involved in the development and recurrence of skin diseases. Further study of its heterogeneity and plasticity will not only enhance our comprehension of diseases, but more importantly, this will facilitate the development of more effective therapeutic approaches.

Author contributions

GL: Writing – original draft, Investigation, Formal analysis, Data curation. ZW: Writing – review & editing, Visualization, Data curation, Conceptualization. SL: Writing – review & editing, Visualization, Supervision, Funding acquisition.

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Conflict of interest

The 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

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Type-2 immunity associated with type-1 related skin inflammatory diseases: friend or foe?

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Chronic inflammatory skin diseases are multifactorial diseases that combine genetic predisposition, environmental triggers, and metabolic disturbances associated with abnormal immune responses. From an immunological perspective, the better understanding of their physiopathology has demonstrated a large complex network of immune cell subsets and related cytokines that interact with both epidermal and dermal cells. For example, in type-1-associated diseases such as alopecia areata, vitiligo, and localized scleroderma, recent evidence suggests the presence of a type-2 inflammation that is well known in atopic dermatitis. Whether this type-2 immune response has a protective or detrimental impact on the development and chronicity of these diseases remains to be fully elucidated, highlighting the need to better understand its involvement for the management of patients. This mini-review explores recent insights regarding the potential role of type-2-related immunity in alopecia areata, vitiligo, and localized scleroderma.

KEYWORDS

type-1 immunity, type-2 immunity, atopic dermatitis, localized scleroderma, alopecia areata, vitiligo

Introduction

The characterization of the diversity of immune cell subsets has extended our understanding of the complexity of the mechanisms driving the development and recurrence of chronic inflammatory disorders and hastened the subsequent use of targeted therapies. Three major types of innate and adaptive cell-mediated effector immunity have been identified (1, 2). While these immune responses are primarily involved in protection against pathogens, their aberrant activation can also be harmful and lead to the development of autoimmunity or to inflammatory or allergic diseases (1, 3).

Type-1 immunity mainly involves innate lymphoid type-1 cells (ILC1), natural killer (NK) cells, CD4 Th1 and cytotoxic CD8 Tc1 cells, mainly inducing interferon (IFN)-γ and tumor necrosis factor (TNF)-α (1). Besides its protective role against intracellular

pathogens such as viruses, it is also implicated in inflammatory diseases such as alopecia areata (AA), localized scleroderma (LS), and vitiligo.

Type-2-associated immune cells include ILC2, Th2 cells, eosinophils, mast cells, basophils, and alternatively activated macrophages, which are known to release cytokines like interleukin (IL)-4, IL-5, IL-13, or IL-31 (3–6). These cells are critical for the defense of the organism against extracellular pathogens (i.e. helminth parasites) and for the maintenance of tissue homeostasis (tissue regeneration and wound repair) (7). However, besides its protective role, pathogenic activation of type-2 immune response contributes to the development of allergic and inflammatory diseases such as asthma, allergic rhinitis, and atopic dermatitis (AD) (5, 8, 9). AD is characterized by skin barrier dysfunction contributing to an aberrant sensitization to environmental allergens (10). In AD, type-2 cytokines like IL-4 and IL-13 are directly implicated in the impairment of epidermal barrier integrity observed in AD lesions by inhibiting the synthesis of key structural proteins such as filaggrin, loricrin, honerin, and involucrin (11–14). The role of type-2 inflammation in AD, and especially that of IL-4/IL-13, is exemplified by the efficacy of therapies targeting these cytokines, e.g. the anti-IL-4Rα antibody dupilumab and the anti-IL-13 antibodies tralokinumab and lebrikizumab for moderate to severe AD (15–30). However, the pathophysiology of AD is more complex with heterogenous phenotypes underlying different endotypes, with the involvement of type-1 (e.g. Th1 cells) and/or type-3 (Th17 and Th22 cells) immune cell subsets (31–35). Likewise, an increasing body of evidence has shown that type-2-associated immune response may also play a role in the development of type1 or type-3-related skin diseases, hence increasing the complexity of disease pathogenesis and patient stratification. This mini-review examines recent insights into the role of type-2 inflammation in type-1-associated skin inflammatory diseases with a focus on LS, AA, and vitiligo (Figure 1).

Localized scleroderma

LS is a rare autoimmune skin disorder characterized by inflammation and fibrosis of the skin, with dense collagen deposition in the dermis and underlying connective tissues (36). Inflammatory patches and/or bands of thickened skin develop on the head and neck region, trunk and extremities. Morphea is the most frequent subtype of LS with onset between 40 and 50 years of age (37). LS is classified into five main types according to the extent and depth of fibrosis: limited, generalized, linear, deep and mixed (37, 38). Its pathogenesis is based on genetic predisposition combined with external triggers such as trauma, repeated friction, and surgery, that induce aberrant inflammatory and profibrotic responses, fibroblasts being a critical factor during the development of the disease (39–41). During the early inflammatory stage of LS, $CD4^+$ T cells, macrophages, and eosinophils infiltrate the skin and adjacent blood vessels (36, 42, 43). Both Th1 and Th17 responses seem implicated in this primary stage, with an increased release of chemokine (C-X-C motif) ligand (CXCL)9/10, IFN-γ, TNF-α, IL-23, IL-17 and transforming growth factor (TGF)-β (36, 44). CXCL9 and CXCL10 serum levels correlate with the disease activity (45, 46). Interestingly, Werner et al. recently identified clusters of inflammatory fibroblasts prone to release CXCL12 or CXCL9/10 in LS lesions. The same study also demonstrated the crosstalk between fibroblasts and infiltrated immune cells (e.g. macrophages and T cells) to perpetuate inflammatory signals in lesions (47). Indeed, inflammatory fibrosis was shown to be dependent on CXCL9 and its receptor CXCR3 in a mouse model of skin fibrosis, thereby confirming the involvement of a type-1 immune response in the early phase of skin fibrosis (48). In addition, the increased expression of several adhesion molecules by endothelial cells, such as vascular cell adhesion molecule 1 (VCAM1), Intercellular Adhesion Molecule1 (ICAM1) and E-selectin, contributes to the recruitment of immune cells in the lesional areas (49).

Fibrosis is a key mechanism defining LS lesions and is characterized by excessive deposition of extracellular matrix (ECM) components such as collagen in the tissue. TGF-β is considered as a major profibrotic factor owing to its effects on fibroblast proliferation, differentiation, migration, and the production of extracellular cellular matrix components (50, 51). However, clinical trials blocking TGF-β produced conflicting results (52, 53).

It has been postulated that as the disease progresses, a shift occurs to a type-2 immune response that is associated with the development of skin fibrosis. Type-2-related cytokines (IL-4, IL-5, IL-6 and IL-13) are increased in the serum and skin of LS patients, and IL-13 serum levels correlate with the number of lesions in LS (54–56). Such type-2 immunity appears to be associated with the fibrotic/sclerotic stage of the disease (36). *In vitro* studies showed that IL-4 and IL-13 induce an excessive production of ECM components such as collagen, periostin, proteoglycan synthesis, and fibronectin by scleroderma and/or normal fibroblasts (57–61). These cytokines also stimulate the production of TGF-β and the synthesis of matrix metalloproteinase (MMP)1, MMP3 and TIMP-1 (a tissue inhibitor of MMP), as well as the proliferation of fibroblasts and their differentiation in myofibroblasts (62–64). Interestingly, the inhibition of type-2 signaling prevents the development of cutaneous fibrosis *in vivo* (65–67). A phase II clinical trial is ongoing to test the efficacy of dupilumab in localized scleroderma patients (NCT04200755).

Alopecia areata

AA is a chronic non-scarring hair loss condition affecting 0.5–2% of the population and resulting from an autoimmune response targeting the hair follicle (68). AA is predominantly driven by a type-1 inflammatory response associated with the production of IFNγ by antigen-specific $CD8^+$ $NKG2D^+$ Tc1 and $CD4^+$ Th1 cells in response to an environmental trigger, such as stress, viral infection, or trauma. This induces the collapse of the immune privilege of the hair follicle leading to its growth arrest (69). IFNγ also contributes to the increased inflammation through the induction of CXCL9/10 by the hair follicle epithelium, leading to the recruitment of $CXCR3^+$ T cells to the bulb (70).

Recent data also suggest the contribution of the type-2 immune response in AA pathogenesis. From a clinical perspective, AA is associated with atopic dermatitis and allergic conditions, and an atopic background increases the risk of developing it (71–75). Loss-

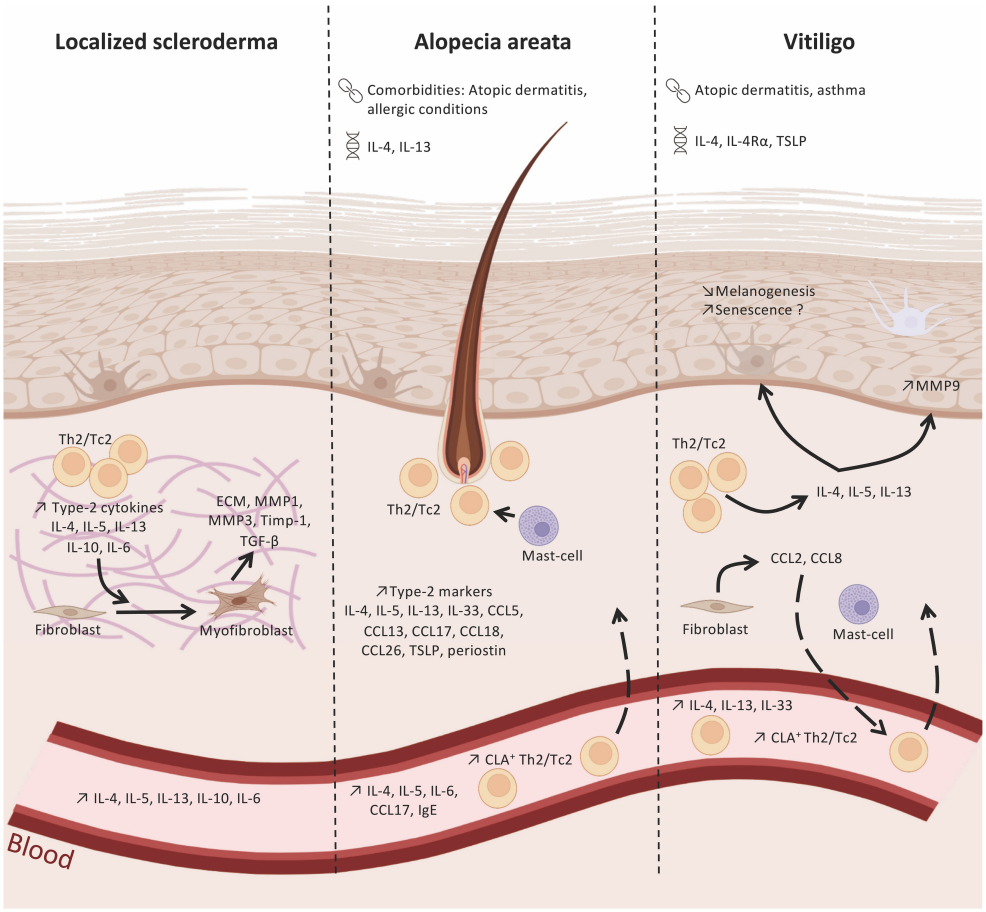

FIGURE 1
Type-2 immunity in localized scleroderma, alopecia areata and vitiligo. Polymorphims with IL-4/IL-13 genes have been identified in alopecia areata (AA) and vitiligo. In addition, these two diseases are associated with atopic dermatitis or allergic conditions. Type-2 immunity cells and markers found in the skin and blood of patients withlocalized scleroderma (LS), AA, and vitiligo, suggesting their role in the immune network of these pathologies. In LS, type-2 cytokines released by Th2 and Tc2 cell subsets (e.g. IL-4, IL-5, IL-13) infiltrating LS lesions promote the differentiation of fibroblasts into myofibroblasts and the production of pro-fibrotic factors, like TGF-β. AA skin lesions display elevated levels of type-2 cytokines and chemokines released by epidermal, dermal, and immune cells that will contribute to the recruitment of Th2/Tc2 and my influence hair loss. The type-2 environment in vitiligo skin may regulate melanogenesis and the loss melanocytes. : comorbidities; polymorphisms. Created with BioRender.com.

of-function mutations in the gene encoding filaggrin are associated with the severity of AA in patients with a history of AD, and genetic studies identified the association of AA with polymorphisms for the genes encoding IL-4 and IL-13 (76–78). An increase in mast cells with a pro-inflammatory phenotype in the perifollicular area of AA patients was reported. These mast cells display an increased degranulation activity and could interact with $CD8^+$ T cells to provide co-stimulatory signals (via 4–1BBL, OX40L, ICAM1) and possibly to present neo-autoantigens (79). In addition, AA skin lesions display an increase in type-2-related cytokines and chemokines, including IL-4, IL-5, IL-13, IL-33, chemokine (C-C motif) ligand (CCL)-5, CCL13, CCL17, CCL18, CCL26, TSLP and periostin (80–83). Interestingly, after intralesional corticosteroid injection, a downregulation of CCL18 was associated with a clinical improvement (82). Levels of IL-4, IL-5, IL-6, IL-13, CCL13, CCL17, CCL22, CCL26, and IgE are also increased in AA patients' sera (83–88). Czarnowicki et al. observed an increase in circulating skin-homing cutaneous lymphocyte-associated antigen $(CLA)^+$ Th2 and CLA^+ Tc2 cell subsets in AA patients compared to healthy controls that correlated with disease activity. In contrast, IFNγ was associated with the chronicity of the disease (89). All these data highlight the putative role of Th2 cells in disease pathogenesis.

However, the use of dupilumab in AA led to conflicting results, some investigations showing a significant improvement while others reporting exacerbation or new onset of the disease (90–100). Patients with an atopic background and high IgE levels exhibited a better response to dupilumab (101). Recent data suggest that non-atopic AA patients display an increase in circulating Tc1 cells while AA patients with concomitant AD show a skewed Th2 profile (102). In addition, the infiltration of $CCR4^+$ Th2 cells around the hair bulb in skin lesions is more extensive in AA patients with AD (102). Altogether, these data suggest that as in AD, different clinical phenotypes and related endotypes likely define AA patients.

Vitiligo

Vitiligo, the most common depigmenting skin disease, is defined by a type-1 skewed immune bias, with the involvement of IFNα, IFNγ, TNFα, CXCL9, CXCL10, and CXCL16 (103–105). Melanocyte loss results from the cytotoxic activity of CD8 T cells and detachment of melanocytes from the basal layer of the epidermis in response to the cytokine microenvironment (106). The perilesional skin of vitiligo patients is characterized by the infiltration of $CXCR3^+$ $NKG2D^+$ melanocyte-specific resident memory CD8 T cells and recirculating memory T cells producing IFNγ and TNFα (107–112). These type-1 cytokines impair the expression of genes involved in melanocyte adhesion, function, and melanogenesis (113, 114). IFNγ and TNFα also induce melanocyte detachment through the production of MMP9 by keratinocytes, which cleaves E-cadherin, a transmembrane glycoprotein important for melanocyte adhesion (115). In addition, these cytokines amplify the local inflammation through the release of CXCL9/10 by epidermal cells (105, 107). The type-1 inflammation is not restricted to the perilesional skin but concerns also the nonlesional skin of vitiligo patients (116).

Recent data suggest the involvement of a more complex cytokine network in disease pathogenesis with the involvement of type-2 cytokines. Epidemiological studies demonstrated the association of vitiligo with atopic diseases driven by a type-2 immune response, like AD or asthma (117–120). Genome wide association studies (GWAS) identified TSLP gene polymorphism in patients with vitiligo (121). Despite the absence of evidence from GWAS, smaller genetic studies identified polymorphisms of the gene coding for IL-4 as a risk factor for developing vitiligo (122, 123). These polymorphisms correlate with an increase in IL-4 and IgE levels in the serum of vitiligo patients. IL-4 receptor (IL-4R)-α and TSLP gene polymorphisms are associated with an increased susceptibility to vitiligo, reinforcing the putative role of type-2 cytokines in vitiligo (121, 124, 125). In addition, IL-4, IL-13, and IL-33 levels are increased in the serum of vitiligo patients (126–129). An increase in mast cells in vitiligo lesions was reported (130, 131). Czarnowocki et al. reported an increase in both circulating skin-homing CLA^+ T cells producing IFNγ or IL-13 in patients. IL-13 levels decreased with vitiligo duration, suggesting its potential role in the early stages of the disease (132). We recently showed that vitiligo skin T cells produce both type-1 and type-2 cytokines, and in particular IL-13 (105). In addition, levels of chemokines that can be associated with a type-2 immune response, such as CCL5, CCL18, CXCL12, or CXCL16, are increased in vitiligo perilesional skin (104, 105, 133). A recent study in a mouse vitiligo model induced by the inoculation of melanoma cells, depletion of regulatory T cells, and excision of the tumor showed that IFNγ induces the secretion of CCL2 and CCL8 by dermal fibroblasts through JAK2/STAT1 signaling, resulting in type-2 cell attraction (134). Indeed, CCL2 is implicated in Th2 polarization and CCL8 in the recruitment of Th2 cells (135, 136). These data suggest the interconnected role of type-1 and type-2 immune responses in the inflammatory environment observed in vitiligo.

So far, the potential impact of type-2-related cytokines on melanocytes has received little attention. IL-4 and IL-13 were reported to inhibit melanogenesis (137, 138). Moreover, IL-13 induces the production of matrix metalloproteinase (MMP)-9 by keratinocytes (115, 139) and may therefore contribute to melanocyte loss in vitiligo together with IFNγ and TNFα. In addition, melanocytes and fibroblasts present a senescence pattern in vitiligo skin (140–142). IFNγ and TNF-α were shown to induce senescence in melanocytes (143, 144), and it would be interesting to evaluate the impact of type-2 cytokines in senescence in vitiligo given that IL-13 can promote senescence in submandibular glands (145). Nonetheless, type-2-related cytokines may also be protective in some subclinical subsets of vitiligo, since dupilumab induced or worsened vitiligo in AD patients (100, 146–148).

Conclusion

Accumulating evidence is underlining the complexity of the cellular and cytokine network involved in the pathogenesis and flares of chronic autoimmune and inflammatory skin diseases. This diversity is likely linked to subclinical phenotypes and associated endotypes, as shown in AD. The role of the type-2 immune response is well characterized in atopic dermatitis and other type-2-related skin diseases. Recent data emphasize its role in other inflammatory skin disorders like vitiligo, AA and LS, which may explain the efficacy of small molecules like JAK inhibitors that target multiple cytokine pathways. In addition, the efficacy of emerging treatments targeting the type-2 response is being investigated, especially the IL-4/IL-13 axis in scleroderma and more recently in AA. The findings may be promising in a clinical subset of patients. Future studies will undoubtedly further decipher the role of the type-2 immune response in these diseases and provide insights into how they are involved in their pathogenesis and how to stratify patients. This may provide much needed guidance on choosing the most appropriate targeted therapy for patients.

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Signaling pathways and targeted therapy for rosacea

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Rosacea is a chronic skin inflammatory disease with a global prevalence ranging from 1% to 20%. It is characterized by facial erythema, telangiectasia, papules, pustules, and ocular manifestations. Its pathogenesis involves a complex interplay of genetic, environmental, immune, microbial, and neurovascular factors. Recent studies have advanced our understanding of its molecular basis, focusing on toll-like receptor (TLR) 2 pathways, LL37 expression, mammalian target of rapamycin (mTOR) activation, interleukin (IL)-17 signaling, transient receptor potential vanilloid (TRPV) functions, and the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways. LL37-associated signaling pathways, particularly involving TLR2 and mTORC1, are critical in the pathogenesis of rosacea. LL37 interacts with signaling molecules such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), nuclear factor kappa B (NF-κB), inflammasomes, C-X-C motif chemokine ligand 8 (CXCL8), mas-related G-protein-coupled receptor X2 (MRGPRX2)-TRPV4, and vascular endothelial growth factor (VEGF). This interaction activates macrophages, neutrophils, mast cells, and vascular endothelial cells, leading to cytokine release including tumor necrosis factor-alpha (TNF-α), IL-6, IL-1β, C motif chemokine ligand (CCL) 5, CXCL9, and CXCL10. These processes contribute to immune response modulation, inflammation, and angiogenesis in rosacea pathophysiology. The IL-17 signaling pathway also plays a crucial role in rosacea, affecting angiogenesis and the production of inflammatory cytokines. In addition, recent insights into the JAK/STAT pathways have revealed their integral role in inflammatory and angiogenic mechanisms associated with rosacea. Rosacea treatment currently focuses on symptom management, with emerging insights into these molecular pathways providing more targeted and effective therapies. Biological agents targeting specific cytokines, IL-17 inhibitors, JAK inhibitors, and VEGF antagonists are promising for future rosacea therapy, aiming for enhanced efficacy and fewer side effects. This review provides a comprehensive overview of the current knowledge regarding signaling pathways in rosacea and potential targeted therapeutic strategies.

KEYWORDS

rosacea, pathogenesis, signaling pathways, targeted therapy, review

1 Introduction

Rosacea is a common chronic skin inflammatory disease affecting 1% to 20% of the global population (1). It is characterized by various signs and symptoms, including erythema, telangiectasia, papules, pustules, and flushing with burning and stinging sensations on the central face (2). Rosacea is categorized into four subtypes: erythematotelangiectatic rosacea (ETR), characterized by persistent erythema and telangiectasia on the central face; papulopustular rosacea (PPR), presenting with persistent facial erythema, papules, and pustules; phymatids rosacea (PhR), marked by thickened skin and an irregular surface texture; and ocular rosacea (3). A key hallmark of rosacea is its hypersensitivity to various stimuli like temperature changes, ultraviolet light (UV), emotional changes, and certain foods such as spicy food (4). Rosacea often impacts the facial area, significantly affecting patients' self-esteem and mental health, and is associated with systemic diseases like hypertension, inflammatory bowel disease, autoimmune disorders, and migraines (5).

Current research indicates that the pathogenesis of rosacea is mainly due to the cross-talk of genetic and environmental factors (4, 6). This includes immune dysfunction, chronic inflammation, microbial imbalances, and vascular neurologic dysfunction (7). Recent molecular studies have identified critical signaling pathways in rosacea, highlighting the roles of toll-like receptor (TLR)2, LL37 production (8), the interleukin (IL)-17 signaling pathway (9), and the LL37- mammalian target of rapamycin (mTOR) and Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways (10, 11). These discoveries are crucial for developing targeted treatments. Currently, the treatments of rosacea are primarily symptombased, with effective solutions still under research (12). This review provides a detailed understanding of the signaling pathways involved in rosacea, as well as the emerging targeted therapeutic strategies.

2 LL37-related signaling pathways

TLRs play a crucial role in recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (13), triggering anti-pathogen responses, including antimicrobial peptide secretion and proinflammatory cytokine and chemokine production (14). TLR2, a primary pattern recognition receptor, is significantly overexpressed in rosacea patients' keratinocytes, contributing to heightened skin sensitivity to various stimuli (15). TLR2 is also expressed in sensory neurons, and the TLR2 signaling pathway contributes to the mechanism of neurological dysfunction in rosacea (16). Numerous studies have confirmed that TLR2 responds to environmental stimuli such as reactive oxygen species (ROS), microbial imbalance, Demodex mites, UVB radiation, and temperature changes (17–19). Glucocorticoids can increase TLR2 expression in epidermal keratinocytes, potentially leading to glucocorticoid-induced rosacea-like dermatitis (20). And these trigger factors can amplify TLR2 expression through enhanced endoplasmic reticulum (ER) stress and activating transcription factor 4 (ATF4) upregulation (16). Upon TLR2 activation, Kallikrein 5 (KLK5) and total serine protease activity are released from keratinocytes, a process reduced by TLR2-deficient mice. TLR2's ability to release KLK5 is calcium-dependent, with TLR2 ligands triggering a calcium influx that increases KLK5 release (15, 21). KLK5 is also mediated by Metalloproteinases (MMPs), which decompose the extracellular matrix (22). MMP2 and MMP9 are associated with the pathogenesis of rosacea, with elevated MMP-9 mRNA levels in rosacea patients' facial skin (17, 23, 24).

Cathelicidin, an antimicrobial peptide (AMP), acts as an endogenous antibiotic (25). It is initially inactive and activated by serine proteases into multiple active peptides. Specifically, KLK5, a trypsin-like serine protease, is key in converting cathelicidin into LL37 by processing its precursor, hCAP18 (human cationic antimicrobial protein of 18 kDa) (26). Research by Mylonas A. et al. have revealed that KLK5 cleaves cathelicidin, producing peptides with increased DNA binding and enhanced induction of type I interferons (IFNs) in plasmacytoid dendritic cells (pDCs) (27). Cathelicidin expression is regulated by vitamin D–dependent mechanisms involving the vitamin D receptor, controlling human cathelicidin in various cell types, as well as vitamin D-independent mechanisms that increase cathelicidin expression in response to external stressors like infections, injuries, or barrier disruption, often coinciding with ER stress (28–30). LL37 is produced via the TLR2-KLK5 pathway in response to stimuli such as temperature increase. Moreover, mTORC1, a serine/threonine protein kinase, regulates cathelicidin expression in keratinocytes through a positive feedback mechanism. LL37 binds to TLR2, activating mTORC1 signaling and increasing LL37 expression in keratinocytes, highlighting mTORC1's vital role in LL37 amplification (10, 31, 32). LL37 is central to rosacea pathogenesis, being overexpressed in rosacea patients' lesional skin (33–35). Intradermal injection of human LL37 in mice models induces inflammatory responses similar to rosacea, making it a key model in rosacea research (36, 37). LL37 has multiple functions, including immune response modulation, inflammation, and angiogenesis (33, 38). It activates mast cells (MCs), keratinocytes, neutrophils, and macrophages, leading to pro-inflammatory cytokine production, leukocyte chemotaxis, MMP expression, and angiogenesis (36, 39–42). LL37-associated signaling pathways are shown in Figure 1.

2.1 LL37- MRGPRX2-TRPV4 pathway in rosacea

LL-37, a potent chemoattractant, activates MCs in the inflammatory cascades. Increased MC concentration and degranulation, with a positive correlation between MC density and rosacea duration (43). In MC-deficient mice, rosacea-like symptoms are absent following LL37 dermal injection (44, 45). Subramanian H. et al. identified LL37's induction of MCs through the Mas-related G-protein-coupled receptor-X2 (MRGPRX2) (46). β-arrestin 2 (βarr2) regulates this via extracellular Signal-Regulated Kinase 1 and 2 (ERK1/2) phosphorylation and nuclear factor kappa B (NF-κB) activation in mice, suggesting potential therapeutic targets in rosacea (47). Sulk M. et al. observed an upregulation of the transient receptor potential vanilloid (TRPV) 4 channel, co-localized with MCs in rosacea patients (48). LL37 directly increases TRPV4 expression in human MCs via MRGPRX2. This elevation in

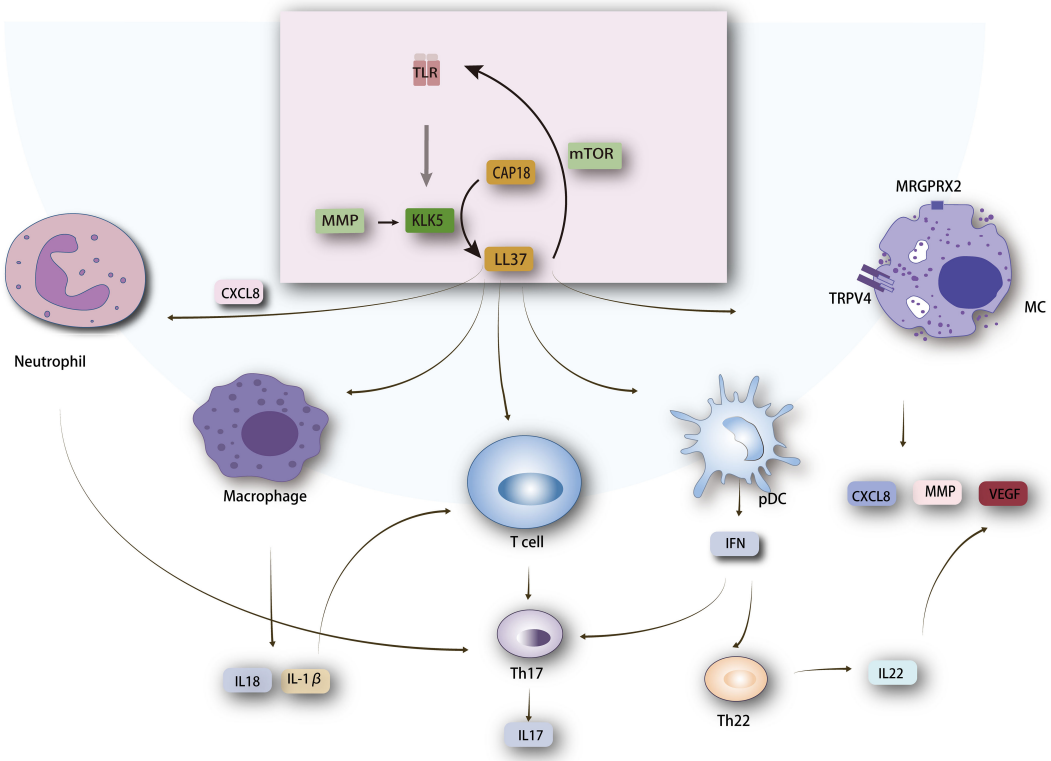

FIGURE 1
Mechanism of LL37 in Rosacea Pathogenesis. LL37 interacts with several key molecules, including Toll-like receptor 2 (TLR2), mechanistic target of rapamycin complex 1 (mTORC1), chemokine (C-X-C motif) ligand 8 (CXCL8), and Mas-related G-protein coupled receptor member X2 (MRGPRX2) linked to transient receptor potential vanilloid 4 (TRPV4). These interactions lead to the activation of various cell types such as macrophages, neutrophils, T cells, mast cells, and plasmacytoid dendritic cells (pDCs). Activation of these cells results in the production of cytokines, playing a critical role in inflammation, immune modulation, and angiogenesis in rosacea. The cytokines produced, such as IL-1β and TNF-α, contribute to the inflammatory responses characteristic of rosacea. The figure showcases the crucial LL37-mediated pathways and their roles in the pathogenesis of rosacea, emphasizing the complex interplay between different cell types and signaling molecules.

TRPV4 likely facilitates greater cation influx, raising intracellular Ca2+ levels and priming MCs for continuous degranulation or transgranulation (49, 50). Activated MCs release various cytokines, including IL-1, transforming growth factor (TGF-β), tumor Necrosis Factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF) (51). Additionally, MMP9 mRNA, a key MC marker, is upregulated in rosacea-affected skin, primarily near blood vessels (45). Neutrophils, which are recruited following mast cell (MC) activation, are a significant source of LL-37. This creates a feedback loop that perpetuates MC activation and chronic cutaneous inflammation in rosacea (52). Therefore, MCs are crucial in cathelicidin-induced skin inflammation through their role in cytokine and bioactive mediator secretion upon stimulation (53).

2.2 LL37-IL1β/IL17 pathway in rosacea

The NF-κB and the mitogen-associated protein kinase (MAPK) signaling pathway are crucial in LL37mediated inflammation (54, 55). LL-37 activates MAPK, leading to phosphorylation of ERK1/2 and p38 kinases (56), and induces NF-κB-mediated gene expression (57, 58). These pathways play a central role in the pathogenesis of rosacea, as evidenced by increased p38 and ERK levels in ocular rosacea tissue (59), upregulated MAPK pathways in PPR lesional tissue (60), and elevated NF-κB activity in rosacea patients' eyelid samples (61). Furthermore, TLR signaling pathways also converge on MAPK and NFκB-dependent gene expression (62). Importantly, the TLR2/Myeloid differentiation factor-88 adaptor protein (MyD88)/NF-κB is implicated in rosacea pathogenesis, as suggested by elevated MyD88 levels in rosacea skin biopsies (63). Moreover, dietary supplementation with n-3 PUFAs has been shown to ameliorate skin inflammation in an experimental rosacea model by inhibiting this pathway (64). Deng Z. et al. noted that LL37 initiates NF-κB activation, possibly through mTORC1 signaling (10). Additionally, UV radiation-induced ROS in keratinocytes activates MAPK and NF-κB pathways, influencing inflammatory signaling (65, 66). These pathways control inflammatory cytokine gene expression in immune cells (67, 68). Specifically, the expression of two NF-κB target genes, namely IL-1α and IL-1β, was elevated in rosacea (60, 69).

LL-37 also enhances the ability to release IL-1β by activating the inflammasome (70). NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) deficiency reduces LL37-induced rosacea-like inflammation (39). NLRP3, an intracellular sensor, is overexpressed in PPR subjects (71). The formation of the NLRP3

inflammasome subsequently leads to the caspase 1-dependent release of the pro-inflammatory cytokines IL-1β and IL-18 (72). IL-1β emerges as a critical mediator in the inflammation development in PPR (60). IL-18, an integral constituent of the IL-1 cytokine family, is heightened in rosacea patients (73). TNF-α signaling also upregulates IL-1β expression (60).

IL-1β serves as a co-stimulator of the proliferation of T-cells and is linked to Th17 lymphocyte differentiation (74). Th17 cells, active in rosacea, release proinflammatory cytokines, prominently IL-17. In rosacea, T-cell-dominated lymphocytes infiltrate affected skin (75), with consistently elevated IL-17 serum levels (76). Thus, IL-17 plays a crucial role in rosacea pathogenesis, particularly in PPR (77, 78). IL-17 has diverse functions. It activates VEGF-induced angiogenesis and expansion, as shown in both *in vitro* and *in vivo* studies (79). Obradovic´ H. et al. found that recombinant mouse IL-17 induces MMP9 expression in mouse myoblast C2C12 cells after IL-17 treatment (80).Furthermore, IL-17 stimulates vitamin-D3-induced LL37 production in keratinocytes (81, 82). Remarkably, LL37 induces genes related to Th1/Th17 polarization (83). IL-17 also prompts the production of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-8, and IL-6 (84). Rosacea skin samples show increased expression of these cytokines (85). Apart from Th17 cells, Th1 cells are also involved in the pathogenesis of rosacea. Th1 cells secrete IFN-γ, a potent macrophage activator that classically activates human macrophages into a pro-inflammatory (M1) phenotype *in vitro* (86). This enhances the interaction between CD4+ T cells and the innate immune system in the disease.

2.3 LL37-CXCL8 interaction in rosacea

LL37 induces the release of C-X-C motif Chemokine ligand (CXCL) 8 (formerly known as IL-8) from keratinocytes, a crucial chemotactic factor for neutrophils in rosacea (57, 87). Transcriptome analyses showed increased CXCL8 expression in rosacea (88, 89). Neutrophil migration is prompted by Demodex folliculorum and its associated bacillus oleronius in rosacea (90). These neutrophil pathways and proteins are central to rosacea's inflammation, with pustule development indicative of neutrophil infiltration (88). Neutrophils play a vital role in microbial defense, neutralizing threats through enzyme release, ROS synthesis, and inflammatory mediator production (91). This influx of neutrophils, in turn, precipitates the secretion of IL-17, thereby establishing a chronic inflammation cycle in rosacea.

2.4 LL37-VEGF axis in rosacea pathogenesis

Angiogenesis, facilitated by VEGF, is central to rosacea's hallmark symptoms of flushing and erythema (92). VEGF serves dual roles in angiogenesis and inflammation (93). In facial redness, VEGF, VEGF-R1, and VEGF-R2 are upregulated in the granular layer and stratum corneum of keratinocytes, as well as in dermal leukocytes including lymphocytes, macrophages, and plasma cells (94, 95). The VEGF polymorphism (+405C/G) is linked to rosacea severity (96). CD31+ cells infiltrates are primary sources of VEGF, driving angiogenesis (97). VEGF production by activated T cells stimulates angiogenesis and promotes Th1 cell differentiation, creating a feedback loop (98, 99). Additionally, UVB exposure activates VEGF signaling, with VEGF-A intensifying vascular sensitivity to UVB (100).

LL37 contributes to angiogenesis in rosacea. It activates endothelial cells (ECs) and VEGF via FPRL1, promoting angiogenesis (101). mTORC1 signaling mediates LL37-induced angiogenesis, with activation noted in ECs of rosacea lesions and LL37-induced rosacea-like mouse models (102). Furthermore, LL37-induced type I IFNs from pDCs, overexpressed during rosacea flare-ups, lead to an increased Th22/Th17 cytokine response (27). Enhanced IL-22 expression and EC sensitization to IL-22 facilitate aberrant angiogenesis (27). Moreover, TLR2 pathway overexpression in keratinocytes augments proinflammatory cytokine and chemokine expression, including IL-8, IL-1β, TNF-α, and C motif chemokine ligand (CCL) 5, CXCL9, CXCL10, and CXCL11 (8). These elevated levels of cytokines and chemokines result in the induction of vascular hyper-reactivity (103).

A recent study investigated the role of Hippo signaling pathway, specifically yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), in rosacea. The study found alterations in these signaling molecules in rosacea patients, suggesting their involvement in the development of new angiogenesis within the skin. Furthermore, the study showed that inhibiting YAP/TAZ reduced VEGF immunoreactivity, a marker of blood vessel formation. These findings suggest that YAP/TAZ may play a role in the mechanisms by which rosacea causes abnormal blood vessel growth (104).

3 JAK/STAT signaling pathway

The JAK/STAT pathways have a wide range of functions on immune responses, cellular proliferation, differentiation, apoptosis, and immunoregulation (105). JAK inhibitors are increasingly used in treating inflammatory skin disorders (106). In LL37-treated HaCaT cells, elevated JAK2 and STAT3 levels suggest a strong connection between JAK/STAT signaling and rosacea's inflammatory response. JAK2/STAT3 activation interacts with TLR2 signaling (107), leading to increased production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-8 (108, 109).

Rosacea's inflammation and immune infiltration are exacerbated by skin barrier disruption, partly due to STAT3-mediated cytokine signaling in keratinocytes (110). STAT3 also regulates degranulation in human and mouse MCs (111, 112). ERK1/2-mediated mitochondrial STAT3 phosphorylation contributes to MC degranulation (113). Blazanin N.et al. observed that acute solar UV exposure activates pSTAT1-related signaling in keratinocytes (114), indicating epidermal-derived STAT1's role in epithelial-immune communication in rosacea (115). The role of IL-17 in increasing VEGF expression via JAK/STAT signaling has been demonstrated in various contexts. IL-17 has been shown to induce reactive astrocytes and upregulate VEGF through JAK/STAT

signaling, as well as up-regulate VEGF in nucleus pulposus cells via the same pathway. These findings suggest that similar mechanisms might be relevant to the inflammatory response in rosacea (116, 117).

4 Cutaneous neuroinflammation and downstream signal pathways in rosacea

Cutaneous neurogenic inflammation (CNI) is widely recognized in rosacea, involving a series of signaling cascades. Ion channels, particularly transient receptor potential (TRP) channels in skin nerve fibers, activate upon stimuli, releasing vasoactive neuropeptides that interact with keratinocytes, immune cells, and blood vessels (118, 119). These neuropeptides exacerbate inflammation and vascular dilation, translating nerve impulses into signals for immune cells. Rosacea is a classic example of CNI, which can be explained by the neurologic hypersensitivity in patients with rosacea (120).

TRPV1, a critical cation channel primarily for Ca2+, is involved in cutaneous neurogenic inflammation and pain (121). TRPV1 expression increases in rosacea, especially in keratinocytes (122), upon stimulation by factors like pH changes, high temperatures, and UVB exposure (123, 124). Activated TRPV1 stimulates sensory neuron C fibers, releasing mediators that contribute to neurogenic inflammation and pain through elevated cytosolic Ca2+ levels. This leads to increased release of neuropeptides such as pituitary adenylate cyclase-activating polypeptide (PACAP), vasoactive intestinal peptide (VIP), VEGF, adrenomedullin, calcitonin gene-related peptide (CGRP), and substance P (SP), all implicated in rosacea pathogenesis (125–127). These neuropeptides collaborate in processes like inflammation, tissue damage, vasomotor disturbances, and increased neurovascular reactivity (128, 129). Abnormal amino acid metabolism, specifically glutamic and aspartic acids, can enhance the formation of erythema and telangiectasia in rosacea-like mouse skin through vasodilatory neuropeptides in peripheral neurons and keratinocytes (130).

CGRP, a potent microvascular dilator, contributes to extensive neurogenic vasodilation and mobilizes inflammatory cells (127). It also modulates cutaneous immunity by affecting NF-κB expression in immune cells (131). SP influences the emergence of edema in rosacea through its interaction with neurokinin 1 receptors and contributes to MCs degranulation, EC proliferation, and localized vasodilation (118, 132). Intradermal PACAP38 administration increases pain perception and skin blood flow, exacerbating rosacea features like facial flushing and edema (125). Mechanistically, PACAP acts as a potent vasodilator and influences vascular responses in human skin (133). It upregulates MC proteases (MMP-1 and MMP-9) and proinflammatory cytokines, including TNF and CXCL2, and may affect the pathway converting hCAP18 into LL37 (134). VIP enhances Th17 cell differentiation, shifting the T-helper cell response towards Th17 (135). In brief, VIP, PACAP, and CGRP act as vasodilators and mediate the production of inflammatory factors through interaction with skin immune cells.

Furthermore, there is notable upregulation of TRPV expression in rosacea, affecting not only neuronal but also non-neuronal cells. Sulk M.et al. observed increased dermal immunolabeling of TRPV2 and TRPV3 and gene expression of TRPV1 in ETR. PPR shows enhanced immunoreactivity for TRPV2 and TRPV4 and increased TRPV2 gene expression (48). Zhou X. et al. identified that TPRV4 also interacts with transient receptor potential melastatin 8 (TRPM8) channels on immune cells or keratinocytes, which is strongly associated with itching in rosacea both in experimental and clinical settings (136).

5 Molecular targeted therapy in rosacea

Rosacea treatment primarily focuses on symptom management, including anti-inflammatory, immunomodulatory, microflora-regulating, and capillary dilation strategies. Common treatments include topical agents (azelaic acid, metronidazole, brimonidine, ivermectin, tacrolimus, pimecrolimus) and oral antibiotics (tetracycline, retinoids) (12, 137). However, increasing concerns over antibiotic resistance and impacts on skin flora indicate a pressing need for more effective and safer therapeutic alternatives (138). Emerging insights into the signaling pathways involved in rosacea mentioned above have led to the exploration of targeted therapies, aiming for improved efficacy and fewer side effects. Table 1 presents current therapeutic targets and corresponding treatments for rosacea. However, the efficacy of these treatments remains challenging to assess and compare due to insufficient clinical studies.

TABLE 1 Summary of key signaling pathways and targeted treatments in rosacea.

Pathway	Targeted molecule	Example	References
LL37-related signaling pathways	TLR2, KLK5, LL-37, MMPs	Retinoids, Azelaic acid, Doxycycline, Carvedilol, Ivermectin	(139–143)
	mTORC1	Rapamycin, Celastrol	(10, 32)
	Th1/Th17-IL17	Secukinumab, Aspirin, Thalidomid	(69, 145, 146)
	VEGF	Topical dobesilate, Tranexamic acid	(97, 147)
JAK/ STAT pathways	JAK2, STAT3	Tofacitinib	(11, 148)

5.1 Targeting TLR2-KLK5- LL37 and mTOR-related pathways

Targeting the TLR2-KLK5-LL37 pathway is currently a key strategy for the clinical treatment of rosacea. Retinoids, azelaic acid, and doxycycline modulate this pathway, reducing KLK5 and cathelicidin expression (139–141). Azelaic acid inhibits serine protease activity, and doxycycline limits KLK5 activity by inhibiting MMP9 (139, 142). Recent studies indicate that Carvedilol and Ivermectin modulate this pathway, contributing to their efficacy in rosacea treatment (143). A vitro study demonstrated that ε-Aminocaproic Acid (ACA) and Superoxide Dismutase 3 (SOD3) are effective in modulating the TLR2-related pathway (65, 144). Topical Rapamycin, an inhibitor of mTOR, has shown clinical effectiveness in treating rosacea. In a controlled study, 18 female rosacea patients were randomized to receive either a placebo or 0.4% FDA-approved rapamycin ointment. The results demonstrated that the group treated with rapamycin experienced significant clinical improvement compared to the placebo group, indicating the potential of mTORC1 inhibition as a therapeutic strategy in rosacea (10). Furthermore, Celastrol and Epigallocatechin-3-gallate (EGCG) also target mTOR-related pathways, exhibiting anti-inflammatory effects (31, 32).

5.2 Targeting Th1/Th17-IL17 in rosacea

The development of biological agents targeting specific cytokines offers a promising approach to treating rosacea. Approved antibodies, including those against IL-1β and IL-17, show potential as novel treatments. Specifically, secukinumab targeting IL-17, a monoclonal antibody primarily used in psoriasis, is under investigation for its effectiveness in treating rosacea. A trial involving 24 patients with papulopustular rosacea assessed the efficacy of secukinumab. The patients received 300 mg of secukinumab weekly for 5 weeks, then monthly for 2 months, the treatment led to significant improvement in papules and overall severity in 17 of the participants, along with enhanced quality of life (145). In addition, Aspirin and Thalidomide have shown potential in moderating Th1/Th17 immune responses, further supporting the strategy of targeting specific cytokine pathways in rosacea (69, 146).

5.3 Targeting VEGF in rosacea

VEGF inhibition has emerged as an effective strategy in rosacea treatment. Topical dobesilate, known for inhibiting angiogenic factors, has been shown effective in treating erythematotelangiectatic rosacea (147). Tranexamic acid, too, has shown efficacy in reducing microvessel density, VEGF expression, and associated inflammatory markers in rosacea patients (97). Additionally, the role of erythroid differentiation regulator 1 (Erdr1) in significantly inhibiting VEGF-mediated angiogenesis has been documented (73).

5.4 JAK/STAT pathway in rosacea

The JAK/STAT pathway plays a crucial role in the pathogenesis of rosacea. Oral tofacitinib, a JAK inhibitor, has demonstrated efficacy in mitigating facial erythema in rosacea. A clinical study with 21 rosacea patients revealed that 71.4% experienced a significant reduction in facial erythema following oral tofacitinib treatment (11). Furthermore, tofacitinib's effectiveness in a case of steroid-induced rosacea underscores its potential, particularly in cases resistant to conventional therapies (148). Additionally, Artesunate has been identified as a promising agent in reducing inflammation through its action on the JAK2/STAT3 pathway (108).

6 Conclusion

This review highlights the complex signaling pathways involved in rosacea and the advancement of targeted therapies. The targeted modulation of the TLR2-KLK5-LL37 and mTOR pathways has shown significant efficacy in clinical settings. VEGF inhibitors have proven beneficial in treating erythematotelangiectatic rosacea. Biological agents, specifically monoclonal antibodies like secukinumab targeting IL-17, have been effective in treatment. The role of the JAK/STAT pathway in rosacea's pathology is significant, with tofacitinib notably successful in reducing facial erythema. Despite these developments, research in targeted therapies for rosacea remains incomplete. Recognizing the complexity of rosacea, which involves multiple signaling pathways, is crucial for future advancements in treatment.

Author contributions

FY: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. LW: Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. DS: Visualization, Writing – review & editing. LZ: Supervision, Writing – review & editing. XW: Data curation, Writing – review & editing. DD: Supervision, Writing – review & editing. XJ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The 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

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Macrophages in inflammatory skin diseases and skin tumors

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Macrophages, as specialized, long-lasting phagocytic cells of the innate immune system, have garnered increasing attention due to their wide distribution and various functions. The skin, being the largest immune organ in the human body, presents an intriguing landscape for macrophage research, particularly regarding their roles in inflammatory skin diseases and skin tumors. In this review, we compile the latest research on macrophages in conditions such as atopic dermatitis, psoriasis, systemic sclerosis, systemic lupus erythematosus, rosacea, bullous pemphigoid, melanoma and cutaneous T-cell lymphoma. We aim to contribute to illustrating the pathogenesis and potential new therapies for inflammatory skin diseases and skin tumors from the perspective of macrophages.

KEYWORDS

macrophage, inflammatory skin diseases, skin tumors, pathogenesis, treatment

1 Introduction

Macrophages are present in all tissues of adult animals (1). They have crucial roles in an organism's biology, including development, maintaining homeostasis, facilitating repair, and reacting to immunological assaults from pathogens. M0 macrophages, as the unmature and inactive form, polarize in different directions depending on the surrounding microenvironment, and form distinguished macrophage subtypes, such as M1 and M2 phenotype (2).

M1 macrophages, also known as classically activated macrophages, can be polarized by lipopolysaccharide (LPS) either alone or in synergism with interferon (IFN)-γ. M1 macrophages are characterized by an enhanced capacity to secrete pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and IL-12. Phenotypically, M1 macrophages exhibit significant levels of cluster of differentiation (CD)68, CD80 and CD86. M1 macrophages play an essential role in promoting inflammation, and display anti-infection and anti-tumoral activity. However, they can also mediate reactive oxygen species (ROS)-triggered tissue impairment, affecting tissue regeneration and wound recovery.

M2 macrophages, also known as alternatively activated macrophages, are polarized by IL-4 and IL-13. They display an anti-inflammatory cytokine profile with elevated levels of

IL-10 and transforming growth factor (TGF)-β. Based on the stimuli, M2 macrophages can be categorized into four subgroups, and they vary in terms of surface markers, released molecules, and biological roles. However, it is important to note that all M2 macrophages share the characteristic of co-express IL-10. M2 macrophages are crucial for clearing parasites, modifying tissues, promoting angiogenesis, and contributing to allergy disorders (3, 4).

Inflammatory skin diseases are a group of diseases resulting from immune system disorders and cause damage to skin tissue, including atopic dermatitis, psoriasis, systemic sclerosis, systemic lupus erythematosus, rosacea, bullous pemphigoid. Macrophages are recognized as significant cellular contributors to persistent inflammation across diverse tissues and illnesses (5). Concurrently, skin tumors, comprising both benign and malignant neoplasms, develop from the skin simultaneously. Nevus and hemangiomas are the most common benign skin tumors, and they are not life-threatening but impact aesthetics. Skin malignancies, including malignant melanoma, basal cell carcinoma, and cutaneous T-cell lymphoma, can be deadly and demand urgent attention. The function of macrophages in the tumor microenvironment (TME) has been extensively researched in many types of tumors, including skin malignancies. Macrophages are crucial in controlling the body's immunological response and metabolism, perhaps contributing to the development of many diseases (4, 6). This review seeks to outline recent discoveries about the role of macrophages in different inflammatory skin diseases and skin tumors.

2 Atopic dermatitis

The symptoms of atopic dermatitis (AD), a chronic inflammatory skin condition, include intense itching and recurrent superficial and spongiotic inflammation (7). A complicated interplay between genetic and environmental variables, including immunological response, skin barrier failure, and pruritus, may be instrumental in the pathogenesis of AD (8). Numerous investigations have revealed a robust correlation between AD and macrophages.

2.1 The characteristic of macrophage in AD

Using molecular imaging approaches, 2,4-dinitrofluorobenzene (DNCB) induced AD-like skin lesions have been observed to exhibit infiltrated-macrophage profile (9). The difference in macrophage polarization between skin samples from AD and psoriasis is evident. M2 macrophages were almost exclusively detected in AD samples. While traditionally regarded as an anti-inflammatory phenotype, recent study suggested that M2 macrophages contribute to the pathogenesis of AD through the secretion of CCL18, thus promoting the continued recruitment of Th2 cells and maintaining inflammation (10).

AD macrophages have lower toll-like receptor (TLR)-2 expression and less release of pro-inflammatory cytokines in response to TLR-2 ligand stimulation when compared to healthy controls. This may be a factor in AD patients' increased vulnerability to *Staphylococcus aureus* skin infections (11). Notably, psoriasis patients also exhibit colonization with *Staphylococcus aureus*. When exposed to *Staphylococcus aureus α-toxin*, macrophages from AD patients generated less C-X-C Motif Chemokine Ligand (CXCL)10 than those from psoriasis patients. Decreased secretion of CXCL10 results in reduced Th1 polarization (12).

A distinct cluster of macrophages expressing C-C Motif Chemokine Ligand (CCL)13 and CCL18 was discovered with single-cell RNA-sequencing in the leukocyte-infiltrated region of the lesional skin in AD. Analysis of ligand-receptor interactions revealed interactions between T cells, dendritic cell (DC)s, fibroblasts, and M2 macrophages that expressed CCL13 and CCL18. This provides a thorough understanding of the immunological milieu in AD (13).

2.2 The pathogenic roles of the macrophages in AD

Macrophages contribute to the development of AD through a variety of processes. An important factor in human AD is CLDN1, a component of epidermal tight junctions. The association between human AD patients' CLDN1 levels and macrophage recruitment has been elucidated by recent research. Mice with reduced CLDN1 expression levels displayed AD-like morphological traits and attracted more macrophages to the skin lesion (14). YKL-40 is a crucial inflammatory marker in type II inflammation. Compared to normal persons, AD patients' skin had a greater level of YKL-40. Subsequent research indicated that the primary source of YKL-40 was dermal macrophages, indicating that macrophages may be involved in the pathophysiology of AD (15).

Macrophages participate in the mechanism of AD itch as well. IL-31 is a type II cytokine linked to pruritus in many dermatologic diseases. For instance, it has been reported that CD206+ M2-like macrophages are the primary producers of IL-31 in recessive dystrophic epidermolysis bullosa (16). M2 macrophages are dominant sources of IL-31 in AD as well. Moreover, AD itch is caused by a sophisticated network of periostin, basophils, thymic stromal lymphopoietin, and IL-31-expressing macrophages (17).

Autophagy of macrophages is essential for immunological regulation and has been linked to the onset of AD. Compared to wild-type mice, autophagy-related gene 5 cKO mice display deficient autophagy activity, lower cutaneous inflammation and decreased M2 macrophage infiltration. Mechanistically, deficiency of autophagy causes CCAAT enhancer binding protein beta to accumulate, which in turn stimulates the production of suppressor of cytokine signaling 1/3, ultimately suppresses the expression of the M2 marker (18).

One hallmark of AD is inflammation-mediated lymphangiogenesis, which is intimately related to macrophage recruitment. Strong macrophage chemoattractant monocyte chemoattractant protein-1 is expressed at high levels by IL-4-stimulated keratinocyte cells. Furthermore, a notable rise in dermal macrophages expressing vascular endothelial growth factor-C, a pro-lymphangiogenic factor, is observed in the AD mice model (19).

Research has also been conducted regarding the role of chemokines related to macrophages in the etiology of AD,

particularly macrophage migration inhibitory factor (MIF). The stratum corneum MIF levels in the skin lesions were found substantially higher compared to unaffected regions in the same patient. MIF provides a helpful gauge to measure the degree of AD locally (20). There is a link between the MIF promoter 173G/C polymorphism and a higher risk of AD (21). MIF promoter polymorphisms, namely the C-173 allele and the C/5-CATT and C/7-CATT haplotypes, were found to be substantially linked to a higher risk of AD in Korean patients (22). The characteristics and pathogenetic roles of the macrophages in AD are summarized in Figure 1.

2.3 Treatments for AD involving macrophages

Traditional Chinese medicine exhibited great potential in treating AD, including Periploca forrestii Schltr saponin and Stellariae Radix. Periploca forrestii Schltr saponin, which was traditionally used to treat rheumatoid arthritis, exhibits substantial potential for therapy in AD by suppressing the expression of both M1 and M2 macrophage markers (23). Stellariae Radix, which was previously used to treat fever and insomnia, successfully inhibited M1 macrophage infiltration in a DNCB-induced AD mouse model. Mechanistically, Stellariae Radix suppressed the production of tumor necrosis factor (TNF)-α, CXC-10, IL-12, and IL-1β and reduced the expression of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) in M1 macrophages (24). A new topical medication for AD, called Nuclear Transport Checkpoint Inhibitor, inhibited the invasion of macrophages, and decreased the proliferation of Ki-67-positive cells (a subset of cells within the basal layer of the epidermis) (25). Naringenin, a flavonoid derived from plants, can reduce AD symptoms by inhibiting the M1-like macrophage phenotype, high mobility group box-1 (HMGB1) cascade, and levels of inflammatory cytokines. Moreover, naringenin can induce anti-inflammatory gene expression through the transformation of the M1 to M2 phenotype, resulting in increased levels of CD36 and IL-10 (26). Dictamnine, a natural alkaloid isolated from the root of Dictamnus albus, hinders DNCB-triggered AD skin inflammation by blocking M1 macrophage differentiation and enhancing macrophage autophagy at inflammation sites. Furthermore, dictamnine decreases the secretion and suppresses the genetic expression of inflammatory molecules (27). However, the curative effect of these potential therapies was evaluated in AD-mouse models and bone marrow-derived macrophages. In the future, we anticipate more large-scale clinical trials to verify these outcomes.

Nemolizumab, a humanized monoclonal antibody against IL-31 receptor A, holds great promise for alleviating pruritus and inflammation in AD patients in many clinical trials (28, 29). Dupilumab is another humanized monoclonal antibody that has gained approval for the treatment treating moderate-to-severe AD.

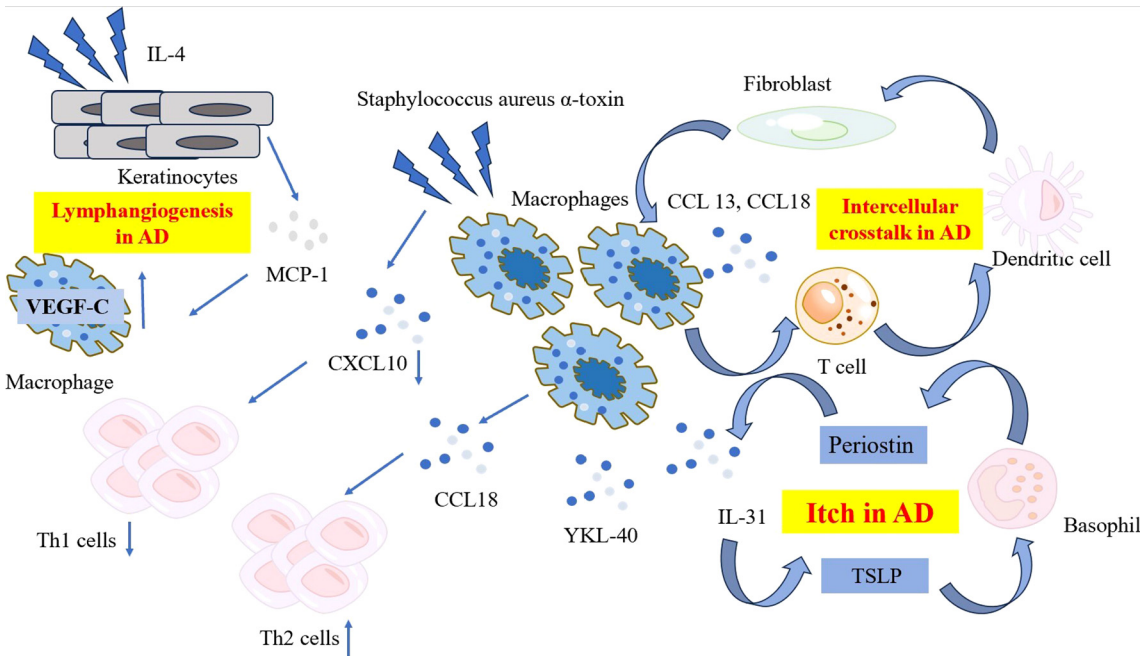

FIGURE 1
The characteristic and pathogenetic roles of the macrophages in AD. Compared to psoriasis, macrophages in AD produce lower level of CXCL10 when exposed to Staphylococcus aureus α-toxin, resulting in reduced Th1 polarization. Instead, macrophages in AD produce high levels of CCL18, recruiting more Th2 cells to affected skin and release YKL-40, an important Th2 marker. A network comprising periostin, TSLP, basophils and macrophage-derived IL-31 contribute to the mechanism of itch in AD. Ligand-receptor interactions data revealed the intracellular crosstalk between CCL13, CCL18-macrohages, T cells, DCs and fibroblasts. Macrophages also get involved in the lymphangiogenesis in AD by expressing significant level of VEGF-C. AD, atopic dermatitis; CCL, C-C Motif Chemokine Ligand; CXCL, C-X-C Motif Chemokine Ligand; DC, dendritic cell; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TSLP, thymic stromal lymphopoietin; VEGF-C, vascular endothelial growth factor-C; YKL-40, Chitinase 3-like 1.

Dupilumab can specifically bind to the IL-4Rα subunit, thereby inhibiting the signal transduction of IL-4 and IL-13, and blocking the Th2 inflammatory response. Both IL-4/13 and IL-31 pathway contributes to AD itch. Recent findings suggest that IL-31 can induce itching independently of IL-4 and IL-13 *in vivo* (30). M2 macrophages are implicated in the pathogenesis of AD pruritus and inflammation through the secretion of IL-31 and Th2 cytokines. However, there is a lack of direct studies addressing the impact of nemolizumab and dupilumab on immune cells, particularly the phenotype and number of macrophages. The janus kinase (JAK) pathway is activated in the signaling transduction of many cytokines relevant to AD. A network meta-analysis has demonstrated that many JAK inhibitors can ameliorate the signs and symptoms of AD, with upadacitinib showing particular efficacy (31). It has been documented that JAK inhibitor can reduce the infiltration of macrophages in lesional sites in allergic contact dermatitis mouse models (32). However, no analogous experiments have been conducted in AD mouse models.

3 Psoriasis

Psoriasis is a prevalent chronic inflammatory skin disorder distinguished by a significant inflammatory presence along with enlarged and distorted blood vessels. Infiltrated macrophages in psoriatic skin lesions are crucial in the advancement of this unregulated skin inflammation.

3.1 The characteristic of macrophage in psoriasis

Analyzed data from the GEO database showed a notable rise in the level of expression of macrophage markers and inflammatory cytokines in lesional tissues as compared to normal tissues in 58 patients with psoriasis (33). Significant variations in the composition of innate immune cells were found between psoriatic plaques and normal skin. There is a notable increase in the quantity of M0 and M1 macrophages in psoriatic skin. Both the count and proportion of macrophages underwent alterations. The abundance of M0 macrophages was linked to the psoriasis severity degree (34, 35). Psoriatic patients had a greater ratio of M1 to M2a macrophage polarization compared to controls (36). The proportion of C-C Motif Chemokine Receptor (CCR) 1+ macrophages increase in psoriasis-affected skin compared to healthy skin, as determined by single-cell RNA sequencing and flow cytometry data. CCR1+ macrophages exhibited elevated expression of genes associated with inflammatory cytokines and chemokines, such as CXCL-8, CXCL-2, and IL-1B (37).

Immune cell composition varies between the early and late stages of psoriatic skin lesions. Neutrophils infiltrated the epidermis in the early phase, but monocytes and monocyte-derived DCs were mostly present in the dermis. During the late phase, there was a temporary rise in the number of macrophages in the dermis (38).

3.2 The pathogenic roles of the macrophages in psoriasis

Several efforts have been undertaken to determine the function of macrophages in the development of psoriasis. The IL-23/IL-17 immunological axis plays an important role in the initiation and progression of psoriasis. A novel pathogenic macrophage subpopulation, triggered by IL-23 and characterized by a unique gene expression profile, has been discovered recently. M (IL-23) produce significant quantities of IL-17A, IL-22, and IFN-γ, contributing to the development of psoriasis-like dermatitis in a mouse model (39). Additionally, the IL-23/IL-17 immunological axis is proposed to play a role in the development of psoriasis by initiating ACT1/TRAF6/TAK1/NF-κB pathway in macrophages (40). Two important autoantigens in psoriasis are LL-37 and ADAMTS-Like Protein 5. It has been observed that ADAMTS-Like Protein 5+ and LL-37+ cells are co-expressed with CD163+ macrophages in both the superficial and deep dermis (41).

Interactions between macrophages and keratinocytes play a significant role in the development of psoriasis. Keratinocytes can interact with macrophages via HMGB1, promoting macrophage inflammatory polarization (42). The interaction between macrophages and exosomes generated from vitamin D receptor-deficient keratinocytes is crucial for the advancement of psoriasis. Exosomes-sh vitamin D receptor markedly enhanced macrophage proliferation and directed their polarization toward the M1 phenotype, while suppressing macrophage apoptosis (43).

Psoriasis is more prevalent and severe in men than in women. A recent investigation has shown that the root cause is linked to estrogen. Estradiol can inhibit the production of IL-1β by macrophages, and IL-1β is necessary for the generation of IL-17A in the psoriasis model. This perspective may clarify the disparity in both the occurrence and seriousness of psoriasis between genders (44).

Macrophages and psoriasis-related comorbidities have also been studied. Psoriasis patients with comorbidities have elevated levels of chitotriosidase compared to those without comorbidities. Chitotriosidase is primarily produced by activated macrophages in reaction to pro-inflammatory signals (45).

Macrophage-related cytokines are also linked to the development of psoriasis. The levels of macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemoattractant protein-1 were considerably elevated in patients with psoriasis vulgaris and positively associated with psoriasis area and severity index score (46). While MIF levels were elevated in the blood, MIF-positive staining in the psoriatic epidermis was notably reduced. MIF mRNA level decreased simultaneously in the psoriatic lesions, supporting this discovery (47). Further investigation is required to understand the disparity in MIF levels between the psoriatic epidermis and the circulation. The -173 GC genotype and the 6C haplotype of MIF polymorphisms are linked to an increased risk of plaque psoriasis in the Mexican population (48). Patients with psoriasis showed significantly lower frequencies of genotypes -794*CATT 5/7 and 7/7, while the CATT*5/MIF-173*C haplotype was more common (49).

3.3 Treatments for psoriasis involving macrophages

Shikonin is an organic matter extracted from the roots of Lithospermum erythrorhizon. Combining Shikonin with methotrexate has been demonstrated to hinder the advancement of psoriasis by controlling the polarization of macrophages. Administration of Shikonin and methotrexate in an imiquimod (IMQ)-induced psoriasis mice model can reduce the expression of F4/80 positive cells and decrease the mRNA levels of M1 macrophage markers (50). The PSORI-CM02 formula, a novel Chinese medicine, has been proven to have an anti-psoriatic effect. It can decrease macrophage infiltration, diminish M1 but increase M2 markers in IMQ-induced psoriasis mice (51). Etanercept, the first anti-TNF inhibitor, blocks the JAK/STAT3 pathway, decreasing the ratio of Th17/Treg and promoting M2 polarization, ultimately relieving psoriasis in mice (52). Application of Mung bean-derived nanoparticles topically can facilitate maintaining the balance of polarized macrophages and inhibit the activation of the NF-κB signaling pathway, leading to a reduction in skin inflammation (53). The pathogenetic roles of the macrophages in psoriasis and treatments involving the M1 phenotype are summarized in Figure 2.

4 Systemic sclerosis

Systemic sclerosis (SSc) is a paradigmatic rheumatic disease characterized by immune dysfunction-driven inflammation affecting multiple organs, finally leads to fibrosis. Skin involvement is among the most prominent manifestations of SSc. Raynaud's phenomenon is the most prevalent skin lesion observed in SSc patients. Other skin lesions of SSc encompass puffy fingers, skin thickening and induration, digital ulcers, and hyperpigmentation. The exact cause of SSc is not well understood yet.

4.1 The characteristic of macrophage in SSc

In the skin of patients with SSc, there is a notable increase in the quantity of CD163+ cells located among collagen fibers when compared to the skin of healthy individuals (54). Macrophage signatures were found to be upregulated in early SSc patients compared to healthy controls. M2 and M1 macrophage signatures were present in 96% and 94% of patients, respectively. Furthermore, M2 and M1 signatures were associated with a higher extent of skin involvement, but also skin thickness progression rate prior to

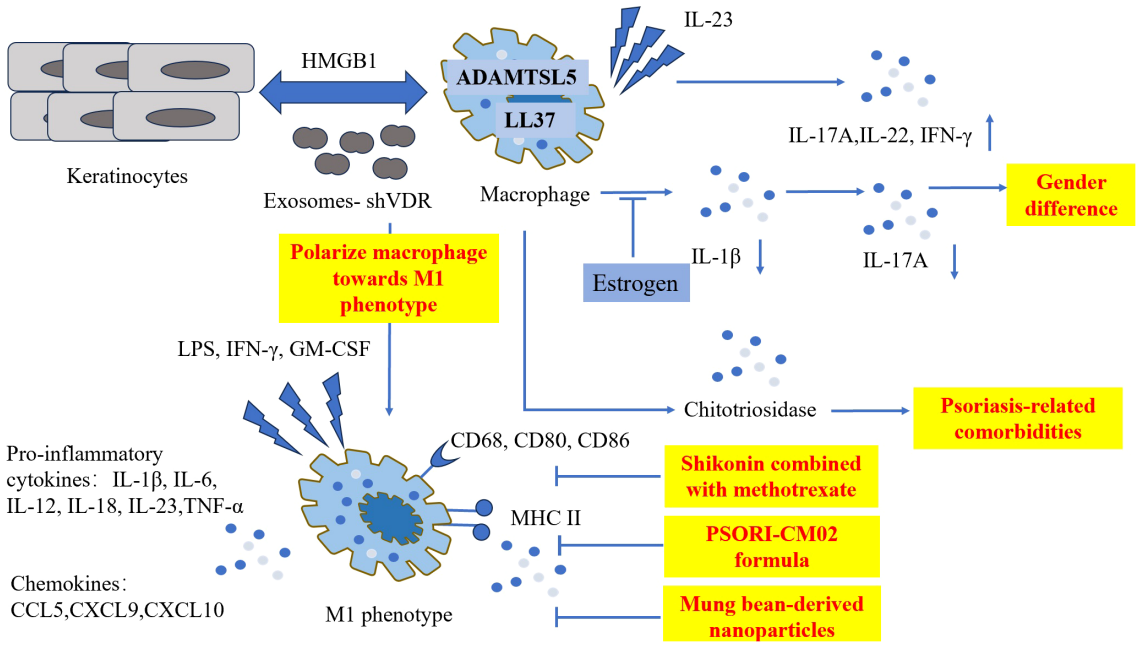

FIGURE 2
The pathogenetic roles of macrophages in psoriasis and treatments involving M1 phenotype. Macrophages co-express with two important psoriasis autoantigens LL-37 and ADAMTSL5. Macrophages triggered by IL-23 produce significant quantities of IL-7A, IL-22 and IFN-γ. Chitotriosidase secreted by activated macrophages is related to psoriasis-related comorbidities. Estrogen suppresses the production of IL-1β, furthermore reducing the level of IL-17A. Keratinocytes interact with macrophages via HMGB1, and exosomes derived from VDR-deficient keratinocytes polarize macrophages toward M1 phenotype, exaggerating the inflammation condition. Shikonin combined with methotrexate, PSORI-CM02 formula, and Mung bean-derived nanoparticles exhibit anti-psoriatic properties by hindering M1 polarization. ADAMTSL5, ADAMTS-Like Protein 5; CD, cluster of differentiation; CCL, C-C Motif Chemokine Ligand; CXCL, C-X-C Motif Chemokine Ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; HMGB1, High Mobility Group Box-1; IFN, interferon; IL, interleukin; LL-37, Cathelicidin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; TNF, tumor necrosis factor; VDR, vitamin D receptor.

biopsy, an independent predictor of mortality (55). Dual phenotypic macrophages were recently identified in SSc disease. SSc patients exhibited elevated proportions of peripheral cells displaying M1, M2, and a combination of M1/M2 phenotypes in comparison to the control group (56). The transcriptome profile of macrophages in SSc shows increased activity in glycolysis, hypoxia, and mTOR signaling, while exhibiting decreased activity in IFN-γ response pathways (57). Single-cell transcriptome data have revealed three specific myeloid cell clusters in diffuse cutaneous SSc, including one macrophage cluster. This cluster expresses Fcγ receptor IIIA at high level, indicating a transition from normal CCR1+ and MARCO+ macrophages (58).

4.2 The pathogenic roles of the macrophages in SSc

Macrophages in SSc exhibit a profibrotic activation profile, meanwhile emit signaling molecules and have surface indicators linked to both M1 and M2 macrophage activation (59). M1 macrophage is associated with the beginning of fibrosis and accelerates its advancement in SSc. Research has shown that LPS-induced M1 macrophage pyroptosis contributes to fibrosis in SSc via the Cathepsin B/NLRP3/GSDMD pathway (60). In addition, ferroptosis presents in the bleomycin (BLM)-induced SSc mice model, where the M1 macrophage upregulates the expression of the ferroptosis driver Acyl-CoA synthetase long chain family member 4 and enhances its susceptibility to ferroptosis (61). Besides M1 macrophage, periostin contributes to the inflammation and fibrosis of SSc by potentially influencing M2 macrophages. Periostin-stimulated macrophages from healthy controls showed a substantial decrease in the proportion of M2 macrophages compared to those from SSc patients. Periostin stimulation led to a considerable upregulation of pro-fibrotic cytokines, chemokines, and extracellular matrix proteins in macrophages at the mRNA level (62).

Macrophages and fibroblasts contribute to the development of SSc by reciprocally activating each other. Macrophages show enhanced secretion of proinflammatory cytokines when stimulated with exosomes generated from fibroblasts of SSc patients. Collagen and fibronectin synthesis is greatly activated in fibroblasts when receiving signals from SSc exosome-stimulated macrophages (63). Co-culture investigations in Transwell experiments also demonstrated that SSc macrophages induce fibroblast activation (59). A self-assembled skin equivalent system was created to investigate the communication between macrophages and fibroblasts in SSc. The outcome provides more evidence supporting the mutual activation that relies partially on TGF-β (64). Depleting B cells has been suggested as a novel strategy for treating SSc, given that B cells can inhibit the differentiation of profibrotic macrophages. The extent of profibrotic macrophage activation induced by B cells is correlated with the fibrosis severity (65).

SSc-interstitial lung disease (ILD) is a complication associated with high morbidity and mortality. Immunohistochemistry analysis showed an accumulation of CD68+ and mannose-R+ macrophages in the lungs of SSc patients. Furthermore, single-cell RNA sequencing investigation of tissue-resident CD14+ pulmonary macrophages in SSc-ILD patients has shown an active profibrotic signature and increased Fibronectin 1 expression (66). Elevated levels of mixed M1/M2 macrophages in the circulation are linked to SSc-ILD, systolic pulmonary artery pressure, and the presence of anti-topoisomerase antibodies, which are established predictors of lung involvement in SSc (67). The upregulation of CCL18 and CD163 in the lungs of patients with SSc-ILD strongly implicates the pathogenetic roles of activated macrophages in this complication. Levels of CCL18 and CD163 are positively correlated with FibMax, an indicator for accessing lung fibrosis progression (68).

Levels of Serum MIF were considerably higher in both limited and diffuse SSc groups compared to healthy controls (69, 70). Microvascular endothelial cells and fibroblasts showed increased production of MIF when exposed to SSc serum, indicating the cellular source of MIF (70). MIF has the potential to serve as biomarkers and prognostic variables for pulmonary arterial hypertension (PAH) secondary to SSc. Patients with PAH related to SSc had elevated levels of MIF in their circulation compared to SSc patients without PAH. Patients with a higher New York Heart Association class exhibited higher levels of MIF (71). The MIF 7C haplotype is linked to an increased risk of SSc in the southern Mexican population and is correlated with increased MIF mRNA levels. MIF is associated with a proinflammatory response in SSc, as it correlates positively with the Th1 and Th17 cytokine profile (72). Except for MIF, Citrullinated vimentin, a biomarker of macrophage activation, was elevated in early diffuse-SSc compared to late diffuse-SSc (73). The characteristic and pathogenetic roles of the macrophages in SSc are summarized in Figure 3.

4.3 Treatments for SSc involving macrophages

Imatinib is a tyrosine kinase inhibitor typically used in the treatment of chronic myeloid leukemia. Notably, imatinib-loaded gold nanoparticles have demonstrated great efficacy in reducing IL-8 secretion, cell viability, and M2 polarization in alveolar macrophages (74). Nintedanib, another tyrosine kinase inhibitor, has shown promising antifibrotic effects in a SSc animal model. The underlying mechanism is associated with impaired M2 polarization of monocytes and reduced numbers of M2 macrophages (75). As for pulmonary fibrosis, an intractable problem in SSc patients, Zhang et al. proposed methyl-CpG-binding domain 2 (MBD2) as a novel therapeutic target. Depletion of MBD2 has been shown to prevent pulmonary fibrosis in a BLM-treated mouse model and to reduce the infiltration of M2 macrophage in the lungs of BLM-treated mice. MBD2 suppresses the SHIP expression and enhances PI3K/Akt signaling, thereby promoting the macrophage M2 phenotype (76). Ruxolitinib, a JAK inhibitor, exhibited anti-fibrosis properties in a BLM-SSc mouse model. *In vitro* experiments have revealed that ruxolitinib enhances macrophage efferocytosis when exposed to IFN, and reduced TGF-β- activated marker in fibroblasts derived from SSc-related pulmonary fibrosis tissues (77).

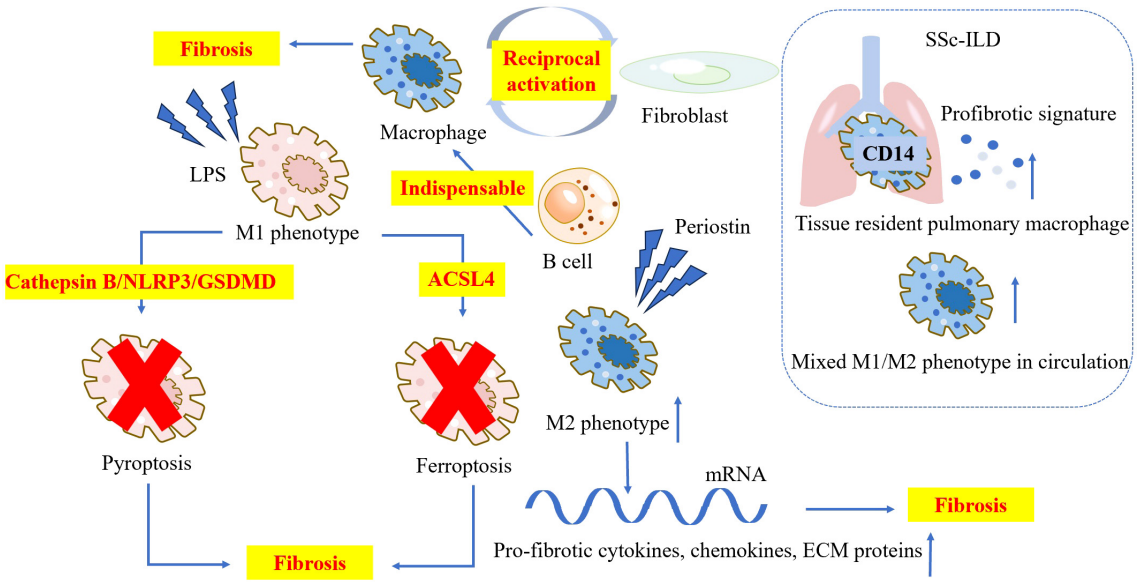

FIGURE 3
The characteristic and pathogenetic roles of the macrophages in SSc. Macrophages and fibroblasts mutually activate each other and contribute to the pathology in SSc. B cells promote the differentiation of profibrotic macrophages, and is indispensable for the progression of SSc. Periostin induces higher ratio of M2 macrophage and upregulates the mRNA level of pro-fibrotic cytokines, chemokines, and ECM proteins. M1 macrophage facilitates fibrosis by pyroptosis and ferroptosis. CD14+ tissue resident pulmonary macrophages in SSc-ILD patients' lungs show an active profibrotic signature. Elevated levels of mixed M1/M2 phenotype macrophages are observed in the circulation of SSc-ILD patients. ACSL4, Acyl-CoA synthetase long chain family member4; CD, cluster of differentiation; ECM, extracellular matrix; GSDMD, Gasdermin D; LPS, lipopolysaccharide; NLRP3, NOD-like receptor thermal protein domain associated protein 3; SSc-ILD, systemic sclerosis-interstitial lung disease.

5 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by complex pathophysiology and genetic susceptibility. The disease is defined by the involvement of multiple systems and organs, recurring flare-ups and remissions, and the emergence of various autoantibodies in the body. Untreated SLE can lead to permanent harm to organs and finally lead to death. Skin lesions are frequently observed in the majority of SLE patients. Nearly half of SLE presents with acute cutaneous lupus erythematosus, characterized by a butterfly-shaped rash over the cheeks and nose. Additionally, SLE patients may exhibit subacute and chronic cutaneous lupus erythematosus. Photosensitivity, alopecia, and oral mucosal ulcers are also frequently observed in SLE patients.

5.1 The pathogenic roles of the macrophages in SLE

Some scientists have suggested that M1 and M2 macrophages have distinct functions in the development of SLE. M1 macrophages exacerbate SLE, whereas M2 macrophages seem to alleviate its effects (78). The involvement of M2 macrophages in SLE is still a topic of debate. Other researchers observed a rise in the presence of CD163+ M2 macrophages in SLE skin and elevated soluble (s) CD163 levels in SLE patient blood specimens. Increased systemic and local CD163 expression indicates that M2 macrophages may contribute to the development of SLE as well (79). Furthermore, M2 macrophages have been suggested to play a role in the development of lupus nephritis. Urine sCD163 is highly associated with the current activity index of renal pathology and several particular pathological characteristics. M2 macrophages are a significant source of increased urine sCD163 levels, indicating its potential for predicting renal pathology (80).

Macrophages release ROS and inflammatory cytokines, which aggravate the inflammatory condition and tissue damage in SLE. Anti- dsDNA antibodies are crucial in the advancement of SLE. Anti-dsDNA antibodies can trigger NLRP3 inflammasome activation by binding to TLR-4 on macrophages, resulting in elevated mitochondrial ROS generation (81). Myeloid-derived suppressor cells may aggravate the IMQ-induced lupus model by enhancing TLR-7 pathway activation in macrophages. Mechanically, Myeloid-derived suppressor cells derived S100 Calcium Binding Protein A 8/9 increased IFN-γ secretion by macrophages, which then stimulated TLR-7 pathway activation in an autocrine manner (82). Activated lymphocyte-derived DNA induces macrophages to polarize toward M2b. M2b macrophages are distinguished by their production of inflammatory cytokines and their role in promoting inflammation condition, which is crucial in the progression of SLE (83). Activated lymphocyte-derived DNA-stimulated macrophages exhibit heightened glycolysis, reduced pentose phosphate pathway activity, and increased glycogenesis in glucose metabolism. The reduced pentose phosphate pathway activity ultimately resulted in increased levels of ROS (84).

Macrophages also play a role in the development of SLE by efferocytosis. Efferocytosis is the phagocytic elimination of apoptotic cells, and individuals with SLE show impairments in

this process (85). The diminished efferocytosis is not an inherent defect but rather dependent on serum, linked to lower levels of C1q, C4, and C3 (86). Genes related to inflammation, autophagy, and signaling are upregulated in macrophages engulfing apoptotic cells from SLE patients (87). Efferocytosis capability differs between male and female mice. Female mice had a more pronounced impairment in macrophage efferocytosis compared to male mice, which could be reversed by administering male microbiota (85). SLE patients have been shown to exhibit elevated levels of urokinase-type plasminogen activator receptor expression. TLR-7 controls urokinase-type plasminogen activator receptor expression through ERK/c-JNK signaling and hinders macrophage efferocytosis (88). Tyro3 is a receptor that plays a role in identifying apoptotic cells in the process of efferocytosis. Autoantibodies targeting Tyro3 have been linked to increased disease activity in SLE and can hinder the ability of macrophage efferocytosis (89). Efferocytosis activity can be restored by co-culturing with human umbilical cord-derived mesenchymal stem cells. This reversal effect has been observed *in vitro* experiments and in SLE patients who underwent umbilical cord-derived mesenchymal stem cells transplantation (90). Bone marrow-derived mesenchymal stem cells release exosomes including miR-16 and miR-21, subsequently stimulate the anti-inflammatory transformation of macrophages. Furthermore, these macrophages exhibit enhanced efferocytosis ability and can be used to alleviate lupus nephritis (91).

5.2 Treatments for SLE involving macrophages

Azithromycin, a macrolide antibiotic, has emerged as a novel medication for SLE. *In vitro* experiments using macrophages that mimic the SLE phenotype have shown a reduction in M1 markers and an increase in M2 markers after azithromycin application, and this effect is dependent on Akt phosphorylation (92). Diffuse alveolar hemorrhage (DAH) is a potentially fatal complication of SLE. Serp-1, a rabbit myxomavirus-encoded serpin, has been shown to prevent the occurrence of SLE-associated DAH in a mouse model by modulating macrophage function. According to Zhuang et al., Serp-1 inhibits DAH by enhancing LXR-regulated M2 macrophage polarization and IL-10 production by KLH4 regulation (93). Additionally, PAM3, a TLR2/1 agonist, has shown promise in the treatment of SLE. It not only induces the differentiation of monocytes into an immunosuppressive M2 phenotype *in vitro* but also reduces disease severity in a lupus-prone mouse model (94).

6 Rosacea

Rosacea is a long-lasting inflammatory skin disorder identified by erythema and pustules. Macrophage infiltration is considered a frequently overlooked characteristic present in all kinds of rosacea (95). A large amount of CD68+ macrophages have been found to infiltrate the rosacea lesions (95, 96). Immune infiltration analysis also suggests that M1 macrophages play a significant role in rosacea (97).

6.1 The pathogenic roles of the macrophages in rosacea

Macrophages have been documented as participants in the deterioration mechanisms of rosacea. Guanylate Binding Protein 5 has been recognized as a crucial regulator of rosacea by promoting M1 macrophage polarization through the NF-κB signaling pathways (98). Elevated levels of the antimicrobial peptide LL-37 are commonly linked to the development of rosacea. LL-37 can enter macrophages' cytoplasm via P2X7 receptor-mediated endocytosis and enhance NLRP3-mediated inflammasome activation in macrophages (99). ADAM-like metalloprotease Decysin-1 is considered to be associated with inflammation. Recent studies show that ADAM-like metalloprotease Decysin-1 may contribute to inflammation in rosacea by influencing the M1 polarization of macrophages (100).

6.2 Treatments for rosacea involving macrophages

Carvedilol, a nonselective beta-adrenoceptor antagonist, is an effective treatment for rosacea. *In vitro* studies have shown that carvedilol can reduce TLR-2 expression in macrophages, leading to decreased kallikrein related peptidase 5 secretion and LL-37 expression (101). Paeoniflorin, a monoterpenoid glycoside with various pharmacological activities, can alleviate rosacea-like inflammatory response by inducing suppressor of cytokine signaling 3 expression and suppressing the LPS-induced upregulation of TLR-2 and LL-37 via the ASK1-p38 cascade in macrophages (96). Artemisinin, the most effective antimalarial drug, decreases the presence of macrophages and immune cells in mice rosacea lesions, furthermore suppresses the production of chemokines associated with immune cells (102).

7 Bullous pemphigoid

Bullous pemphigoid (BP) is a deadly autoimmune dermatological disorder marked by initial red lesions and the subsequent formation of subepidermal blisters. The pathology of BP is linked to autoantibodies that target two hemidesmosomal proteins: BP180 and BP230.

7.1 The pathogenic roles of the macrophages in BP

There is a significant occurrence of CD163+ tissue-associated macrophages in BP. The increased levels of sCD163 in the serum of patients with BP compared to healthy individuals confirmed the activation of CD163+ tissue-associated macrophages. Chen et al. demonstrated that mice with macrophage deficiency were resistant to blister formation induced by pathogenic antibodies. In contrast, mice lacking T cells or B cells did not exhibit this resistance, indicating that macrophages, rather than T and B lymphocytes,

play a pivotal role in the development of subepidermal blisters in experimental BP. Macrophages can facilitate the infiltration of neutrophils, a key step of experimental BP formation, and this mechanism relies on the activation or degranulation of mast cells (103). BP M2 macrophages showed a notable increase in both mRNA expression and production of CCL18 when exposed to IL-4 or IL-13 (104). Nuclear receptor related 1 belongs to the orphan nuclear receptor family and can regulate inflammation in both directions. Nuclear receptor related 1 is highly expressed in a specific group of cutaneous macrophages in patients with BP. This particular subgroup of macrophages in skin lesions is distinguished by elevated TNF levels and reduced expression of the anti-inflammatory marker CD163L1 (105).

7.2 Treatments for BP involving macrophages

Minocycline, a conventional medication for BP, has been shown to reduce the production of Th2 chemokines by M2 macrophages, thereby preventing the recruitment of Th2 cells and eosinophils to lesional skin in BP. While both CCL18 and CCL22 are Th2 chemokines implicated in BP, minocycline selectively suppresses the production of CCL18. The precise mechanism behind this selective effect remains to be elucidated (106). Dipeptidyl peptidase-4 inhibitors are associated with a higher incidence of BP. However, the concurrent use of lisinopril, a medication used to treat hypertension and heart failure, may counteract this risk. Lisinopril is capable of inhibiting the upregulation of matrix metalloproteinase and angiotensin-converting enzyme-2 in macrophages, thus exerting a mitigating effect on dipeptidyl peptidase-4 inhibitor-induced BP (107). T-cell immunoglobulin and mucin domain-3 is a well-recognized immune checkpoint molecule. Elevated levels of T-cell immunoglobulin and mucin domain 3 in macrophages within the affected skin of BP patients suggest its potential as a target for future immunotherapeutic interventions (108).

8 Melanoma

Melanomas are malignant tumors originating from melanocytes that can appear on any part of the body. Tumor-associated macrophages (TAMs) and other innate immune cells are crucial in chronic inflammatory processes that support tumor growth and advancement. M1 macrophages have immunostimulatory, anti-tumorigenic, and anti-angiogenic properties, while M2 macrophages support tumor growth and angiogenesis.

8.1 The characteristic of macrophage in melanoma

Studies have shown that invasive melanomas have a greater quantity of CD68+ and CD163+ TAMs in comparison to benign nevi (109). TAMs in melanoma are a diverse and constantly changing group, with a subset of unpolarized CD68+/CD163−/iNOS− macrophages consistently existing (110). Different stages of melanomas display distinct macrophage constituents. During the initial phase of malignant melanoma, the number of M1 intratumoral macrophages is lower than that of the M2 population. As the disease advanced, M1 macrophage recruitment was quickly and increasingly surpassed by an upsurge in M2 TAMs (111).Macrophages' function differs based on their location. Stromal macrophages have a unique transcriptional profile compared to those found in tumor nests, as they are reprogrammed to take on DC activity (112). The quantity and composition of macrophages are associated with the outcome of melanoma. High numbers of CD68+ macrophages inside tumor cell nests are linked to recurrence, while a low proportion of CD163+ macrophages in the tumor stroma is related to recurrence and, in initial melanomas, also with poor overall survival (109). The state of macrophage polarization is linked to the level of lymphocytic infiltration in melanoma, which also impacts the prognosis (110).

8.2 The pathogenic roles of the macrophages in melanoma

Increasing evidence has revealed that macrophages are implicated in melanoma migration. CD163+ macrophages found within the tumors are associated with the development of metastases (113). Angiogenesis is a crucial step in the preparation of lymph nodes for melanoma metastasis. Exosomes from melanoma cells stimulate the generation of granulocyte-macrophage colony stimulating factor in pre-metastatic lymph nodes. Granulocyte-macrophage colony stimulating factor could activate hypoxia-inducible factor (HIF)-1α in M1 macrophages and HIF-2α in M2 macrophages. HIF-1α stimulates new blood vessel formation, whereas HIF-2α contributes to the structural normalization of newly formed blood vessels (114). TAMs promoted endothelial cell movement, tube creation, and tumor development through TAM-derived adrenomedullin. Adrenomedullin possess endocrine and paracrine activities simultaneously. The paracrine effect is mediated by the endothelial NOS signaling pathway, while the autocrine effect induces macrophages to polarize toward the M2 phenotype (115).

Tumor cells and TAMs interactions are crucial for initiating tumor cell motility. TAMs can transmit cytoplasmic modules to tumor cells, enhancing tumor cell motility and dissemination (116). Another hypothesis for metastasis mentions the fusion of macrophages with tumor cells (MTFs). After being injected subcutaneously into nude mice, cultivated MTFs spread and formed metastatic tumors at remote locations. The cultivated MTFs consistently displayed pan-macrophage markers, M2 polarization markers, and melanocyte-specific markers (117). HMGB1 has a significant role in the growth and spread of murine melanoma. HMGB1 is secreted by melanoma tumor cells as a consequence of hypoxia, and could increase M2-like TAMs accumulation and an create an IL-10-rich TME (118). CD34- melanoma-initiating cells rely on M2 macrophages for their survival and growth. This discovery provides additional confirmation that macrophages play a role in the distant spread of melanoma (119).

8.3 Treatments for melanoma involving macrophages

Transitioning the polarization state of TAMs from the tumor-favoring M2 phenotype to the anti-tumor M1 phenotype is a promising strategy in oncotherapy. Chemotherapy occupies an important component position in combination treatments of melanoma. Doxorubicin-loaded polysaccharide hydrogels have demonstrated effective polarization of TAMs toward the M1 phenotype (120).

In addition to traditional chemotherapy drugs, researchers are now exploring new methods by regulating macrophage polarization to treat melanoma. TLR-7/8 agonists, such as resiquimod (RES) and telratolimod, can induce the polarization of macrophages toward the M1 phenotype. Bexarotene (BEX), a highly affinity selective retinoid X receptor, can reduce M2 polarization. A dual macrophage polarizer was created by mixing BEX with RES to enhance the M1 phenotype while inhibit the M2 phenotype. This combination exhibited incomparable inhibitory effects on B16F10 cells (121). Tumor-associated adipocyte exhibits a transformed pro-tumorigenic characteristic which can attract monocytes and stimulate their transformation into the M2 phenotype. Telratolimod is encapsulated within the lipid droplets of adipocytes and is intended to be discharged at the tumor site. Injecting drug-loaded adipocytes boosted tumor-inhibiting M1 macrophages in primary and distant tumors, halting tumor growth in a melanoma model (122). These innovative treatments have demonstrated anti-tumor effects in animal and cell models, but they have not yet been implemented in clinical practice.

9 Cutaneous T-cell lymphoma

Cutaneous T-cell lymphoma (CTCL) is a rare kind of lymphoma originating in the skin, and consists of a collection of subtypes with different clinical manifestations, histological features, and prognosis. Mycosis fungoides (MF) and Sézary syndrome (SS) are the two main types of CTCL (123). While CTCL may progress slowly in its initial stages, it can result in considerable morbidity and mortality as it proceeds (124).

9.1 The characteristic of macrophage in CTCL

A prominent subtype of M2 TAM expressing PD-1 has been found in CTCL TME, and playing an immunosuppressive role. Lenalidomide is an immunomodulatory drug typically used in treating hematological malignancies. Anti–PD-L1 combined with lenalidomide induces functional changes in TAMs, thereby enhancing phagocytic activity and impairing migration of M2-like TAMs and augmenting T cell proliferation to improve antitumor immunity. Combining anti-PD-L1 and lenalidomide treatment induces a functional transition from a PD-1+ M2 phenotype toward a proinflammatory M1 phenotype *in vitro*. Meanwhile, this transformation enhances phagocytic activity by blocking NF-κB and JAK/STAT (125).

The polarization state of macrophages in CTCL TME is not static. Granulomatous MF shows a transition of macrophage polarization from M1 in the initial phases to M2 in the later stages (126). The quantity of macrophages varies depending on the tumor stage, with a notably greater amount of CD68+ macrophages in the tumor-stage compared to early-stage folliculotropic MF (127).

Granulomatous slack skin is a very uncommon type of CTCL distinguished by a high quantity of macrophages. Macrophages in granulomatous slack skin are divided into three distinct subpopulations with unique transcript characteristics (128):

- The CD163+/CD206+ cluster displays a TAM M2-like phenotype and expresses markers involved in T-cell interaction and tumor progression.
- The apolipoprotein C1+/APOE+ cluster has a non-M1 or -M2 phenotype and may be associated with lipid metabolism.
- The CD11c+/lysozyme+ cluster demonstrates an M1-like phenotype and expresses matrix metalloproteinase-9 strongly.

9.2 The pathogenic roles of the macrophages in CTCL

The interaction between malignant T cells and macrophages is extensively studied in CTCL TME. A subtyping system has been created using the genetic characteristics of malignant T cells and the surrounding TME that promotes tumor growth (129). The interaction between malignant CTCL cells and CCL13+ macrophages has been demonstrated to promote tumor growth by increasing S100 Calcium Binding Protein A9 levels and activating NF-κB (130). Similar intercellular communications have been observed in the transformed CTCL tumor ecosystem. Malignant T cells that express MIF interact with macrophages, and B cells that express CD74 are also involved in this interaction (131).

Macrophage enrichment has a role in creating an immunosuppressive TME. Elimination of M2-like TAMs using liposomes containing clodronate (the first-generation bisphosphonate treating osteoporosis) has been demonstrated to postpone the progression of CTCL (132). Furthermore, the expressions of vascular markers also decrease by macrophage exhaustion, suggesting macrophages are implicated in both the advancement of CTCL and neoangiogenesis. CCR2 inhibitor, which hinders the movement of monocytes through CCR2, can lead to the reduction of macrophages. Mice treated with CCR2 inhibitor showed significantly reduced tumor sizes and weights compared to the control group, providing more evidence of the adverse impact of macrophages in CTCL (133).

Macrophages play a predictive role in the progression of CTCL, with the quantities of CD163+ cells in affected skin and serum sCD163 levels correlating with disease advancement (134). Another study suggests that the CD163/CD68 ratio should be used to evaluate TAMs instead of focusing on the total TAM count. A high ratio of CD163/CD68 in tumor stage MF and SS suggests M2 polarization of TAMs, which is associated with tumor advancement. Serum levels

of sCD163 and CCL22 can indicate M2 load and may serve as indicators for evaluating disease progression (135). However, there is still no consensus on the relationship between CD163+ cells with tumor progression. Some researchers suggest that the proportion of CD206+ cells, as opposed to CD163+ cells, increases in correlation with tumor advancement (136).

9.3 Treatments for CTCL involving macrophages

BEX has been authorized for the treatment of relapsed CTCL after at least one prior systemic therapy. BEX's clinical benefits are partly attributable to its ability to decrease the synthesis of CCL22 by M2 TAMs (137). IFNs are efficacious in treating advanced-stage MF, potentially by influencing M2 TAMs as well. Mechanistically, IFN-α2a and IFN-γ reduce CCL17 and CCL18 expression and synthesis, while raising CXCL10 and CXCL11 levels in M2 macrophages (138).

10 Conclusion and prospect

Numerous immune cells get involved in the pathogenesis of inflammatory skin diseases and skin tumors. In this review, we aim to understand the pathogenesis from the perspective of macrophages. Due to their complex functions and dynamic polarization states, macrophages are extensively implicated in the occurrence of AD, psoriasis, SSc, SLE, rosacea, BP, melanoma and CTCL. The mechanism of macrophages in these conditions is multifaceted, including intercellular interactions (macrophages and B cells, T cells, keratinocytes, basophils and fibroblasts), cell death (ferroptosis and pyroptosis), and cell functions (autophagy and efferocytosis). Additionally, multiple signaling pathways and molecules, such as exosomes, ILs, CCLs, CXCLs, are also involved.

In the future, we anticipate that more macrophage-related indicators can be developed to assess the disease severity, prognosis and complication occurrence and to guide more precise treatment. Furthermore, targeting the number and polarization state of the macrophages holds promise for the exploration of new therapeutic approaches. For example, M2 macrophages are considered to play immunosuppressive roles in the TME. Research may focus on depleting M2 macrophages or converting them to an anti-tumoral M1 phenotype within the TME with safe medications. The investigation of macrophages in inflammatory skin diseases and skin tumors remains a vibrant research area and we are confident that patients will benefit from these advancements in the future.

Author contributions

S-HL: Writing – original draft, Writing – review & editing. JZ: Writing – review & editing. Y-GZ: Writing – review & editing.

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Conflict of interest

The 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

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