IMMUNOLOGY OF PSORIATIC DISEASE

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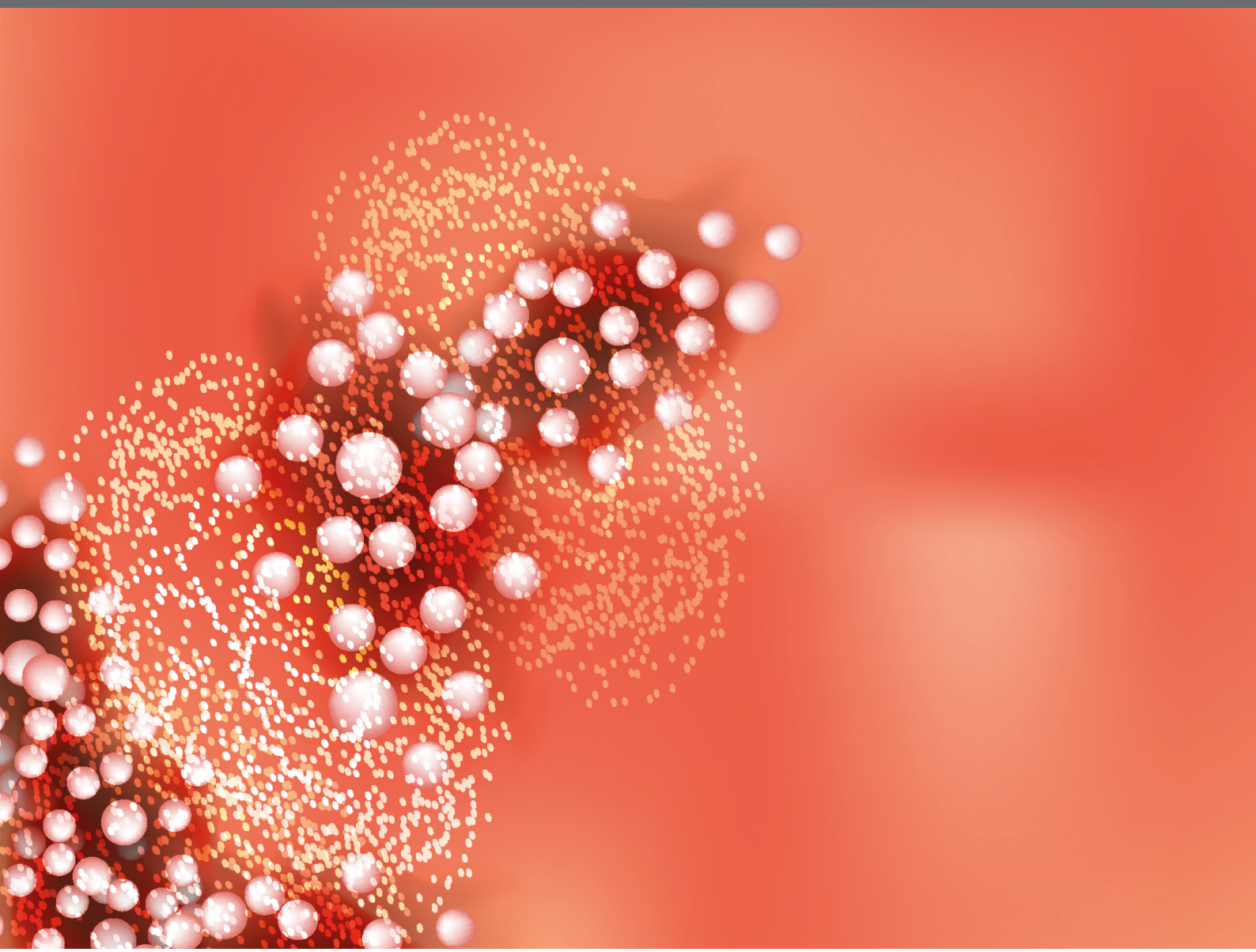

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IMMUNOLOGY OF PSORIATIC DISEASE

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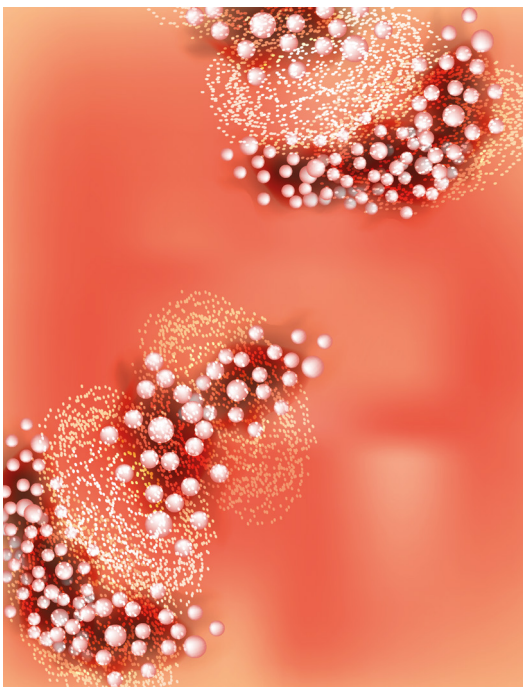

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Psoriasis is a chronically relapsing inflammatory skin disorder affecting about 2% of the worldwide population. The disease is associated with important systemic manifestations, including cardiovascular comorbidities and metabolic syndrome. In addition, about 30% of patients develop joint inflammation known as psoriatic arthritis (PsA).

Our knowledge on the pathogenesis of psoriasis has dramatically expanded in the last decade, suggesting the existence (or co-existence) of both auto-immune and auto-inflammatory components. Skin lesions develop from a complex interplay between keratinocytes, vascular endothelium, dendritic cells, and T cells, generating a self-sustaining inflammatory cycle. Within this cycle, epidermal CD8+ T lymphocytes specific for self-antigens may represent the major autoimmune mechanism.

Despite the recent progress in the comprehension of the pathogenesis of psoriasis many questions remain open, ranging from the plaque-initiating events to the characterization of the autoimmune /autoinflammatory components of the disease. The mechanisms that link cutaneous psoriasis to its extra-cutaneous and systemic manifestations also remain vague.

In this Research Topic we invited top scientists to summarize the front-line research in the field of immunology of cutaneous psoriasis and its systemic and joint manifestations.

Our intention was to integrate the pillar concepts of psoriasis immunopathology with the most novel insights, aiming at providing an advanced view of this rapidly evolving and fascinating field.

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Editorial: Immunology of Psoriatic Disease

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Keywords: psoriasis, psoriatic arthritis, metabolic syndrome, cardiovascular comorbidities, autoimmune/autoinflammatory disease, inflammation, skin immunopathology, cytokines

Editorial on the Research Topic

Immunology of Psoriatic Disease

Psoriasis is a chronic recurrent T cell-mediated inflammatory skin disease with a strong genetic predisposition. The disease is associated with joint manifestations (psoriatic arthritis, PsA), developing in about 30% of patients, and with comorbidities such as metabolic syndrome.

Genome-wide scans provided the first insight into the pathogenesis of psoriasis and showed that the HLA-C*06:02 allele in the psoriasis susceptibility locus 1 on chromosome 6 accounts for up to 50% of disease heritability. Other gene variants associated with psoriasis are involved in the IL-23/IL-17 axis, $CD8^+$ T cells differentiation, antigen processing, NF-κB/IL-1/TNF axis, and type-I interferon response (1).

The picture that emerges is that psoriasis is a complex disease with autoimmune and autoinflammatory components that involves the interplay between keratinocytes, microvascular endothelium, dendritic cells (DCs), and T cells, generating a self-sustaining inflammatory cycle around the TNF/IL-23/IL-17 axis. Epidermal $CD8^+$ T lymphocytes producing IFNγ and IL-17 may represent a major autoimmune mechanism in disease pathogenesis (2, 3).

Many questions remain unanswered and new scenarios open up based on the increasing evidence that has been recently provided. The mechanisms leading to the initial manifestations of psoriasis remain uncertain, and the exact characterization of the autoimmune and autoinflammatory responses occurring in psoriasis patients, as well as the mechanisms linking skin with extra-cutaneous and systemic manifestations await clarification.

In this Research Topic, we invited scientists to summarize the latest advances on immunological mechanisms of psoriatic disease. The topic starts with three manuscripts exploring the possible autoimmune nature of psoriasis and its extra-cutaneous manifestations. Prinz reviews the refined approach that led to the characterization of autoreactive $CD8^+$ T cells specific for melanocyte-derived ADAMTLS5 antigens presented by HLA-C*06:02, strengthening the evidence of an autoimmune component in psoriasis pathogenesis. HLA-C appears central to the promotion of organ-specific T cell responses due to its ability to present positively charged skin self-antigens.

Then, Frasca et al. describe their original research on the characterization of autoantibodies to neutrophil LL-37 antimicrobial peptide in patients presenting PsA. The authors show that autoantibodies to LL-37 are elevated in PsA synovial fluid and correlate with clinical inflammatory markers. Although antibody formation may represent a secondary effect of the priming of LL37-specific T helper cells, these findings provide new insights in the autoimmune aspects linking psoriasis and its extra-skin manifestations.

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In the next article, De Jesús-Gil et al. overview the translational studies showing microbial trigger of skin-homing CLA^+ cells. The authors provide evidence that the CLA^+ fraction of circulating T cells secrete IL-17A, IL-17F, and IL-9 when stimulated with *Streptococcus pyogenes*. In addition, they report that CLA^+ T cells in psoriasis patients respond to skin *S. pyogenes* and *C. albicans* extracts, suggesting a relationship between memory T cells and environmental microbes. Finally, the authors underline how CLA^+ T cells activated by streptococcal antigens in tonsils could migrate to the skin where they recognize keratin-derived self-antigens presenting homology with streptococcal proteins.

In this context, Casciano et al. outline the conceivable sequence of T cell-mediated events of the psoriatic inflammatory cascade. These include an initial phase in which autoreactive $CD8^+$ T cells could play a major role, a second phase involving both self-reactive and polyclonal $CD4^+$ T amplified by the IL-23/IL-17A axis, and an antigen-independent downstream recruitment of circulating $CXCR3^+$ T cells. In this view the egress of T cells from the skin and their recirculation through the blood could represent a link between cutaneous psoriasis and its systemic and joint manifestations.

The topic proceeds with four articles addressing the complex cytokine and cellular networks in psoriasis. Schön and Erpenbeck uncover the importance of the IL-23/Th17 axis in the cross-talk between innate and adaptive immune responses, including new players such as IL-17-producing innate lymphoid cells and unconventional γδT cells. The authors further analyze the T cell-neutrophil-keratinocyte loop leading to the amplification of the immune responses, particularly referring to the role of neutrophil extracellular traps. These latter may modulate the immune system by reducing the activation threshold of T cells and by favoring the presentation of neutrophil autoantigens (e.g., antimicrobial peptides) to antigen presenting cells (APCs).

Further exploring the cytokine network in psoriasis, Brembilla et al. review the presence and function of cytokines belonging to the IL-17 family, focusing on the role of isoforms other than IL-17A. The functions of IL-17F and IL-17C are highlighted, as well as a recently discovered role for IL-17E (IL-25). This latter, over-produced by keratinocyte in psoriasis, promotes the activation of macrophages, leading to the amplification of the psoriatic inflammation and the recruitment of innate immune cells, such as neutrophils, to the skin.

With reference to other keratinocyte-derived cytokines, Bridgewood et al. report a role for IL-36γ in the induction of IL-23 and TNFα by macrophages, as well as in endothelial activation and angiogenesis.

The topic continues with the manuscript from Albanesi et al., highlighting the role of keratinocytes in the establishment and amplification of the psoriatic inflammation. In response to a trigger, keratinocytes participate to the early phase of disease by releasing β-defensins, S100 proteins and antimicrobial peptides that in turn activate APCs to initiate an adaptive response. Epidermal cells may also be a source of autoantigens such as neolipids recognized by CD1a-restricted T cells and keratins cross-recognized by streptococcal-specific T cells. Once activated, keratinocytes function as amplifier of the inflammation by producing chemokines and cytokines, particularly those belonging to the IL-1 family, such as IL-36γ (see articles from Schön and Erpenbeck; Bridgewood et al.)

The next three manuscripts describe novel disease patho-mechanisms. Dolcino et al. investigate the long non-coding RNA (lncRNA) signature of PsA patients. lncRNAs control gene expression at multiple levels and are emerging as new players in autoimmune disease. The authors integrated this information with gene expression and microRNA data from the same cohort, using protein-protein interaction network and pathway enrichment analysis. They identify a restricted set of lncRNAs modulated in PsA that target genes highly expressed in the disease, such as genes involved in inflammatory response and bone remodeling. These lncRNAs were in addition involved in the modulation of lipid metabolism and mTOR pathways, suggesting their link to disease comorbidity.

Next, Chimenti et al. review the role of oxidative stress in psoriasis and PsA. A focus is given to mediators released from mast cells and mitochondrial activity (e.g., tryptase and cytochrome c), and their role as amplifiers of the inflammatory loop.

Finally, Mylonas and Conrad provide an interpretation of paradoxical psoriasis, an immunologically different form of psoriasis induced in some patient following anti-TNF treatment. In classical psoriasis, the initial release of IFNα activates myeloid DCs to produce TNFα and IL-23. TNFα subsequently maturates DCs and limits IFNα production via a negative feedback loop, leading to activation of IL-23-dependent T cell responses. In patients developing paradoxical psoriasis, the therapeutic blockade of TNF inhibits DC maturation, leading to a sustained IFNα production and the occurrence of an inflammation independent of T cell.

The three articles that follow discuss the association of psoriasis with cardiovascular comorbidity.

First, Boehncke travels through the cellular and molecular events at the basis of this association: genetic predisposition, skin inflammation, shared pathogenetic mechanisms. While all these aspects are likely involved, none explains why psoriasis might actually be regarded as an independent risk factor for cardiovascular diseases. The framework of the "psoriasis march" represents the possible missing link: psoriasis is a systemic inflammatory condition leading to insulin resistance and endothelial dysfunction, resulting in increased vascular stiffness and atherosclerosis.

Then, Sajja et al. provide a detailed view of the immunological mechanisms shared by psoriasis and atherogenesis. Activation of Th1 and dysfunction of Treg cells emerge as a main feature. Additional data sustain a role for innate immune cells, in particular the low-density granulocytes (LDGs). These cells have an enhanced capacity to from extracellular traps, which represents a source of autoantigens on one hand, and a direct cause of endothelial injury and cardiovascular plaque rupture on the other hand.

Therapies for psoriasis targeting these shared mechanisms may potentially prevent the rate of cardiovascular events. Treatments aimed at reducing inflammation, such as methotrexate and IL-1β neutralization, provided a proof of

this concept. In this context, Caiazzo et al. show that none of the recently-licensed biologics (e.g., anti-IL-12/23 and anti-IL-17 agents) significantly impacts the cardiovascular risk, despite the analysis is biased by a short follow-up period. Overall, these reviews underline that an intimate link actually exists between psoriasis and cardiovascular diseases. This justifies a comprehensive approach to the management of psoriasis, including proper advice and a regular screening and monitoring for cardiovascular factors.

The next three manuscripts explore novel therapeutic avenues. As of now, inhibition of IL-17A proved to be the most effective approach, demonstrating the key role of Th17 cells in clinical settings. Tang et al. embrace the rationale of inhibiting the development of Th17 cells (with logic similar to that behind the use of anti-IL-23 agents), rather than neutralizing IL-17A. This could be more beneficial since it would result in the inhibition of multiple pro-inflammatory cytokines. The originality of their vision relies on the use of small molecule antagonist targeting RORγt, the master regulator of Th17 cells.

Besides targeting Th17 cells, the possibility of restoring the functionality of Treg cells for therapeutic purpose may represent an attractive idea. Chen et al. report that the beneficial effect of PSORI-CM02 formula, a traditional Chinese medication based on the combination of five herbs, relies on the amplification of Treg in disfavor of Th17 cells.

In the last article of the collection, Buerger approaches the question of the therapeutic intervention from a different angle, and drives the reader into the intracellular epidermal processes induced by the immunological network. Focus of the review is the role of the mTORC1 cascade as regulator of the epidermal homeostasis, independently of its known function as immune-modulator. While active during cellular proliferation, keratinocytes need to switch off the mTORC1 pathway to proceed into terminal differentiation and cornification, partially via the activation of an autophagy response. Cytokines such as IL-17A and TNF aberrantly activate mTORC1 resulting in hyperproliferation and the arrest of the differentiation process. In this respect, mTOR could be seen as a novel therapeutic target for psoriasis.

In conclusion, this collection well proves how our comprehension of the patho-physiology of psoriasis has radically changed in the last decade. Constantly more gaps are getting filled, and multiple novel mechanistic insights added to our previous knowledge, spanning from the autoimmune nature of the disease to the possible causes underlying extra-cutaneous manifestations and comorbidity. A hard work awaits however scientists and clinicians in the future, as patient deserve increasingly more specific and efficacious therapeutic or even curative options.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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MINI REVIEW
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Human Leukocyte Antigen-Class I Alleles and the Autoreactive T Cell Response in Psoriasis Pathogenesis

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Psoriasis is a complex immune-mediated inflammatory skin disease characterized by T-cell-driven epidermal hyperplasia. It occurs on a strong genetic predisposition. The human leukocyte antigen (HLA)-class I allele *HLA-C*06:02* on psoriasis susceptibility locus 1 (PSORS1 on 6p21.3) is the main psoriasis risk gene. Other HLA-class I alleles encoding HLA molecules presenting overlapping peptide repertoires show associations with psoriasis as well. Outside the major histocompatibility complex region, genome-wide association studies identified more than 60 psoriasis-associated common gene variants exerting only modest individual effects. They mainly refer to innate immune activation and the interleukin-23/$T_{h/c}$17 pathway. Given their strong risk association, explaining the role of the HLA-risk alleles is essential for elucidating psoriasis pathogenesis. Psoriasis lesions develop upon epidermal infiltration, activation, and expansion of $CD8^+$ T cells. The unbiased analysis of a paradigmatic Vα3S1/Vβ13S1-T-cell receptor from a pathogenic epidermal $CD8^+$ T-cell clone of an *HLA-C*06:02*$^+$ psoriasis patient had revealed that HLA-C*06:02 directs an autoimmune response against melanocytes through autoantigen presentation, and it identified a peptide form ADAMTS-like protein 5 as an HLA-C*06:02-presented melanocyte autoantigen. These data demonstrate that psoriasis is an autoimmune disease, where the predisposing HLA-class I alleles promote organ-specific inflammation through facilitating a T-cell response against a particular skin-specific cell population. This review discusses the role of HLA-class I alleles in the pathogenic psoriatic T-cell immune response. It concludes that as a principle of T-cell driven HLA-associated inflammatory diseases proinflammatory traits promote autoimmunity in the context of certain HLA molecules that present particular autoantigens.

Keywords: psoriasis, pathogenesis, autoreactive T cells, human leukocyte antigen association, HLA-C*06:02, T-cell receptor, autoimmunity, autoantigens

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INTRODUCTION

Psoriasis is a complex T-cell mediated skin disease. Skin lesions are characterized by sharply demarcated heavily scaling inflammatory plaques which result from T-cell driven epidermal hyperplasia. T cells infiltrating psoriasis skin lesions display a T-helper/cytotoxic cell ($T_{h/c}$) 17 phenotype producing the $T_{h/c}$17 signature cytokines interleukin (IL)-17A, IL-22, and IFN-γ (1, 2). They promote keratinocyte proliferation, accumulation of neutrophilic granulocytes, and the production of antimicrobial peptides and other inflammatory cytokines and chemokines. Activation and differentiation

Abbreviations: TCR, T-cell receptor; GWAS, genome-wide association study; HLA, human leukocyte antigen; MHC, major histocompatibility complex; ADAMTSL5, ADAMTS-like protein 5.

of the lesional psoriatic $T_{h/c}17$ response is maintained by IL-23 that is produced by local dendritic cells and keratinocytes. By today, blocking IL-17A or IL-23 represents the most efficient treatment modality (3).

Psoriasis is multifactorial and involves the interaction of individual genotypes with environmental, infectious, and lifestyle factors. The human leukocyte antigen (HLA)-class I allele *HLA-C*06:02* is the main psoriasis risk gene (4–6). Functional clustering of common variants associated with psoriasis highlighted the roles of interferon signaling and the NF-κB cascade and of regulatory elements related to $CD4^+$ and $CD8^+$ T cell maturation, development, and activation including the IL-23 pathway and $T_{h/c}17$ differentiation (7). Thus, understanding psoriasis pathogenesis has to explain the role of HLA-C*06:02 within the complex genetic background predisposing to psoriasis. Therefore, this review will focus on the functional implications of the main HLA psoriasis risk gene, *HLA-C*06:02*.

HLA-CLASS I ASSOCIATION OF PSORIASIS: THE MAIN GENETIC RISK

The most significant association signal observed in psoriasis genome-wide association studies that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ (8) was observed within the major histocompatibility complex (MHC) region (7, 9–13). The highly polymorphic nature and density of genes and the extensive linkage disequilibrium that exists within the MHC, however, had initially hampered the identification of the causal gene that confers psoriasis susceptibility in the HLA region. Sequence-based methods in large samples finally proved *HLA-C*06:02* as the main psoriasis risk gene (4, 6). *HLA-C*06:02* defines early onset, severity and familial clustering of psoriasis (14–16). Unlike HLA-class II-associated autoimmune diseases psoriasis shows no evidence of interactions between different HLA alleles. *HLA-C*06:02* contributes a non-additive risk effect and represents a true HLA-class I risk gene (17).

Interpreting HLA associations has to consider that the frequency of alleles can differ between populations. The prevalence and incidence of psoriasis shows ethnic and geographic variations. A relatively high prevalence of psoriasis in European countries and in the USA (0.5–6.5%) contrasts with a low prevalence in East Asian countries (0.2–0.3%) (18, 19). Given the strong risk effect of *HLA-C*06:02*, the ethnic allele frequency spectra of *HLA-C*06:02* may at least partially explain the heterogeneity of psoriasis in different ethnic populations. This highlights the need to use population-specific reference panels made by deep sequencing to impute MHC alleles and amino acids (8). The strong psoriasis risk of *HLA-C*06:02* allele has been validated in worldwide populations including Europeans (17, 20), East (6, 21), and South Asians (22), with odds ratios of as high as 3.0–10.0. HLA fine-mapping analysis using the HLA imputation method successfully identified multiple other less obviously associated HLA-class I and class II variants that confer psoriasis risk independently from *HLA-C*06:02* (20). Aside from *HLA-C*06:02* psoriasis associated with *HLA-C*12:03*, *HLA-C*07:01*, *HLA-C*07:02*, *HLA-C*07:04*, *HLA-B*27*, and *HLA-B*57*. Further associations were seen with *HLA-B* amino acid positions 9, 67, and 116, *HLA-A* amino acid 95, and *HLA-DQα1* amino acid position 53 which are all localized within the HLA antigen binding region (6, 20, 21, 23). Although *HLA-C*06:02* and the AA position 67 of *HLA*-B are shared between Caucasian and Chinese populations, other independent HLA-risk variants differ between the two populations. *HLA-A*02:07*, which corresponds to the cysteine residue at HLA-A position 99, shows a strong association in Chinese but is very rare or absent in Europeans, whereas *HLA-B*07* shows a strong association in Caucasians while it is very rare in Chinese. The other HLA variants are common in both Caucasian and Chinese, but show population-specific associations, *HLA-A*02:01* for Caucasian and the AA positions 114 and 144 of HLA-A for Chinese. These population-specific effects contribute significantly to the ethnic diversity of psoriasis prevalence (12).

The Japanese population has unique characteristics. Psoriasis prevalence in Japan is one of the lowest compared with worldwide populations (0.1–0.3%) (24–27), and *HLA-C*06:02* is almost absent within the Japanese population (<0.5%) (21). Due to low allele frequency in the Japanese population, the impact of *HLA-C*06:02* on psoriasis susceptibility in Japanese psoriasis patients was less apparent compared with that seen in other populations (2.3% in psoriasis cases and 0.4% in controls). Still, a Japanese-specific reference panel showed increased odds ratios for *HLA-C*06:02*, *HLA-C*12:02*, and *HLA-C*07:04* in the Japanese psoriasis population (21).

Interestingly, several of the psoriasis-associated HLA-class I alleles are also significantly increased in Crohn's disease (*HLA-C*06:02*, *HLA-C*12:02*) and ulcerative colitis (*HLA-C*12:02*, *HLA-C*07:02*) (28). *HLA-B*27* predisposes for ankylosing spondylitis, inflammatory bowel disease, and psoriasis arthritis, creating an overlapping HLA-class I risk pattern although no autoantigens have been identified in these diseases.

FUNCTIONAL ASPECTS OF HLA-CLASS I ALLELES IN PSORIASIS

Genetic variation in HLA genes within the MHC locus is associated with many immune-mediated inflammatory diseases (IMIDs): virtually any autoimmune condition is associated with particular HLA-class I or class II alleles (29–31). For most of these diseases, the HLA association explains more disease risk than any other gene locus. While IMIDs share many of the non-HLA loci, the associated HLA-class I and/or class II alleles are usually disease specific (32). This attributes the HLA alleles with a high degree of disease specificity and pleads for a direct causal role in the pathogenesis of the respective IMID. The association with *HLA-C*06:02* in psoriasis is particularly intriguing because only 3 of more than 12,000 different HLA-class I alleles show a strong disease linkage: *HLA-B*27* with ankylosing spondylitis, *HLA-B*51* with Behçet's disease, and HLA-*C*06:02* with psoriasis.

Human leukocyte antigen-class I molecules are expressed by all nucleated cells. They present peptide antigens of usually 8–10 amino acids to αβ T-cell receptors (TCRs) of $CD8^+$ T cells (33). The antigenic peptides are derived from cytoplasmic proteins, i.e., proteins produced within the cells. They are processed from the

parent proteins by the proteasome for loading into the peptide-binding groove of the HLA-class I molecules. The complex of peptide and HLA-class I molecule is transported to the cell membrane for recognition by $CD8^+$ T cells (34, 35). Accordingly, an HLA-class I-restricted immune response must be directed against a particular target cell which expresses the antigenic protein within the cytoplasm.

Human leukocyte antigen alleles are extremely polymorphic. Sequence variation is concentrated in the α1 and α2 domains that contain the binding sites for peptide antigens and interact with the TCR. HLA polymorphisms result in variable peptide-binding grooves. They contain two or three specific acceptor sites or pockets which bind specific amino acid side chains of peptides and thus define the spectrum of antigenic peptides a particular HLA molecule can present (35, 36). Due to the diverse acceptor sites, different HLA molecules select different peptide repertoires for presentation (37, 38), although the binding specificities may overlap (39, 40). Because the peptide residues in between the anchors may be flexibly occupied a single HLA-class I molecule can theoretically display between $6 \times 20^{5-7}$ different decamer peptides (36, 41, 42).

The amino acid motifs of peptides presented by HLA-C*06:02 and several other HLA-class I molecules have recently been characterized in detail (43–45). Nonamer peptides presented by HLA-C*06:02 show select amino acids at the anchor residues 2 and 9, and a potentially secondary anchor at residue 7 (**Table 1**). The dominant amino acids are arginine at residue 2, leucine, valine, and less preferred isoleucine and methionine at residue 9, and arginine at residue 7. As a particular feature, HLA-C*06:02 has very negatively charged pockets defining peptides with large positively charged amino acids at residue 2 (B-pocket) and 7 (E-pocket) and thus selects a distinct repertoire of positively charged peptides. The studies of Di Marco et al. (43) and Mobbs et al. (44) further provided an estimate of the spectrum of self-peptides presented by HLA-C*06:02 and other HLA-C molecules. Depending on the experimental approach peptide elution identified between 1,000 and 3,000 different self-peptides from HLA-C*06:02 expressed in a B-cell line (43, 44). Thus, a substantial part of the cellular proteome should be immunologically visible to $CD8^+$ T cells (44).

According to the peptide-binding pattern *HLA-C*06:02* was assigned to the same HLA supertype as other psoriasis-associated HLA alleles including *HLA-C*07:01*, *HLA-C*07:02*, and *HLA-B*27* (43). All of them utilize the same anchor residues and present peptide repertoires that may partly overlap (**Table 1**) (43–45). HLA-C*06:02 further shares the strong negative charge of the E-pocket with HLA-C*12:03 (44) which has several similar functional domains and peptide-binding pockets as HLA-C*06:02 (46) and constitutes another potential HLA-risk allele for psoriasis and psoriatic arthritis (20, 23). Thus, several psoriasis-associated HLA-class I molecules have overlapping peptide-binding properties and might replace each other in conferring psoriasis risk. Due to the strength of association, *HLA-C*06:02* may be considered the representative HLA-risk allele within this spectrum and should therefore be particularly suitable for analyzing the role of HLA in psoriasis pathogenesis.

TABLE 1 | Amino acids at anchor residues 2 and 9 of peptide antigens and ADAMTS-like protein 5 (ADAMTSL5) presented by psoriasis-associated human leukocyte antigen (HLA) molecules.

HLA molecule	Residue 2	Residue 9
HLA-C*06:02	R/Y/K[a]	L/V/I/Y/F/M
HLA-C*07:01	R/T/N	L/F/Y/M
HLA-C*07:02	R/Y/K	L/Y/F/M
HLA-B*27	R	L
ADAMTSL5 peptide	R	L

[a]*One letter amino acid code; font size approximately reflects the relative frequency of the respective amino acid observed at this position in peptides eluted from the HLA molecules according to Ref. (44–45).*

THE TARGET CELL AND AUTOANTIGENS OF AN HLA-C*06:02-RESTRICTED PATHOGENIC PSORIATIC T CELL RESPONSE

Heritability is an estimation of how much variation in a disease can be explained by particular genetic variants. Genetic information then has to be combined with functional analysis to allow for a precise definition of the particular role of certain genes in disease. The actual immunological role of HLA-class I molecules suggests that HLA-C*06:02 may predispose to psoriasis by presentation of an autoantigen from a skin-specific cell population. Identification of target cell and potential autoantigens of the psoriatic immune response therefore appeared as the major challenge for clarifying psoriasis pathogenesis.

This approach requires the TCRs of the pathogenic T-cell response. The paired α- and β-chains of TCRs define both HLA restriction and fine peptide specificity of a T cell (47). Because of the high diversity of the human TCR repertoire any two T cells expressing the same αβ-TCR heterodimer likely arose from a common progenitor T cell (48). In response to antigen stimulation, T cells become activated and undergo clonal expansion at the site of antigen exposure. In the obvious absence of infections, expanded TCR clonotypes are commonly viewed to characterize those T cells relevant for the pathogenesis of IMIDs (48, 49).

Psoriasis lesions develop upon epidermal infiltration and activation of $CD8^+$ T cells (50, 51). Marked oligoclonality of the T-cell populations within psoriatic skin lesions indicated that psoriatic T-cell activation is driven by locally presented antigens (52–56). Lesional T-cell clones were strictly associated with psoriatic skin lesions and reappeared in relapsing psoriasis (52, 56). T-cell clonality was definitely proven by single cell TCR analysis and particular evident for $CD8^+$ T cells in lesional epidermis (52, 54). Accordingly, the clonal TCRs likely characterized those $CD8^+$ T cells which mediate psoriatic inflammation. To identify potential targets of the pathogenic psoriatic T-cell response we expressed the paired α- and β-chains of clonal $CD8^+$ T cells from the psoriatic T-cell infiltrate along with human CD8 α and β chains in a T-hybridoma cell line that reports on TCR signaling through the expression of super green fluorescence protein under control of NFAT (**Figure 1**) (57, 58). Consequently, these TCR hybridomas carried the specificity of the pathogenic psoriatic

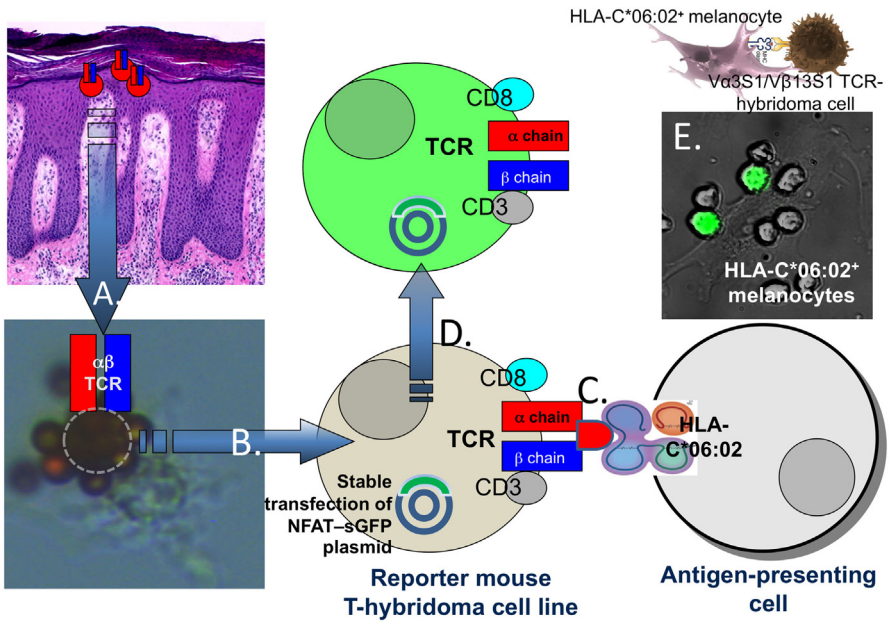

FIGURE 1 | Proof of the HLA-C*06:02-restricted autoimmune response against melanocytes in psoriasis. Following separation of epidermis and dermis of lesional biopsies from HLA-C*06:02$^+$ psoriasis patients CD8$^+$ T cells (A) were isolated from epidermal cell suspensions using magnetic beads coated with CD8 antibodies. The arrow "A" points to a CD8$^+$ T cells (encircled) isolated from lesional psoriatic epidermis which is rosetted by magnetic beads with a melanocyte attached. T-cell receptor (TCR) α and β chain mRNA of single T cells was transcribed into cDNA and sequenced by a newly developed method for single cell TCR analysis (54), cloned into expression plasmids and (B) expressed in a TCR$^{\alpha-\beta-}$ mouse reporter T-hybridoma cell line stably transfected with a plasmid for super green fluorescent protein under control of NFAT (57–59). The TCR hybridoma cells were cocultured with various primary cell types or cell lines either positive or negative for HLA-C*06:02 (C). Upon TCR ligation, the hybridoma cells produce green fluorescent protein which was visualized by UV-fluorescence microscopy or FACS analysis (D). (E) Shows the activation of the Vα3S1/Vβ13S1-TCR hybridoma by spindle-shaped HLA-C*06:02$^+$ primary melanocytes in a coculture experiment.

T cells and allowed for an unbiased, i.e., hypothesis-free analysis of the immunologic targets of the pathogenic psoriatic immune response.

Using a paradigmatic Vα3S1/Vβ13S1-TCR from an epidermal CD8$^+$ T-cell clone of an *HLA-C*06:02*-positive psoriasis patient, we could identify melanocytes as HLA-C*06:02-restricted target cells of the psoriatic immune response (**Figure 1**) (59). By means of plasmid-encoded peptide libraries, we then determined the amino acid pattern of HLA-C*06:02-presented peptide ligands of the Vα3S1/Vβ13S1-TCR. Nonamer peptides that ligated the Vα3S1/Vβ13S1-TCR displayed arginine at residues 2 and 7, and leucine at residue 9 and thus corresponded to the conserved amino acid pattern which is preferentially presented by HLA-C*06:02 and other psoriasis-associated HLA-class I molecules (**Table 1**) (43–45). Screening the human proteome and the transcriptome of melanocytes with this particular amino acid pattern identified several peptides from natural human proteins which ligated the Vα3S1/Vβ13S1-TCR. Only a peptide from ADAMTS-like protein 5 (ADAMTSL5), however, was immunogenic in the context of the full-length parent protein and unlike the other peptides could be generated through proteasomal cleavage and NH_2-terminal ERAP1 trimming. Knock-down and mutation experiments finally confirmed the role of ADAMTSL5 as melanocyte autoantigen (59). Blood lymphocytes of more than two-thirds of psoriasis patients but not healthy controls responded to ADAMTSL5 stimulation by production of the psoriasis key cytokines, IL-17 or IFN-γ (59). These data proved psoriasis as a true T-cell mediated autoimmune disease. They indicate that HLA-C*06:02 predisposes to psoriasis by mediating an autoimmune response against melanocytes through autoantigen presentation. The pathogenic psoriatic Vα3S1/Vβ13S1-TCR now represents a unique opportunity for understanding the immunopathogenesis not only of psoriasis but also of mechanisms of autoimmunity in general, since it is still unique in medical research: In no other autoimmune disease a similar approach has yet been successful to identify target cells and autoantigens.

The unbiased analysis of a pathogenic psoriatic TCR differs from other hypothesis-driven approaches for the identification of psoriatic autoantigens. Some of them were based on sequence homologies between proteins from keratinocytes and *S. pyogenes* (60, 61), a major infectious psoriasis trigger (62). The pleiotropic multifunctional 37 amino acid molecule LL37, which is generated by extracellular cleavage of the C-terminal part of the 170 amino acid Cathelicidin antimicrobial peptide (63) was proposed a potential autoantigen because LL37 peptides induced strong T-cell responses in psoriasis (64). Verification of the potential autoantigenic character for all these potential autoantigens was based on peptide stimulation assays. Several of the candidate peptides had been chosen according to HLA-C*06:02 anchor motifs. The insights into TCR polyspecificity, however, would predict that peptides designed this way will likely induce T-cell activation irrespective of pathogenic relevance (42). Furthermore, some

of the proposed LL37 peptides did not contain the appropriate anchor amino acid residues for binding to HLA-C*06:02 (44). Without confirming that an HLA-class I-presented peptide can be generated from the parent protein by antigen processing and presentation pathways within the target cell, a role as autoantigen for $CD8^+$ T cells should therefore be interpreted with care.

DISCUSSION

Defining the functional role of the main psoriasis risk gene, *HLA-C*06:02*, allowed for re-defining the architecture of the pathogenic psoriatic immune response. It proposes an HLA-centered pathogenetic model for psoriasis and other HLA-associated IMIDs where a particular HLA allele represents the causal risk gene (65). In psoriasis, HLA-C*06:02 facilitates a T-cell mediated skin-specific autoimmune response. The identification of melanocytes as organ-specific autoimmune target cells of an HLA-C*06:02-restricted immune response and of ADAMTSL5 as a melanocytic autoantigen using a pathogenic psoriatic Vα3S1/Vβ13S1-TCR provided direct experimental evidence for the autoimmune nature of psoriatic inflammation, and it explained why psoriatic inflammation primarily affects the skin (66). The predisposing HLA allele appears as an essential precondition for a tissue- and antigen-specific autoimmune response by its capacity for presentation of select autoantigens. By itself, however, autoantigen presentation is likely not sufficient for causing disease onset but requires the additive effects of common gene variants in genes which provide the costimulatory signals for activation of the actual autoimmune response. The list of common risk gene variants in psoriasis affecting proinflammatory pathways, peptide epitope trimming, IL-23 signaling and $T_{h/c}17$ differentiation is long. It includes genes related to type I interferon signaling (*ELMO1*, *TYK2*, *SOCS1*, *IFIH1/MDA5*, *RNF114, IRF4*, *RIG1/DDX58*, *IFNLR1/IL28RA*, and *IFNGR2*), activation of NF-κB pathways (*TNFAIP3*, *TNIP1*, *TYK2*, *REL, NFkBIA*, *CARD14*, *CARM1*, *UBE2L3*, and *FBXL19*), N-terminal antigen trimming (*ERAP1*), $CD8^+$ T-cell differentiation (*ETS1*, *RUNX3*, *TNFRSF9*, *MBD2*, and *IRF4*), and the IL-23/IL-17A axis (*IL23R*, *IL12B*, *IL12RB*, *IL23A*, *IL23R*, *TYK2*, *STAT3*, *STAT5A/B*, *SOCS1*, *ETS1*, *TRAF3IP2*, *KLF4*, and *IF3*). These genetic traits may augment responsiveness of innate immune mechanisms, provide a proinflammatory environment, and generate sufficient costimulatory signals which may finally exceed the thresholds for activation, differentiation and maintenance of the pathogenic autoreactive T-cell response in psoriasis (65, 66). Overall, these insights support that proinflammatory genetic traits may promote autoimmunity in the presence of the appropriate HLA molecules which present a particular autoantigen.

AUTHOR CONTRIBUTIONS

JP has drafted and written the manuscript.

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Anti-LL37 Antibodies Are Present in Psoriatic Arthritis (PsA) Patients: New Biomarkers in PsA

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Psoriatic arthritis (PsA) is a chronic inflammatory arthritis associated with psoriasis. A third of psoriatic patients develop PsA *via* unknown mechanisms. No reliable diagnostic markers are available for PsA, or prognostic biomarkers for PsA development in psoriasis. We previously uncovered a pro-inflammatory role for cathelicidin LL37 in lesional psoriasis skin. LL37 binds nucleic acids and stimulates plasmacytoid/myeloid dendritic cells (pDC, mDCs) to secrete type I interferon (IFN-I) and pro-inflammatory factors. LL37 becomes an autoantigen for psoriatic Th1-Th17/CD8 T cells. Anti-LL37 antibodies were detected in systemic lupus erythematosus, an autoimmune disease characterized by neutrophil-extracellular-traps release (NETosis) in target organs. LL37 can be substrate of irreversible post-translational modifications, citrullination or carbamylation, linked to neutrophil activity. Here we analyzed inflammatory factors, included LL37, in PsA and psoriasis plasma and PsA synovial fluids (SF)/biopsies. We show that LL37 (as a product of infiltrating neutrophils) and autoantibodies to LL37 are elevated in PsA, but not OA SF. Anti-LL37 antibodies correlate with clinical inflammatory markers. Anti-carbamylated/citrullinated-LL37 antibodies are present in PsA SF/plasma and, at lower extent, in psoriasis plasma, but not in controls. Plasma anti-carbamylated-LL37 antibodies correlate with PsA (DAS44) but not psoriasis (PASI) disease activity. Ectopic lymphoid structures, and deposition of immunoglobulin-(Ig)G-complexes (IC) co-localizing with infiltrating neutrophils, are observed in PsA and not OA synovial tissues (ST). Activated complement (C5a, C9), GM-CSF and IFN-I are up-regulated in PsA and not OA synovia and in PsA and psoriasis plasma but not in HD. C9 and GM-CSF levels in PsA SF correlate with clinical inflammatory markers and DAS44 (C9) and with anti-carbamylated/citrullinated-LL37 antibodies (GM-CSF and IFN-I). Thus, we uncover

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a role for LL37 as a novel PsA autoantibody target and correlation studies suggest participation of anti-LL37 antibodies to PsA pathogenesis. Notably, plasma antibodies to carbamylated-LL37, which correlate with DAS44, suggest their use as new disease activity markers. GM-CSF and complement C5a and C9 elevation may be responsible for autoantigens release by neutrophils and their modification, fueling inflammation and autoreactivity establishment. Finally, targeting GM-CSF, C5a, C9 can be beneficial in PsA.

Keywords: Psoriatic arthritis, psoriasis, LL37, autoantibodies complement, neutrophils

INTRODUCTION

Psoriasis is a systemic inflammatory and autoimmune skin disease of unclear etiology affecting 1–3% of individuals worldwide (1). Red and scaly plaques caused by the hyperproliferation of skin epithelial cells characterize plaque psoriasis, the most common form (1). Notably, up to 30% of psoriasis patients develop Psoriatic arthritis (PsA) (2), a type of spondyloarthritis characterized by enthesitis, dactylitis peripheral arthritis and axial involvement. Diagnostic criteria of PsA are primarily clinical and based on the Classification Criteria for Psoriatic Arthritis (CASPAR) and include evidence of psoriasis, absence of rheumatoid arthritis (AR), and the exclusion of other seronegative arthritis (3). Thus, no reliable serological markers are available to identify PsA, as for RA. Histologically, PsA is characterized by lining layer hyperplasia, innate immune cell activation, T and B-lymphocytes infiltrated synovial tissues, and synovial angiogenesis (4). As for RA, a role of B cells and autoantibodies has been suggested in the pathogenesis of PsA (5). Indeed, although the presence of rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) are characteristic of RA, and uncommon in PsA (6), the detection of ectopic lymphoid structures in PsA synovia (7) suggests production of antibodies against local autoantigens. Notably, anti-carbamylated peptide autoantibodies (anti-CarP Abs) have been recently identified not only in RA (8), but also PsA plasma (9). Currently, no reliable diagnostic biomarkers distinguish PsA from psoriasis and no prognostic markers are available to predict the development of PsA in psoriasis patients.

Cationic antimicrobial peptides (AMP) including the cathelicidin LL37 are aberrantly produced by psoriatic keratinocytes and released by degranulating neutrophils or during neutrophil extracellular trap formation (NETosis) (10–12). LL37 has the ability to bind nucleic acids and induce the production of pro-inflammatory cytokines and type I interferon (IFN-I), by plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs) via TLR7/8/9 triggering (13, 14). Moreover, LL37 has been recognized as self-antigen for psoriatic autoreactive T-cells that are detected in circulation or in lesional skin (15). Both CD4 and CD8 T lymphocytes respond to LL37 and LL37-specific CD4 T cells belong to Th1/Th17 sub-populations. Interestingly, in a systemic autoimmune disease such as systemic lupus erythematosus (SLE), where LL37-DNA complexes are highly released during NETosis, LL37 becomes the target of pathogenic autoantibodies (12, 16). Although neutrophils can infiltrate psoriatic skin (17), and a study suggests that NETosis is possibly occurring in skin lesions (18), whether LL37 becomes the target of autoantibodies in psoriasis patients has not been investigated. Notably, there are few reports that show expression of LL37 in inflamed synovia (19). However, whether and how LL37 plays a role as autoantigen in PsA is still unknown.

In this picture, with the idea to: (1) identify the pathogenic players in PsA, (2) discriminate the immunological pathways that are in common or are distinct between psoriasis and PsA, and (3) identify new disease activity markers for PsA, we have investigated the presence of LL37 and related autoantibodies in PsA and psoriasis patients and analyzed their correlations with clinical parameters and inflammatory factors.

RESULTS

LL37 and Autoantibodies to LL37 Are Present in PsA Synovia

In order to investigate the putative role of LL37 in PsA, we firstly analyzed whether LL37 was measurable in the synovial compartments. As shown in **Figure 1A**, higher levels of LL37 were detected in the synovial fluids (SF) of PsA (median: 0.153, IQR: 0.114) compared to control osteo-arthritis (OA) patients (median: 0.1, IQR: 0.036), $p = 0.031$, by ELISA assay. Laser scanner confocal microscopy of synovial biopsies from patients affected by early PsA showed a consistent staining for LL37, coupled to high staining for myeloperoxidase (MPO), the typical marker of neutrophils, in the lining and sub-lining areas of the synovial membranes (**Figure 1B**). This suggested that LL37 was present in PsA synovial tissues (ST) as the product of neutrophils, although a contribution of other cells might not be excluded. In contrast, only occasionally neutrophils and LL37 positivity were detectable in control OA synovia (**Figure 1B**). Neutrophil-derived antimicrobial peptides, including LL37, have been shown to become target of circulating autoantibodies in SLE patients (12, 16) and, to date, one study reported ectopic lymphoid tissues in PsA synovia (7). Thus we assessed the presence of anti-LL37 antibodies in SF of PsA and control OA patients. Although the antibody levels did not reach a statistical significance between PsA (median: 0.348, IQR: 0.233) and OA SF (median: 0.26, IQR: 0.126), $p = 0.28$, by setting a cut-off (as in **Figure 1A**) we found that autoantibodies to LL37 were present in 7 out of 19 PsA SF (37%) (**Figure 2**).

It is reported that LL37 can become a substrate for post-translational modifications such as citrullination and carbamylation (20, 21). Of note, carbamylated proteins can be the

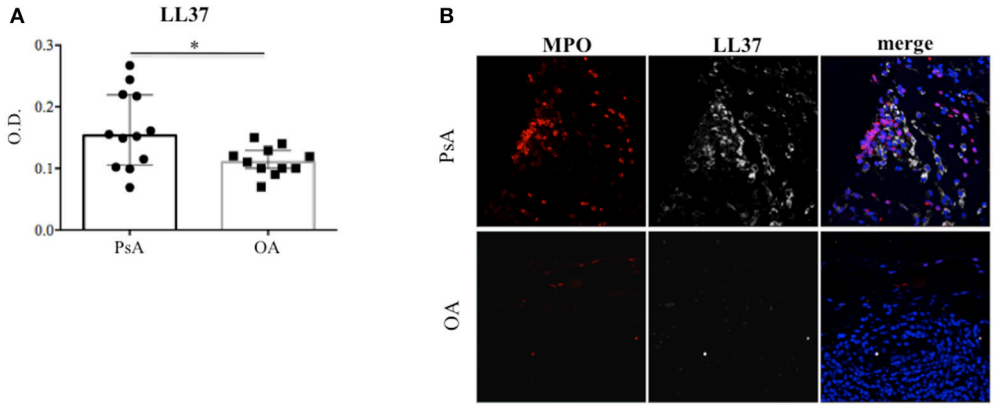

FIGURE 1 | LL37 is expressed in synovial compartment of PsA. **(A)** LL37 was measured by ELISA in synovial fluids of PsA ($N = 12$) and control OA patients ($N = 11$), and LL37 levels are shown as median with Interquartile Range (IQR). P-value is calculated by two-tailed Mann-Whitney U test $^{*}p < 0.05$. **(B)** Confocal microscopy images of synovial tissues of PsA and OA patients stained for myeloperoxidase (MPO; red), LL37 (gray) (original magnification 63x). For PsA, 1 representative staining of 7 patients is shown. For OA, 1 representative staining of 4 patients is shown.

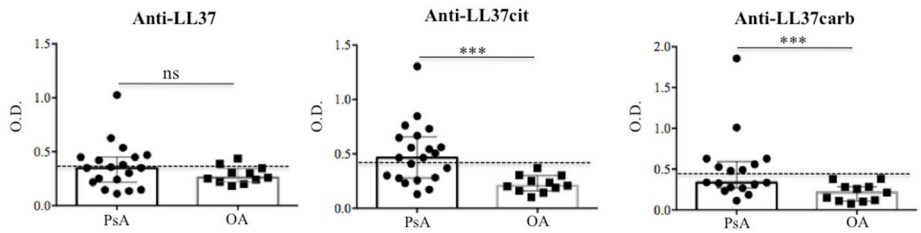

FIGURE 2 | Anti-LL37 antibodies are present in synovial fluids of PsA. Synovial fluids of PsA and OA ($N = 11$) patients were analyzed by ELISA for the presence of anti-LL37 (PsA, $N = 19$), anti-LL37cit (PsA, $N = 21$) and anti-LL37carb (PsA, $N = 17$). Antibody levels are shown as median with Interquartile Range (IQR). *P*-value is calculated by two-tailed Mann-Whitney U test, $^{***}p < 0.0001$. The mean+2 SD (standard deviation) of OA antibody reactivity to native LL37 or modified LL37 was used as cut-off (dotted line).

targets of autoantibodies in PsA (9). In this context, we wondered whether we could also detect antibody reactivity toward either citrullinated (LL37cit) or carbamylated (LL37carb) LL37 or both in PsA SF.

Interestingly, anti-LL37cit antibodies were higher in PsA SF (median: 0.464, IQR: 0.379; 12 out of 21: 57%) than in OA SF (median: 0.207, IQR: 0.14), $p = 0.0008$; similarly, anti-LL37carb antibodies were higher in PsA SF (median: 0.335, IQR: 0.321; 8 out of 17: 47%) than in OA SF (median: 0.214, IQR: 0.174), $p = 0.004$, (**Figure 2**). Of note, there was no significant correlation between anti-LL37 antibody reactivity to native and either citrullinated or carbamylated LL37, suggesting that anti-LL37cit and anti-LL37carb antibody reactivity is probably not due to cross-reactivity but is likely specifically directed toward modified LL37. This indicates that pathways of protein modification are likely to be activated in PsA synovia. Next, we investigated possible correlation between anti-LL37-autoantibody reactivity and clinical parameters such as inflammatory markers and disease activity (DAS44). We found that levels of anti-LL37 antibodies to native protein correlated with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), swollen joints and DAS44 (**Figure 3**). In contrast, no significant correlation was observed between the same clinical data and antibodies to LL37cit or LL37carb. Altogether, these results suggest that LL37 can become an autoantigen in PsA and the antibody response to LL37 in SF may represent a marker of inflammation and disease activity.

Anti-LL37 Autoantibodies Are Detected in Circulation of PsA and Psoriasis Patients

In order to establish whether the presence of anti-LL37 antibody reactivity was a local phenomenon or whether the same reactivity was systemically detectable, we assessed plasma of PsA, psoriasis and control healthy donors (HD), for antibodies to native LL37, LL37cit, and LL37carb by ELISA. Anti-LL37 antibody reactivity to the unmodified peptide was below the cut-off both in PsA and psoriasis (not shown). However, anti-LL37cit antibodies were higher in PsA plasma (median: 0.544, IQR: 0.495; 11 out of 29: 32%) than in HD plasma (median: 0.394; IQR: 0.16), $p = 0.007$. Anti-LL37carb antibodies were higher in PsA (median: 0.66, IQR: 0.439; 18 out of 32: 52%) than in HD plasma (median: 0.158, IQR: 0.099), $p = 0.0001$ (**Figure 4A**). No statistical significance was observed between anti-LL37cit plasma levels of PsA vs. psoriasis plasma (median: 0.49, IQR: 0.478), $p = 0.3$. In contrast, the

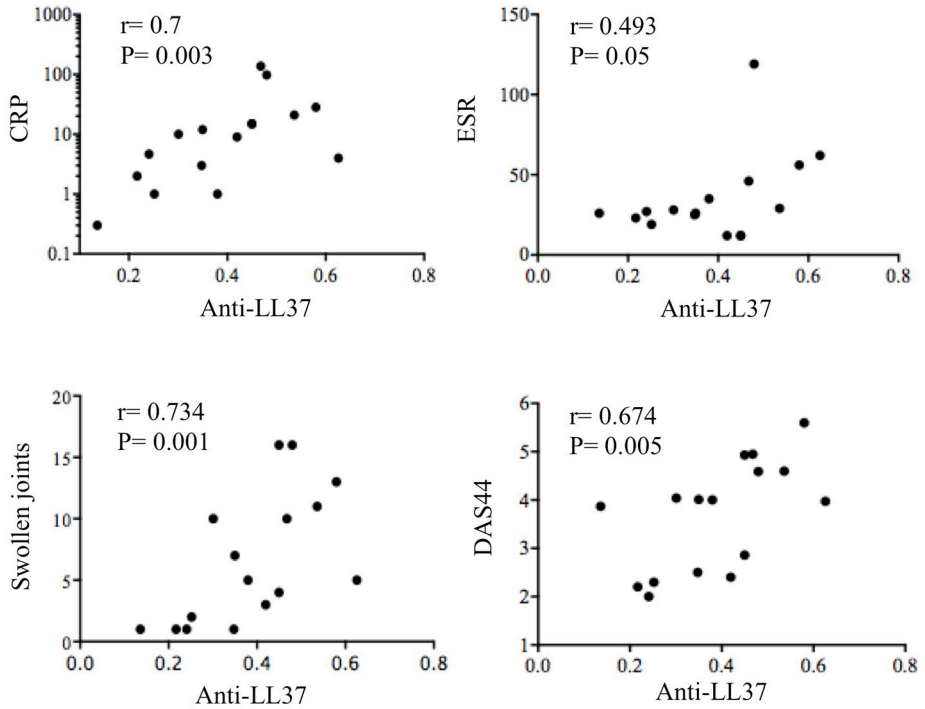

FIGURE 3 | Anti-LL37 levels in SF PsA correlate with disease activity and inflammation markers. SF PsA levels of anti-LL37 Abs ($N = 16$) measured by ELISA as shown in **Figure 2** were correlated with Disease Activity Score (DAS44), C reactive Protein (CRP), Eritrocyte Sedimentation Rate (ESR) and number of swollen joints. The correlation between the two variables was assessed by Spearman correlation coefficient.

anti-LL37carb levels of PsA plasma were significantly higher also when compared to psoriasis plasma (median: 0.43, IQR: 0.47), $p = 0.02$. By setting a cut-off we found that 5 out of 17 (29%) psoriasis patients were positive for anti-LL37cit or anti-LL37carb antibody reactivity (**Figure 4A**). However, levels of anti-LL37cit of psoriasis plasma were not significantly higher compared to HD plasma ($p = 0.15$); in contrast, levels of anti-LL37carb antibodies of psoriasis plasma were significantly higher compared to levels of HD plasma ($p = 0.0001$). Moreover, we observed a significant positive correlation between plasma anti-LL37carb antibodies and disease activity (DAS44) in PsA (**Figure 4B**), in line with recent evidences reporting presence of antibodies to carbamylated proteins in plasma of PsA patients (9). No correlations were apparent between plasma anti-LL37carb or anti-LL37cit antibodies and clinical inflammatory parameters in PsA. Antibody reactivity to LL37cit and LL37carb did not show any correlation with psoriasis activity score index (PASI) in psoriasis patients. These data suggest that measurement of serum levels of anti-LL37carb, but not anti-LL37cit antibodies, may be used as a marker of disease activity in PsA and not in psoriasis.

PsA Synovia Show IgG-Immune Complex Deposition

The deposition of immune complexes (IC) in inflamed tissues is a pathogenic mechanism described in various autoimmune diseases (22, 23). The presence of autoantibodies in synovial fluids and plasma of PsA patients prompted us to investigate the deposition of IgG-IC in ST of PsA, and in OA as control, by confocal microscopy. We visualized the presence of IC in ST of PsA (**Figure 5**) but not of control OA patients (not shown). Interestingly, some of the IC^+-synovial cells were infiltrating neutrophils, as demonstrated by a consistent co-localization of IgG and LL37 staining in cells with the typical polymorfo-nuclear shape (**Figure 5,** inset). IgG staining was detectable also in LL37-negative cells, probably macrophages and/or fibroblasts. These results indicate that IgG are detectable in ST of early PsA patients and that they may represent deposit of immune complexes in PsA synovia; these IgG seemed to include ANCA-like antibodies, suggesting that, among the various antibody specificities, anti-LL37 antibodies may be present and target neutrophils.

Ectopic Germinal Center-Like Structures Are Detected in Synovia of Early PsA

Ectopic lymphoid structures resembling germinal centers have been characterized in chronically inflamed tissues in RA and their presence was associated with an antigen driven B cell response (24–27). To date, only one study addressed the presence of ectopic lymphoid structures in PsA ST (7). We addressed whether lymphoid-like structures might develop in PsA ST already at early disease stage and tried to more finely characterize them: we selected PsA patients with early stage of psoriatic arthritis and naïve to any disease modifying anti-rheumatic drugs (DMARD). We analyzed, by immunohistochemistry (IHC), the presence of infiltrating T and B lymphocytes and presence of

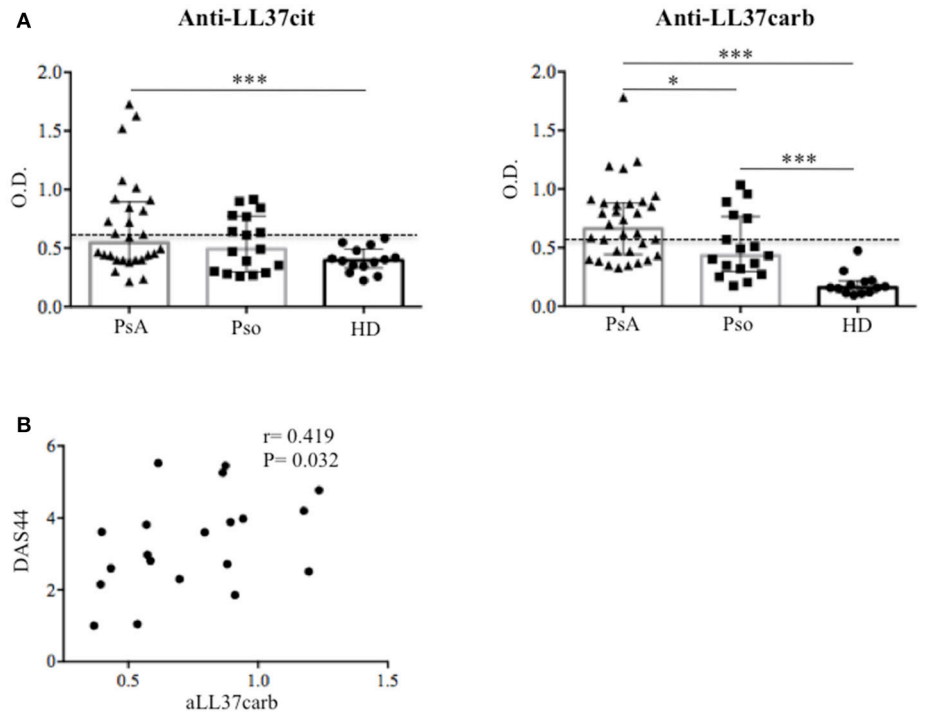

FIGURE 4 | Autoantibody reactivity to citrullinated and carbamylated LL37 in plasma of PsA and Pso patients. **(A)** Levels of anti-LL37cit ($N = 29$) and anti-LL37carb ($N = 32$) were measured in plasma of PsA, Pso (without PsA, $N = 17$) and healthy donors (HD, $N = 14$) by ELISA. Antibody levels are shown as median with Interquartile Range (IQR). *P*-value is calculated by two-tailed Mann-Whitney U test, $^{*}p < 0.05$, $^{***}p < 0.0001$. The mean of HD antibody reactivity+2 SD was used as cut-off (dotted line). **(B)** Levels of PsA anti-LL37carb were correlated with Disease Activity Score (DAS44) by Spearman correlation coefficient and the significant *P* value by two-tailed Mann-Whitney U test.

$CD21^+$ or $CD23^+$ follicular dendritic cells (FDC), together with the expression of the activation/proliferation marker Ki67 and Bcl6, the latter expressed by GC B cells and involved in the development of $CD4^+$ T-follicular helper cells during the GC reaction [Tfh; (28–30)]. As shown in **Figure 6**, we observed the presence of $CD3^+$ T cells and $CD20^+$ B cells aggregates in the ST of early naïve PsA that were associated to $CD21^+$ and $CD23^+$ FDC. The presence of a lower staining for $CD23^+$ cells as compared to that for $CD21^+$ cells appears in line with other works reporting that the complement receptor CD21 represents a more stable phenotypic marker of FDC (31, 32). Moreover, we found a wide expression of the cell proliferation marker Ki67 and presence of $Bcl6^+$ cells located in the T and B cell aggregates. Altogether these data indicate the presence of organized extra-nodal lymphoid structures in PsA synovia and suggest that such structures can develop at very early stages of disease. Indeed, anti-LL37 antibody reactivity was detectable in PsA synovial fluids and plasma of individuals with early stage disease.

Activated Complement And GM-CSF Are Present in PsA Synovial Compartments and in Circulation

The complement system is activated at tissue site where there is antibody deposition, as described in systemic lupus erythematosus (SLE) and RA (33, 34). Complement C5a has been described either as a potent granulocyte chemoattractant (35) or as a priming factor for neutrophil degranulation (36). Moreover C5a, in the presence of GM-CSF, activates neutrophils to undergo NETosis accompanied by release of granules (37). Other complement members, such as C5b-C9, are involved in the activation of citrullination pathways. This is mediated by the peptidyl arginine deiminase (PAD) activation via Ca^{++} influx, following formation of the Membrane Attack Complex (MAC) that induces pore formation in leukocytes (38). Since we observed presence of IgG in the PsA synovial compartment and antibody reactivity to both native and citrullinated LL37, we assessed the content of C5a, C9, and GM-CSF in SF of PsA and control OA patients and, for comparison, in plasma of PsA and psoriasis patients.

As shown in **Figure 7A**, we found a significantly higher concentration of C5a in PsA SF (median: 3590, IQR: 2234) as compared to OA SF (median: 2426, IQR: 1065), $p = 0.007$; C9 was also higher in PsA SF (median: 4727, IQR: 1665) than OA SF (median: 2884, IQR: 2399), $p = 0.001$. GM-CSF was up-regulated in PsA SF (median: 94.45, IQR: 187) as compared to OA SF (median: 1.79, IQR: 13.21), $p = 0.001$. Moreover, C5a in PsA plasma was higher (median: 24309, IQR: 13863) than in HD plasma (median: 16107, IQR: 17310), $p = 0.013$; C9 in PsA plasma was higher (median: 18542, IQR: 7425) than in HD (median: 11292, IQR: 6023), $p = 0.0009$; GM-CSF in PsA plasma was

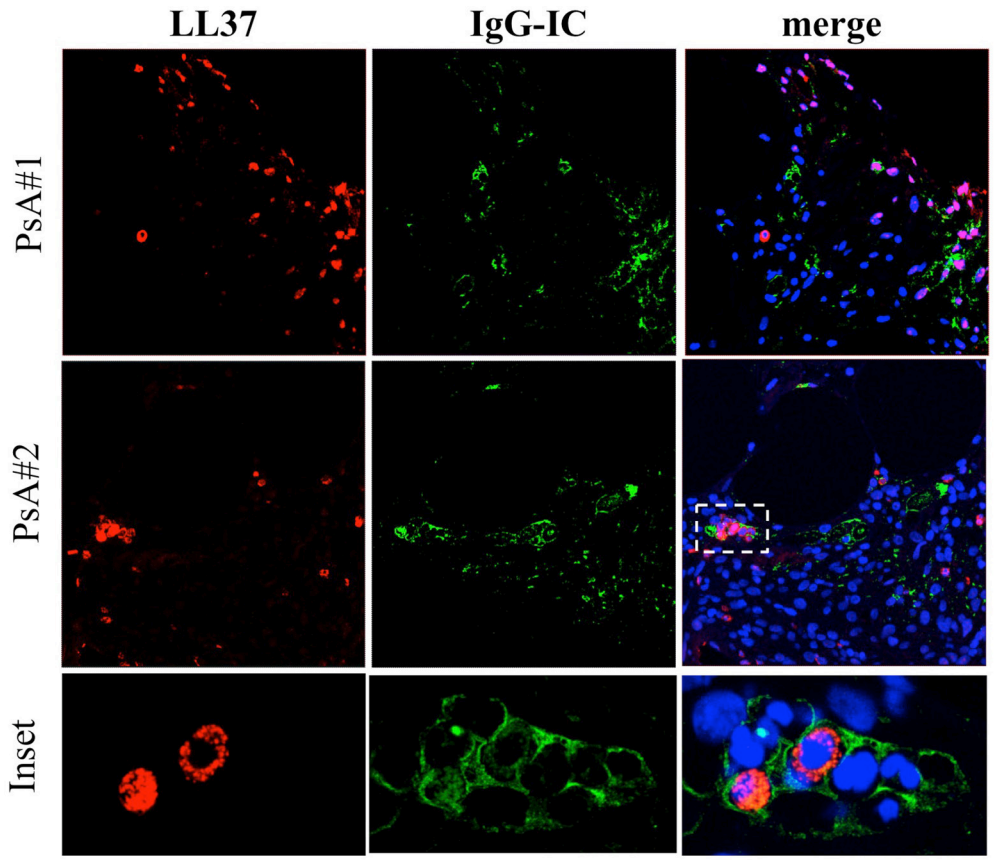

FIGURE 5 | IgG-immune complexes deposition and colocalization with neutrophil-derived LL37 in synovia of PsA. Confocal microscopy images of synovial tissues of two PsA patients stained for LL37 (red), IgG-IC (green) (original magnification, 63x). The dotted white line in PsA#2 indicates the inset of the corresponding picture of the bottom panel. Data are representative of 7 PsA patients.

higher (median: 2.79, IQR: 29.87) than in HD plasma (median: 2.79, IQR: 0), $p = 0.001$ (**Figure 7B**). Of note, the same factors were elevated in the plasma of psoriasis patients. Indeed, the concentration of C5a (median: 19741, IQR: 17432), $p = 0.05$, C9 (median: 21526, IQR: 17432), $p = 0.0001$ and GM-CSF (median: 2.79, IQR: 0), $p = 0.05$, were higher in psoriasis as compared to HD plasma, although the elevation of GM-CSF and C5a were at the limit of the statistical significance (**Figure 7B**). By comparing PsA plasma to psoriasis plasma, only C9 levels were significantly higher in psoriasis than in PsA, $p = 0.02$. C9 levels in synovial fluids, but not in plasma, strongly correlated with disease activity (DAS44; $r = 0.773$, $P = 0.001$; $N = 16$) and various inflammatory parameters: CRP ($r = 0.734$, $P = 0.008$), swollen joints ($r = 0.726$, $P = 0.01$) and tender joints ($r = 0.729$, $P = 0.009$). GM-CSF levels correlated with both anti-LL37cit and anti-LL37carb antibodies in PsA plasma (**Figure 8B**) and with anti-LL37carb (but not with anti-LL37cit) in PsA SF (**Figure 8A**). Moreover, GM-CSF correlated with C5a in PsA plasma (**Figure 8B**). Finally, C5a levels significantly correlated with both anti-LL37cit and anti-LL37carb antibodies in plasma (**Figure 9**), and showed a correlation with DAS44 in PsA, although this correlation was at the limit of the statistical significance ($P = 0.05$; **Figure 9**). Of note, we observed no correlation between circulating C9 or C5a levels and PASI in psoriasis patients. In line with the presence of C9 in PsA SF, we have visualized an abundant presence of C9 in ST of PsA as compared to OA patients by confocal microscopy (**Figure 10**). C9 staining partially colocalized with neutrophils (**Figure 10**).

These results and the relative correlation analyses suggest a participation of activated complement and GM-CSF to antibody reactivity development in PsA and suggest a link between presence of these factors and posttranslational modification of self-proteins in PsA (namely citrullination and, in particular, carbamylation).

An IFN-I Signature Is Present in SF of PsA Patients

Given that immune complexes can induce IFNα (13–15) and an IFN-I signature is present in several immune mediated diseases (SLE, RA), we assessed the presence of IFNα in the synovial compartment of PsA and OA patients.

IFNα was measurable by ELISA assay in 8 out of 20 (35%) PsA SF (median: 1.7, IQR: 63.5) and not in OA SF (median: 1.5, IQR: 0), $p = 0.0001$ (**Figure 11A**). To confirm this, we

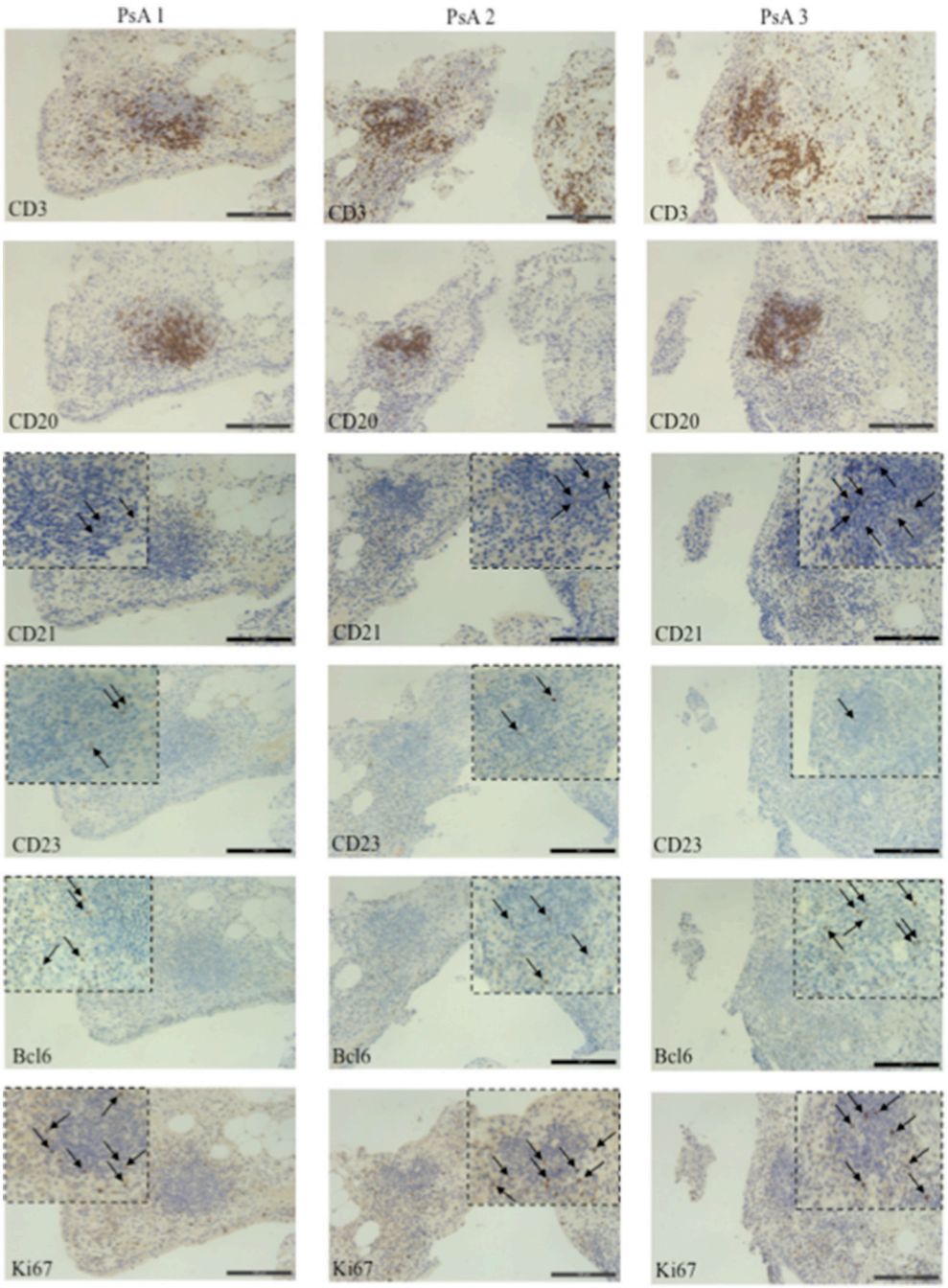

FIGURE 6 | Germinal Center-like structures in synovial tissues of PsA patients. IHC for CD3, CD20, CD21, CD23, Bcl6, and Ki67 (all DAB, brown) of synovial tissue from three early, naive to DMARDs treatment PsA patients (original magnification, 20x). The black arrows indicate a positive staining for CD21, CD23, Ki67, and Bcl6. The dotted black line indicates the insert of the corresponding picture at 40x magnification.

stained PsA and OA ST with a specific surrogate marker of local IFN-I production, the protein MxA (39, 40). MxA staining was extensively found in all PsA ST analyzed (**Figure 11B**), but not in OA ST. MxA staining was distributed either on neutrophils or other unidentified cell types. Although MxA was in part distributed in the vicinity of LL37, the levels of SF IFNα did not correlate with LL37 or anti-LL37 antibodies, with disease activity and/or clinical inflammation markers.

Altogether these findings suggest IFN-I pathways are activated in PsA synovia, and, since ST analyzed by confocal microscopy belong to early diagnosed PsA, an IFN-I signature can represent an early event in disease onset.

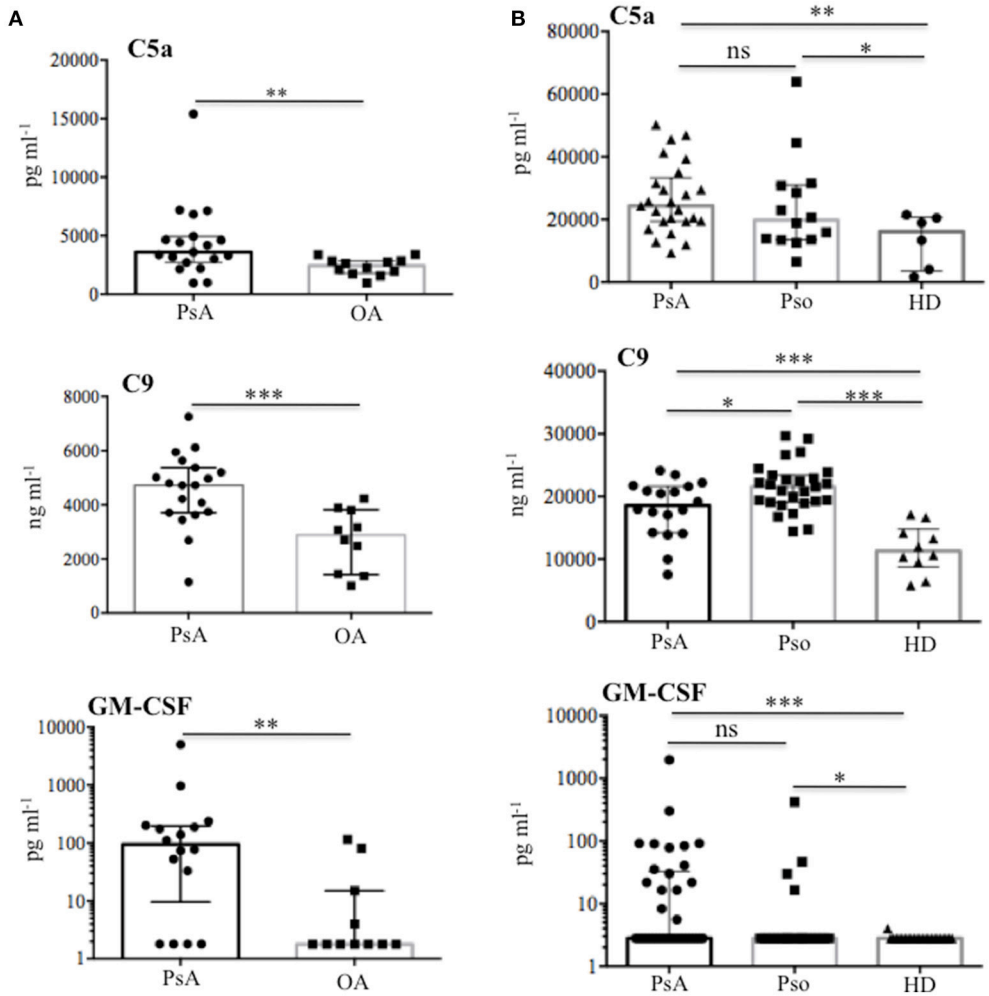

FIGURE 7 | Complement C5a, C9, and GM-CSF in SF and plasma of PsA and Pso patients. **(A)** Content of C5a ($N = 19$), C9 ($N = 19$), and GM-CSF ($N = 21$) were measured by ELISA in SF of PsA and OA ($N = 12$). *P*-value is calculated by two-tailed Mann-Whitney U test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$. **(B)** Plasma levels of C5a in PsA ($N = 25$) and Pso (without PsA, $N = 14$), PsA C9 ($N = 18$), and Pso ($N = 25$), PsA GM-CSF ($N = 25$) and Pso ($N = 16$) were assessed by ELISA. All data in **(A)** and **(B)** are shown as as median with Interquartile Range (IQR). *P*-value is calculated by two-tailed Mann-Whitney U test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

DISCUSSION

PsA develops in 20–30% of psoriasis patients, and skin lesions of psoriasis develop 5–10 years before PsA (41). However the mechanisms linking psoriasis to the development of PsA are still elusive. More generally, no reliable serological markers are available for PsA diagnosis as compared to RA, and PsA pathogenesis is not elucidated.

Results of this study suggest novel factors, some of which are also implicated in psoriasis pathogenesis (13, 15, 36), included the antimicrobial peptide LL37, that seem at work in the pathogenesis of PsA and are potential biomarkers of inflammation/disease status. First of all, we found that LL37 is highly up regulated in SF of PsA patients as compared to control OA. A previous study showed the presence of mRNA for LL37 in inflamed synovial membranes, however analysis of the protein on PsA synovial tissues and fluids were not addressed (42). We found that LL37 is abundantly expressed in PsA ST by confocal microscopy. Since the ST included in the present study belong to very early (<8 months from disease diagnosis) PsA patients, naïve to any pharmacological treatment, these findings indicate an early up regulation of LL37 in PsA ST. Second, we show for the first time that LL37 becomes the target of autoantibodies, representing a novel autoantigen in PsA which sustains the idea of PsA as an autoimmune disease (9). Of note, these anti-LL37 antibodies have been found in synovia and plasma of patients with a very early disease (disease duration between 3 and 10 months) suggesting the development of autoimmunity as an early event. Noteworthy, the patient cohort analyzed in this study includes mainly early PsA patients, in that the mean of disease duration is 11 months. Most intriguing, the presence of autoantibodies to native LL37 in SF well correlates with several inflammatory makers (CRP, ESR, swollen joints count) and disease activity (DAS44) in the

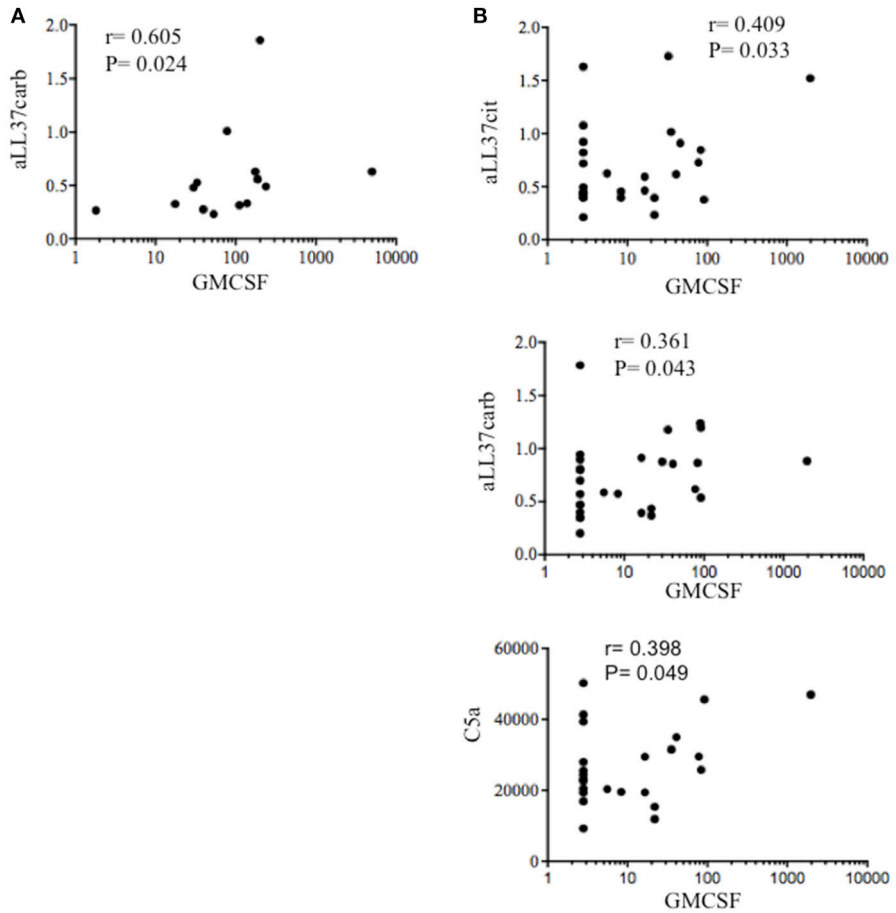

FIGURE 8 | GM-CSF correlates with complement and anti-LL37 antibodies in PsA. **(A)** Spearman correlation analysis was performed between levels of GM-CSF and anti-LL37carb in SF PsA ($N = 17$). **(B)** Spearman correlation analysis between GM-CSF and C5a, anti-LL37cit, and anti-LL37carb in plasma of PsA patients ($N = 23$).

patients analyzed, suggesting a pathogenic role of anti-LL37 antibodies in PsA. These observations fit with the detection of extra-nodal germinal centers (GC)-like structures in PsA ST, which shows that these lymphoid ectopic structures are present in early and naïve ST of PsA. This suggests that the production of autoantibodies occurring at early stages may start as a local phenomenon. Indeed, we visualized for the first time the presence of rare FDC ($CD21^+$ and $CD23^+$ cells) together with evidences of cell proliferation ($Ki67^+$ cells) and markers of both GC B cells and follicular T helper cells ($Bcl6^+$ cells), which suggests that these ectopic lymphoid aggregates can be functional in early PsA. Extranodal lymphoid structures have been identified in other autoimmune or chronic inflammatory diseases such as RA, Sjogren's syndrome, autoimmune thyroid disease, multiple sclerosis or chronic infections (32, 43–45). In these structures lymphocytes are organized and aggregated to form B cell follicles and T cell areas. The functional activity of GC is dependent on the presence of a network of FDC, which retain antigens on their membranes in the form of IC leading to B cell maturation (46). In addition, we show that early synovia also present tissue IgG-IC deposition. Interestingly, IgG staining by confocal microscopy shows co-localization with LL37 in tissue infiltrating neutrophils. These findings suggest an important role of neutrophils as a source of local autoantigens in PsA and may indicate these cells as the target of autoantibody aggression. Neutrophils deliver the content of their granules by degranulation and/or NETosis, phenomena favored by C5a and/or GM-CSF (47, 48) stimulation. Our data show the abundant presence of these factors in SF and also in circulation of PsA patients. Thus C5a and GM-CSF can initiate PsA pathogenesis by attracting and activating neutrophils to deliver their factors by degranulation or in the form of NET (37, 49). NET release of LL37 has been described in SLE where anti-LL37 antibodies are indeed generated (12). Neutrophils have been indicated as a source of autoantigens also in RA (50, 51). The suggestion that C5a and GM-CSF play a role in pathogenic pathways that ultimately lead to autoimmunity via neutrophil activation is supported by the fact that GM-CSF levels in the synovial compartments correlate with autoantibody reactivity. This correlation has been found, in particular, with the presence of anti-LL37 antibodies that react to the post-translational modified versions of LL37 (LL37cit and LL37carb),

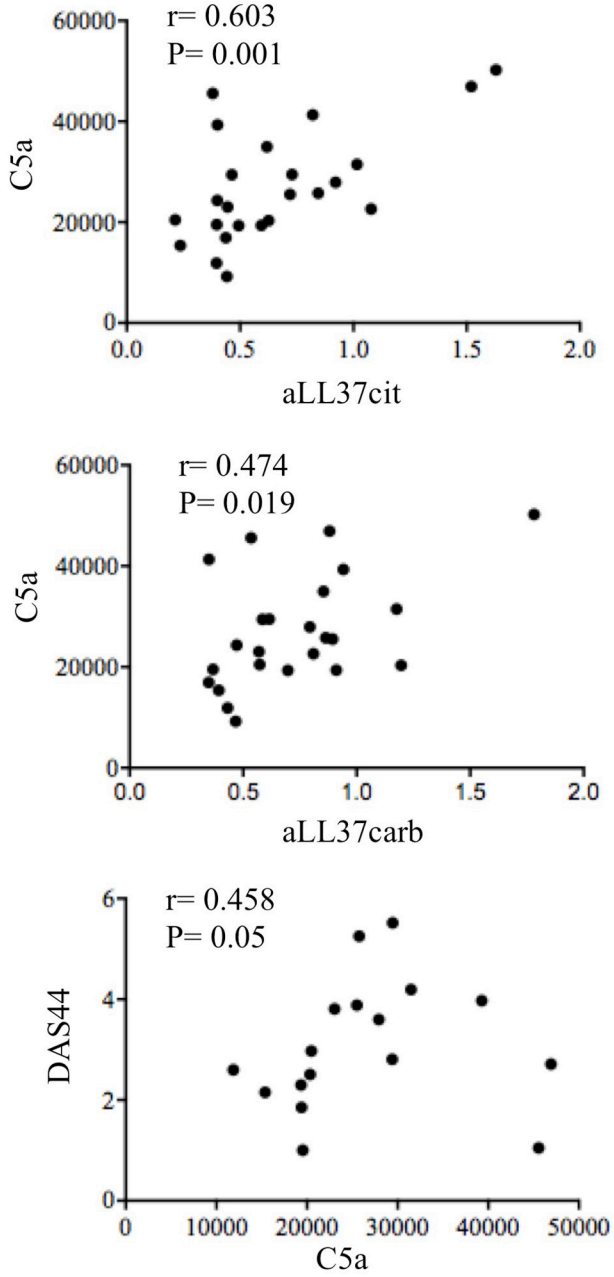

FIGURE 9 | Circulating C5a correlates with anti-LL37 antibodies and disease activity. Spearman correlation analysis was performed between levels of C5a with anti-LL37cit ($N = 24$), anti-LL37carb ($N = 24$) and DAS44 in plasma of PsA ($N = 17$).

whereas a good correlation has been also found between anti-native LL37 antibodies and inflammatory parameters in SF. The reason for this discrepancy is unclear at present, and we are not able to explain it completely. However, it is possible that the determination of reactivity to the native or modified LL37 forms may depend on the specific affinity of the tested autoantibodies, as well from the LL37 preparations used in the assay. Crucial can be their degree of citrullination or carbamylation that is high (see Methods) and may not exactly reflect degree of such modifications *in vivo*. Indeed, LL37 carbamylation and citrullination have been observed *in vitro* and not *in vivo* so far (20, 21).

However, the detected reactivity to both citrullinated and carbamylated LL37 is intriguing and deserves further investigation to understand whether reactivity to the modified LL37 is really distinct from reactivity to the native peptide and the site where this autoreactivity is firstly generated. An important finding is that anti-LL37 antibodies were below the detection limit in PsA circulation, whereas antibodies to modified LL37 were consistent, which is difficult to explain. However, the observation that a percentage of PsA patients respond to carbamylated LL37 and, most importantly, that the magnitude of the antibody reactivity significantly correlates with disease activity (DAS44), suggests to use this immunological parameter as a disease marker in PsA, distinct from psoriasis. Indeed, although also psoriasis patients show antibody reactivity to LL37carb, psoriasis activity index (PASI) did not correlate with this type of antibodies, and levels of anti-LL37carb in psoriasis is significantly lower than that observed in PsA plasma. Our data also support previous findings that PsA patients have high levels of circulating antibodies recognizing carbamylated proteins (9).

The importance of post-translational modification of LL37 in PsA in breaking immunological tolerance to native LL37 is not clarified by our data. However, the fact that antibody reactivity against an autoantigen that is the substrate of both citrullination and carbamylation (20, 21) is induced, reinforces the assumption that neutrophils are crucial player. Indeed, we found strong neutrophil infiltrate in ST of PsA, with abundant presence of MPO, whose activity is strictly connected to production of cyanate (52), which, in turn, favors carbamylation of self-proteins during neutrophilic inflammation. Citrullination is another self-protein modification classically ascribed to neutrophils activity, namely NETosis (50). Although it is matter of debate whether citrullination always occur during NETosis, it is also possible to hypothesize that hypercitrullination phenomena may occur due to MAC activation (38). In this regards, our data show a high presence of activated complement in PsA and C9 detection in tissues. Thus, out data suggest that anti-neutrophil cytoplasmic (ANCA)-like antibodies, such as anti-native LL37, can deposit in ST early during the disease course, where they target neutrophils and activate complement: we assume that abundant neutrophil proteins are released and undergo citrullination and/or carbamylation. This phenomenon activates specific autoantibodies, included anti-LL37 antibodies reacting to the modified antigen, which amplifies a pathogenic pernicious loop. The action of LL37 antibodies can also favor the activation of an IFN-I signature in tissues via TLR7/8/9, as shown in SLE (12), given the binding capacity of LL37 for self-nucleic acids. Cells other than pDCs can also contribute the IFN-I signature observed in PsA synovial, via this mechanism. Moreover, some kind of NETosis (included live NETosis; 37, 39) is likely to occur in PsA (our unpublished observations) as in RA (38), and this may also explain the IFN-signature that we have observed (MxA staining in synovial tissues). Levels of IFNα were also elevated in some PsA SF, although IFNα did not correlate with

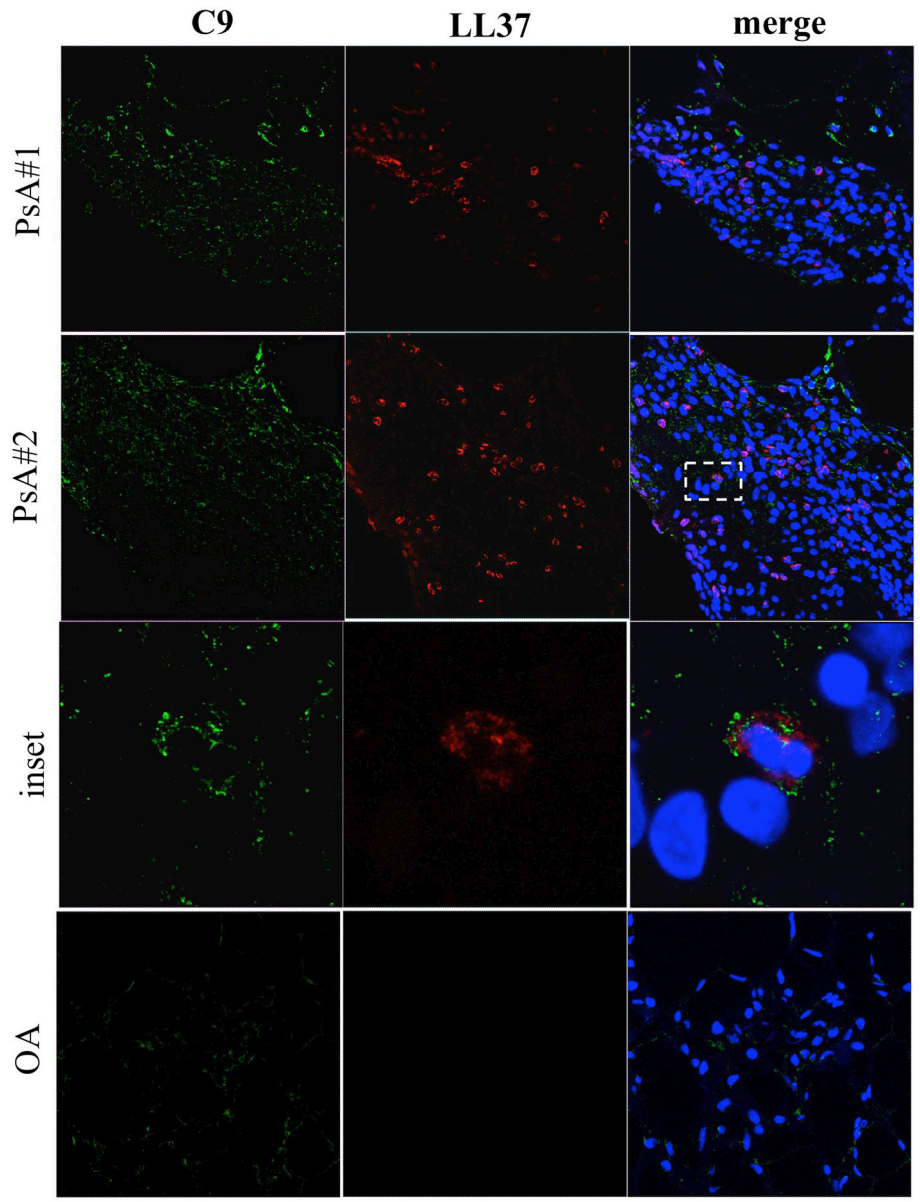

FIGURE 10 | C9 staining in synovial tissues of PsA. Confocal microscopy images of synovial tissues of two PsA and one OA patients stained for LL37 (red) and C9 (green) (original magnification, 63x). The dotted white line in PsA#2 indicates the inset of the corresponding picture of the lower panel. Data are representative of 7 PsA patients and 4 OA subjects.

autoantibodies or disease activity. However, this finding lead us to speculate that, in PsA, IFN-I and NET may directly favor the generation of autoantibodies, at least in memory B cells (16).

Finally, our data showing the presence of complement component C9 in ST and peripheral blood of PsA patients are intriguing, since levels of C9, especially in SF, strongly correlate with all disease parameters (clinically relevant inflammatory parameters and disease activity, as DAS44). Thus, these data lend support to the hypothesis that complement activation is an important player in PsA, and its targeting may be beneficial (53).

In conclusion, the data of the present study shed light on possible new players/mechanisms in PsA pathogenesis (LL37, anti-LL37 antibodies, NETosis, self-protein modifications) while reinforcing involvement of previously suspect players (activated complement, carbamylation, GM-CSF) in the disease. We report that, (i) LL37 behaves as a novel B cell autoantigen in PsA, also in its modified form (LL37cit and LL37carb), (ii) pathways of protein carbamylation and citrullination are likely activated in PsA, (iii) degranulating/netting neutrophils are players in the release of the autoantigens (for instance LL37), under the effect of inflammatory factors, such as GM-CSF and C5a. IgG-immune

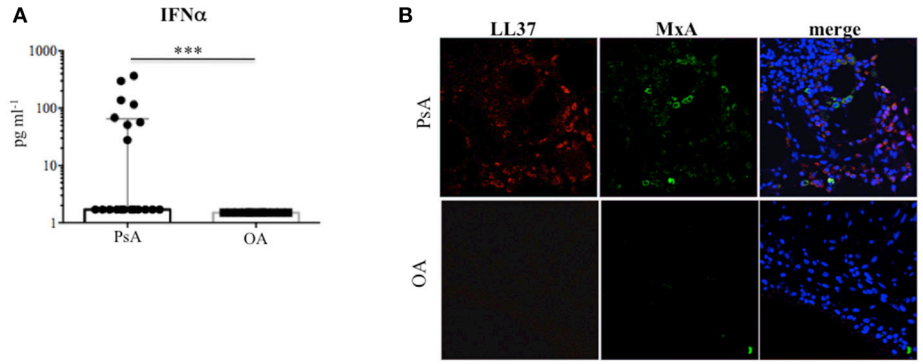

FIGURE 11 | Type I IFN in the synovial compartment of PsA. **(A)** IFNa was measured by ELISA in SF of PsA ($N = 20$) and OA ($N = 12$) subjects. Data are shown as median with Interquartile Range (IQR). *P*-value is calculated by two-tailed Mann-Whitney U test ***$p < 0.001$. **(B)** Confocal microscopy images of synovial tissues of one PsA and one OA patient stained for LL37 (red) and MxA (green) (original magnification, 63x). Data are representative of 7 PsA patients and 4 OA subjects.

complexes formation and their deposition in PsA ST can fuel this vicious circle and also induce an IFN-I signature (12). Since we have shown through our study that some of the characteristics found in PsA are present also in psoriasis patients without PsA (circulating complement, anti-LL37carb antibodies, GM-CSF), longitudinal follow-up of psoriasis patients with antibody reactivity to LL37 and higher complement and GM-CSF levels may be highly informative about the evolution of psoriasis into a more systemic inflammation that eventually develop to PsA. This may allow the identification of prognostic biomarkers for PsA development in psoriasis patients. Finally, almost all patients with PsA have psoriasis and we have shown that LL37 is a CD4 and CD8 T cell autoantigen in at least 46% of psoriasis patients (15). LL37-specific-T cells and their Th1/Th17 secretion pattern correlate with PASI (15). Approximately, the same percentage of PsA patients show CD4 and CD8 T cells proliferating to LL37, which is reasonable (our unpublished data). Whether these PsA T cells exhibit a different phenotype (cytokines secreted, homing receptors and/or T-helper cells markers) that may explain the development of PsA in psoriasis-affected patients in comparison to psoriasis-only patients is under investigation.

MATERIALS AND METHODS

Study Population

The study included thirty-two Caucasian patients 18 years old or older, rheumatoid factor and ACPA negative, affected by PsA according to the CASPAR classification criteria for Psoriatic Arthritis, and recruited from the Rheumatology Unit of the University of Rome Tor Vergata, Sapienza University of Rome, and the Institute of Rheumatology of Fondazione Policlinico Universitario A. Gemelli IRCCS - Catholic University of the Sacred Hearth of Rome.

Patients were treated with csDMARDs (conventional synthetic Disease-Modifying Anti-Rheumatic drugs) in 43% (14/32) and with bDMARDs (biological-DMARDs) in 12% (4/32). Joint disease activity was measured with the number of tender and swollen joint, the pain-VAS (visual analogue scale) and the GH (global health) and by using the Disease Activity Score 44 (DAS44) with the evaluation of the CRP (C-Reactive Protein) and of the erythrocyte sedimentation rate (ESR). Skin disease activity was assessed using PASI (Psoriasis Area Severity Index). Clinical and demographic data of PsA, Pso, and osteoarthritis (OA) subjects are summarized in **Table 1**. Chronic plaque psoriasis diagnosis was based on a confirmed diagnosis for at least 6 months before. Criteria of exclusion: pustular, erythrodermic, and/or arthritis form of psoriasis; history of drug-induced psoriasis; clinically significant flare of psoriasis during 12 weeks prior; concurrent or recent use of any biologic or systemic therapy; received non-biologic systemic psoriasis therapy or phototherapy (including psoralen and ultraviolet A, PUVA), ultraviolet B (UVB) within the previous 4 weeks; or had topical treatment within the previous 2 weeks prior.

Age- and sex-matched healthy donors (HD; $N = 14$) served as normal control (none of them exhibited psoriatic skin or joint symptoms) and were obtained from Blood Center of the Policlinico Umberto I, Roma. Age- and sex-matched OA patients ($N = 12$) served as disease control (**Table 1**).

The study was carried out according to the Declaration of Helsinki and conducted in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines. The study protocols were approved by ethic committee of the University of Rome Tor Vergata, Catholic University of the Sacred Hearth of Rome and Sapienza University of Rome. All patients provided written informed consent before participating in any study-related activities.

Reagents and Carbamylation

LL37 peptide was purchased from Proteogenix (F); citrullinated-LL37 (LL37cit), citrullinated at all five arginin position, was from Anawa. Carbamylation of LL37 (LL37carb) was obtained by incubating LL37 with 1M potassium cyanate (Sigma Aldrich) at 4°C for 3 days, followed by extensive dialysis against PBS as described (54). To verify the carbamylation reaction, a dot-blot assay was performed. Briefly, LL37carb were spotted onto a nitrocellulose membrane (GE Healthcare Life Sciences). After

TABLE 1 | Data are expressed as mean ± standard deviation unless otherwise specified; range of possible values are indicated in square brackets.

Demographic and clinical data	PsA patients ($n = 32$)	PsO patients ($n = 24$)	OA patients ($n = 12$)
Male sex (N/%)	19 (59)	11 (13)	7 (58)
Age (years)	54.3 ± 11.9	51.1 ± 12.1	45.7 ± 15.7
Pso (N/%)	22 (69)		
PsA disease duration (months)	11 ± 5	0 (0)	
Pso disease duration (months)	20 ± 10	15.3 ± 10	
ESR (mm/h)	16.6 ± 10,1		
CRP (mg/dl)	1.6 ± 2.2	2.2 ± 2.6	
N. of tender joints	7.2 ± 9.1		
N. of swollen joints	5.4 ± 3.7		
Pain VAS	4.75 ± 6.69		
GH	4.6± 2.3		
DAS44	2.41 ± 16.1		
PASI	3.4 ± 2.1	14.6 ± 7.2	
ACPA positivity	0 (0)		
IgM/IgA-RF positivity	0 (0)		
csDMARDs (N/%)	14 (43)	22 (91.6)	
bDMARDs (N/%)	4 (12)	15 (62.5)	

PsA, Psoriatic Arthritis; Pso, Psoriasis; OA, Osteoarthritis; DAS44, Disease Activity Score 44; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ACPA, Anti-Citrullinated Peptide Antibodies; RF, Rheumatoid Factor; VAS, visual analoghe scale; GH, global health; csDMARDs, conventional synthetic Disease-modifying antirheumatic drugs; bDMARDs, biological Disease-modifying antirheumatic drugs.

blocking in PBS containing 0.05% Tween 20 (PBS Tween) and 5% non-fat dry milk, the membrane was incubated with a polyclonal anti-carbamyl-lysine antibody (Cell Biolabs, Inc., San Diego, CA, USA) and anti-LL37, O/N at 4°C. The next day, after washings with PBS Tween, peroxidase-conjugated goat anti-rabbit IgG (BioRad Laboratories, Richmond, CA, USA) were used as second antibodies and the reactions were developed with 3-3′ diaminobenzidine (Sigma Aldrich).

Antibodies against LL37, (Mab137), was provided by the Antibody Facility of University of Geneva (CMU; CH). Rabbit polyclonal anti-LL37 was from Innovagen. Both were used for immunofluorescence staining in parallel with appropriate control antibodies.

Human Samples

Synovial fluids (SF) were collected from active knee PsA and age- and sex-matched patients with knee osteoarthritis (OA). Exclusion criteria for SF analysis were local intra-articular corticosteroid injection within 5 weeks before SF aspiration. SF was collected via joint aspiration in association with therapeutic arthrocentesis. Approximately 2–4 ml of SF was collected in sodium heparin-coated Vacutainer^{TM} tubes (Becton-Dickinson); samples contaminated with blood were discarded. Immediately after collection, samples were centrifuged at 1,000g for 15 min at 4°C, and the resulting supernatants were stored at −80°C. Plasma from PsA, psoriasis and HD subjects were collected following standard protocol and stored at −80°C. Synovial tissues were collected from Consecutive patients fulfilling the classification criteria for Psoriatic Arthritis [PsA; (55)] undergoing ultrasound guided synovial minimally invasive tissue biopsy following the published protocol using a 14G needle (Precisa 1410-HS Hospital Service Spa, Italy) (56). Once collected ST specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin for Immunohistochemistry (IHC) or immunofluorescence staining.

Immunohistochemistry (IHC)

Synovial tissue (ST) sections were stained with IgG1 mouse anti-human monoclonal antibody for CD20 (clone L26; at 1.2 μg/ml) or IgG1 mouse anti-human monoclonal antibody for CD3 (clone LN 10; at 1.0 μg/ml) or IgG2a mouse anti-human monoclonal antibody for CD21 (clone 2G9; at 0.34 μg/ml), IgG1 mouse anti-human monoclonal antibody for CD23 (clone 1B12; at 1 μg/ml), IgG1 mouse anti-human monoclonal antibody for Ki67 (clone K2; at 1.2 μg/ml), or IgG2b mouse anti-human monoclonal antibody for Bcl6 (clone LN22; at 1 μg/ml) (all from Leica Biosystem, Newcastle, UK) by immunostainer BOND MAX III (Leica, Newcastle, UK). IHC for CD20, CD3, CD21, CD23, Ki67, and Bcl6 was performed as follows: 3-μm-thick sections were prepared from formalin-fixed paraffin-embedded tissue blocks and were dried in a 60°C oven for 30 min. The sections were placed in a Bond Max Automated Immunohistochemistry Vision Biosystem (Leica Microsystems GmbH, Wetzlar, Germany) according to the following protocol: firstly, tissues were deparaffinized and pre-treated with the Epitope Retrieval Solution 1 (CITRATE buffer) or Solution 2 (EDTA-buffer) at 98°C for 10 min according to the manufacturer's instructions. After washing, peroxidase blocking was carried out for 10 min using the Bond Polymer Refine Detection Kit DC9800 (Leica Microsystems GmbH). Tissues were again washed and then incubated with the primary antibody for 30 min. Subsequently, tissues were incubated with polymer for 10 min and developed with DAB-Chromogen and finally counterstained with hematoxylin (57).

Confocal Microscopy

Three-μm-thick sections in paraffin of human PsA and OA synovia were stained after deparaffination in xilene (5 min, two times), followed by passages in: absolute ethanol (3 min), 95% ethanol in water (3 min), 80% ethanol in water (3 min), 70% ethanol in water, and antigen retrival (5 min at 95°C in 10 mM sodium citrate, pH 6.0). Slides were saturated with blocking buffer (PBS, 0.05% tween 20, 4% BSA) for 1 hour at room temperature. Specimens were stained with a polyclonal rabbit anti-LL37 (Innovagen), rabbit anti-MPO (Abcam), mouse anti-MxA (Novus Bio), monoclonal mouse anti-LL37 (Mab137), polyclonal rabbit anti-human C9 (ATLAS). The following antibodies were used: donkey anti-rabbit IgG AlexaFluor-568 or-647, anti-mouse AlexaFluor-647 and an anti-goat AlexaFluor-488 (Abcam). After washing, slides were mounted in Prolong Gold anti-fade media containing a DNA dye (DAPI) (Molecular Probes). CLSM observations were performed with a Leica

TCS SP2 AOBS apparatus, using a 63x/1.40 NA oil objective. Acquisition of images was performed by a Leica confocal software 2.6 (Leica, Germany).

ELISA

Anti-LL37 antibodies against LL37, LL37cit and LL37carb, were measured by ELISA as previously described (12). Briefly, 96-well flat-bottom plates are coated with 2 $\mu g\ ml^{-1}$ of LL37, citrullinated-LL37 or carbamylated-LL37 in carbonate buffer (0.1M $NaHCHO_3$, pH 9.6) overnight and washed five times with 0.1% Tween-20 in PBS. This washing buffer was used for washing at all steps. The blocking buffer containing 4% Bovine serum albumin (BSA, Sigma) in PBS was used for at least 1 h (or overnight) to saturate unspecific binding sites. After washing, plasma were diluted 1:100 in PBS 4% BSA followed by 1 hour incubation with a horseradish peroxidase—conjugated goat anti-human IgG (Sigma-Aldrich) diluted 1:5,000 in PBS. Synovial fluids were diluted 1:20. The color was developed with 3,3′, 5,5′-tetramethylbenzidine (TMB) substrate (Sigma-Aldrich). The reaction was stopped by adding 50 µl of 2N H_2SO_4 and absorbance determined at 450 nm with a reference wavelength of 540 nm. The cut-off (for both LL37 and related antibodies) was identified by calculating the mean of controls (HD or OA) and by adding 2 standard deviations of the mean: cut-off = mean (HD or OA)+2SD.

To detect IFNα and GM-CSF, plasma and SF were diluted 1:5 and measured by ELISA from MabTech, accordingly to the manufacturer protocol. Plasma and SF content of complement C5a was measured by ELISA from MyBioSource (USA) according to the manufacturer protocol. C9 in plasma and SF was measured by ELISA from Biomatik (USA) according to the manufacturer protocol. LL37 levels of plasma and SF were measured by the Human antibacterial peptide LL37 ELISA Kit (Cusabio, China).

Statistical Analysis

Data were expressed as medians with Interquartile Range (IQR). Differences between median values were determined by two-tailed Mann-Whitney U test ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Correlation analyses were performed by Spearman rank-correlation test. The cut-off value was determined by using the mean + 2SD (Standard Deviation) of the control subjects (HD or OA). Statistical analysis was performed by using GraphPad Prism software version 6 (San Diego, CA, USA).

AUTHOR CONTRIBUTIONS

LF conceived the research with RL, directed and supervised the research with the RL, performed staining of tissue biopsies for confocal microscopy, analyzed and interpreted the data, performed statistical analyses, wrote the manuscript. RaP performed most ELISA experiments and helped with statistical analysis. MC, GF, RoP delivered the clinical ethical and patient-related aspect of the project and provided clinical samples (plasma and SF). SA, BT, LP, EG delivered the clinical ethical and patient-related aspect of the project and obtained clinical samples (plasma, SF and ST biopsies). LaB, AE performed immunohistochemistry on ST. EB, AG, LuB, BM delivered the clinical ethical and patient-related aspect of the project and obtained clinical samples of psoriasis patients and patients' data (plasma). SEA provided patients samples and clinical data, performed ELISA. FS acquired and analyzed most confocal microscopy images. MF acquired some confocal images. IP processed blood samples of HD and patients. FRS, CA, FC, GV delivered the clinical ethical and patient-related aspect of the project and obtained clinical samples and patients data (plasma, SF). TC performed protein carbamylation. AC delivered the clinical ethical and patient-related aspect of the project and obtained clinical samples of psoriasis patients and clinical data (plasma). RL conceived the research with LF, directed and supervised the research with the LF, performed analysis of confocal microscopy, performed ELISA, analyzed and interpreted the data, performed statistical analyses, wrote the manuscript.

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CLA+ T Cell Response to Microbes in Psoriasis

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Streptococcus pyogenes throat infection is a clinically relevant trigger of both guttate and chronic plaque psoriasis, and it provides an ideal context in which to study the pathogenesis of these diseases using an antigen-dependent approach. Circulating cutaneous lymphocyte-associated antigen (CLA) positive (+) memory T cells are a subset of peripheral lymphocytes whose phenotype and function are related to immunological mechanisms in the skin. These cells are considered peripheral biomarkers of T-cell-mediated skin diseases. The coculture of autologous epidermal cells with CLA+ T cells from psoriasis patients activated by *S. pyogenes* allows the reproduction of the *ex vivo* initial molecular events that occur during psoriatic lesion formation. With cooperation of autologous epidermal cells, *S. pyogenes* selectively activates CLA+ T cells both in guttate and plaque psoriasis, inducing key mediators, including an IL-17 response. Here, we explore potential new mechanisms of psoriasis development including the influence of HLA-Cw6 on *S. pyogenes* CLA+ T cell activation in guttate psoriasis, the relevance of IL-9 on microbe induced IL-17 response in guttate and plaque psoriasis, and novel effector functions of *Candida albicans*. This review will summarize recent knowledge of psoriatic mechanisms elicited by microbes that have been studied through an innovative translational perspective based on CLA+ T cell-mediated cutaneous immune response.

Keywords: psoriasis, cutaneous lymphocyte-associated antigen, homing, *Candida albicans*, *Streptococcus pyogenes*

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INTRODUCTION

Molecular studies of psoriasis lesions and patients have allowed translational research to generate potent and novel therapies (1). However, our understanding of the influence of environmental factors on the psoriatic cutaneous immune response is still limited (1). Several microorganisms, including bacteria, fungi, and viruses, have been postulated to be potential triggers and/or exacerbating factors of psoriasis (2). Bacterial genome DNA sequencing in psoriasis is an area of great interest, some microorganisms have been identified but their functional relevance for psoriasis is still to be determined. Psoriasis can be classified as early or late onset (3). The former is associated with the HLA-Cw6 allele, streptococcal throat infection, and a higher tendency to be generalized

(4, 5). Interestingly, patients with this type often present a more intense inflammatory lymphocytic infiltrate and are more likely to receive biological therapy (6). All these observations suggest that the presentation of psoriasis is associated with the present bacterial infection. *Streptococcus pyogenes* throat infection is a well-characterized infectious trigger of gutatte psoriasis (GP) and chronic plaque psoriasis (CPP). More than 60 years ago, it was reported that two-third of GP patients present an acute sore throat 12 weeks before the skin eruption (7). Similarly, CPP can also be triggered by *S. pyogenes* throat infections (8), and interestingly, CPP patients have a higher incidence of recurrent sore throats compared with controls (9, 10). The presence of *S. pyogenes* has been detected in the blood of both GP and CPP patients (11). In addition, tonsillectomy can be a useful therapeutic intervention in CPP patients with a history of streptococcal-associated exacerbations (12). It has been proposed that psoriasis tonsillar CLA^+ T cells (13) activated by streptococcal antigens migrate to the skin where they react to antigens that share sequence homology with the streptococcal proteins (14). However, other microbes may also participate in psoriasis. Fungal cutaneous infections caused by *Candida albicans* have been associated with exacerbation of the disease and a higher frequency of intestinal *C. albicans* isolation in psoriasis patients than controls has been reported (2), although the mechanisms involved in *C. albicans*-induced psoriasis remain poorly characterized.

Interestingly, microbes such as *C. albicans* induce type I interferon response and, type I interferon production by plasmacytoid dendritic cells in skin has been stated to be an important trigger for psoriasis development (15). However, clinical efficacy blocking antibodies against IFN-α have not shown clinical efficacy in psoriasis (16), rising questions about the translational relevance of this mechanism.

In this review, we cover the current state of the art in psoriasis immunopathogenic mechanisms brought out by disease-related microorganisms, such as *S. pyogenes* or *C. albicans*. We focus on cutaneous immune response mediated by CLA^+ T cells and how these microbes affect T cell activation and production of clinically relevant cytokines.

CIRCULATING CLA^+ T CELLS AND THE STUDY OF THE CUTANEOUS IMMUNE SYSTEM

The immune responses of T cells during cutaneous chronic inflammation in psoriasis involve a subset of memory T lymphocytes that can be distinguished from other memory T cells by the surface expression of the cutaneous lymphocyte-associated antigen (CLA) antigen. This antigen is a cell surface carbohydrate that allows the identification of memory T cells that belong to the cutaneous immune system. CLA is an adhesion molecule expressed by 15% of circulating T cells in humans, and by most (>90%) skin-infiltrating T cells, contrary to other inflamed organs (17). In addition to several ligands for chemokine receptors (CCR10, CCR4, CCR6, and CCR8), CLA binds to E-selectin and together with the interaction between the very late antigen-4/vascular cell adhesion protein-1 and lymphocyte function-associated antigen-1/intercellular adhesion molecule-1, forms a code bar system enabling skin lymphoid infiltration (18). The relevance of circulating CLA^+ T cells in the cutaneous immune response lies not only in the skin-seeking capacity of these cells but also in their functional relation to the immune response that occurs in inflamed cutaneous lesions. This feature is derived from the recirculating capacity of these cells between skin lesion and blood during cutaneous inflammation (18, 19). The antigen-specific response and phenotype of circulating CLA^+ T cells has been studied in many human skin conditions. CLA^+ T cells respond to antigens, allergens, or superantigens involved in disease by triggering T cell-mediated skin diseases, such as psoriasis, atopic dermatitis, and contact dermatitis (18). Furthermore, the phenotype and function of these cells are related to the clinical status of the patient, thereby explaining why circulating CLA^+ T cells are considered peripheral cell biomarkers of T cell-mediated cutaneous disease in humans (18). Using CLA^+ T cells from psoriasis patients and healthy controls, our group explores the influence of microbes on cutaneous immune response in psoriasis.

CLA^+ T CELL ACTIVATION BY *S. pyogenes* IN PSORIASIS INDUCES IL-17 AND IL-9 RESPONSES

Studying the antigen-specific immune response of CLA^+ T cells induced by clinically relevant triggers of psoriasis may allow the identification of the translational mechanisms involved in psoriasis. The stimulation of autologous coculture CLA^+ T cells and epidermal cells with *S. pyogenes* leads to an inflammatory immune response that shows the hallmarks of psoriasis. By contrast, the same stimulation of CLA^- cells from the same patient or cultures using CLA^+/CLA^- T cells from healthy controls does not have this effect (20). The CLA^+ T cell response in this model is related to the clinical response of patients in terms of anti-streptolysin O levels, PASI, and duration of flare in GP (21), and to anti-streptolysin O in CPP (20). This activation is determined by the presence of autologous epidermal cells (lesional/non-lesional) and MHC class I and class II presentation. Supernatants of *S. pyogenes*-activated cocultures of CLA^+ T cells and epidermal cells induce epidermal hyperplasia upon intradermal injection in mouse skin (20). IL-17A and IL-17F production is probably the most relevant effect of *S. pyogenes* on CLA^+ T cells in psoriasis. The influence of *S. pyogenes* through the response of these cells and the relevance of IL-17 production in GP have been extensively studied (21). In $HLA\text{-}Cw6^+$ patients whose GP flare is associated with a pharyngitis episode, the Th17-associated response is greater than that exerted by samples from GP patients not associated with pharyngitis. In fact, significantly higher levels of IL-17A, IL-17F, and even IL-6, which participates in Th17-differentiation, were found (21). Thus, the observed response of psoriasis memory T cells to *S. pyogenes* seems to be restricted to CLA^+ T cells, leading to IL-17 production. This cytokine is a key driver of psoriasis, and its

neutralization in patients, or receptor blockade improves the skin condition (1).

The cytokine IL-9 is involved in chronic inflammation and has recently been associated with psoriasis (22, 23). We have demonstrated how *S. pyogenes* preferentially induces IL-9 production during the coculture of autologous CLA$^+$ T cells and epidermal cells in psoriasis but not in healthy controls. IL-9 is produced in the same culture conditions in which IL-17A and IL-17F are detected in a time-dependent manner. IL-9 production is dependent on MHC class I and class II presentation, and it preferentially prolongs CLA$^+$ T cell survival. Higher amounts of IL-9 were detected in psoriasis patients than in healthy controls, but no differences were observed between GP and CPP patients (24). IL-9 has been associated with increased IL-17A production in an animal model of psoriasis (23). Since *S. pyogenes* induces both IL-9 and IL-17A, we examined the interaction between these two cytokines in CLA$^+$ T cells. To this end, we blocked IL-9 function using a neutralizing. A 50% reduction in IL-17A production, but not IFN-γ, was found when was IL-9 was neutralized in CLA$^+$ cells activated by *S. pyogenes*.

Our studies have shown that, in CPP patients without clinical evidence of *S. pyogenes* infection, only CLA$^+$ T cells respond to this microbe in comparison to healthy controls. This observation indicates that psoriasis patients present an adaptive immune response to *S. pyogenes* through IL-17A, IL-17F, IL-9, and IFN-γ production (20, 21, 24) and suggests that *S. pyogenes* modulates the response of the CLA$^+$ T cells that maintain psoriatic lesions, i.e., pyogenes infection has been describe to participate in CPP infection, since higher levels of IgG against *S. pyogenes* proteins are detected in psoriasis patients in comparison to healthy controls (25). Some studies have reported the presence of the genera *Streptococcus* in normal and psoriatic skin (26) and the isolation of *S. pyogenes* in the skin of GP patients (4), probably leading to cutaneous immunization and a CLA$^+$ T cell-restricted response in psoriasis.

CLA$^+$ T CELL RESPONSE TO *C. albicans* IN PSORIASIS

The cutaneous adaptive immune response to *C. albicans* infection is mediated by a Th17 profile since Th17 cells are essential for anti-fungal barrier immunity (27). Patients with Th17 deficiencies have an increased susceptibility to candidiasis (28), and CD45RA$^+$ human T cells may lead to an increase in the number of IL-17 and IFN-γ-producing cells (29). Cutaneous candidal infections have been reported in association with psoriasis exacerbation (30); however, the mechanisms by which *C. albicans* induces psoriasis are poorly understood (2). In psoriasis, *C. albicans*-derived superantigens may induce an expansion of lymphocytes expressing the T-cell receptor variable region beta 5.1 (31). Like in the case of *S. pyogenes*, CLA$^+$ T cells, together with autologous epidermal cells, preferentially respond to *C. albicans* extract by inducing IL-9, IL-17A, and IFN-γ production in psoriasis. This response appears to be restricted to CLA$^+$CD4$^+$ memory T cells since CD4-depleted CLA$^+$ memory T cells do not respond to this microorganism in a coculture model with psoriasis cells (24). These results are in line with the expected immune response to *C. albicans* in the skin. However, the observed preferential response of CLA$^+$ T cells in psoriasis suggests an adaptive immune response to *C. albicans*, underlying its importance as a relevant antigen likely to be involved in triggering the disease.

INFLUENCE OF *S. pyogenes* AND *C. albicans* ON IL-17 ADAPTIVE IMMUNE RESPONSE IN PSORIASIS

The precise mechanisms by which environmental factors trigger psoriasis are not well understood (1). Biological therapies have revealed the clinical relevance of the IL-23/IL-17 axis in this skin disease. Thus, environmental factors that contribute to fueling the IL-23/IL-17 response may induce the condition. The observation that CLA$^+$ T cells in psoriasis patients respond to skin *S. pyogenes* and *C. albicans* extracts indicates a relationship between memory T cells and environmental microbes. Such preferential sensitization to these microorganisms in psoriasis can be either at the tonsillar level in psoriasis through the abnormal generation of CLA$^+$ T cells or at the skin level, since the presence of both *S. pyogenes* and *C. albicans* in psoriatic lesions (13). The CLA$^+$ T cell response to these microbes is based on IL-17A, IL-17F, IL-9, and IFN-γ production. This response indicates that these skin-homing cells will migrate to psoriatic lesions and thus that they may be involved in the local inflammatory response. IL-17A and IL-17F are clinically validated mediators of psoriasis. *S. pyogenes*-driven IL-9 production through CLA$^+$ T cells supports IL-17A production in human lymphocytes, since *in vitro* neutralization of IL-9 reduces IL-17A production by 50% (24).

A current model of IL-17 production in psoriasis considers that some autoantigens, such as LL-37 and ADAMTS-like protein 5, would activate T17 cells (32), initiating the immune circuit of the psoriasis pathogenetic mechanism in the disease. Also, IL-23 production by inflammatory dendritic cells favors the generation and maintenance of the T17 phenotype in psoriasis. Interestingly, regarding the possible interplay between *C. albicans* and *S. pyogenes* and the IL-23/Th17 axis, it has been recently shown that *C. albicans* stimulates dendritic cells to release IL-23 (33). There is a complex interplay between these two microbes and CLA$^+$ T cells in psoriasis; however, the influence of microbes in psoriasis may be more complex that originally believed since microbiota studies demonstrate the presence of a range of microorganisms in psoriatic lesions (34). The functional relevance of these microorganisms for the disease has not been determined (35).

In summary, the observations made to date suggest that circulating CLA$^+$ T cells in psoriasis patients produce increased amounts of IL-17A, IL-17F, and IL-9, in comparison to healthy controls, when activated by *S. pyogenes* (**Figure 1**). Interestingly, the response to *C. albicans* is restricted mainly to CLA$^+$ T cells in cocultures with autologous epidermal cells in psoriasis with a similar cytokine profile. Psoriatic lesions produce several chemokines to attract skin-seeking CLA$^+$ T cells (21) with IL-17 capacity to the skin with potential to induce IL-17-dependent autoantigens and promote and maintain lesion activity. The study of the cutaneous immune response of CLA$^+$ T cells allows us to gain insight into how environmental factors, such microbes, shape psoriasis inflammation.

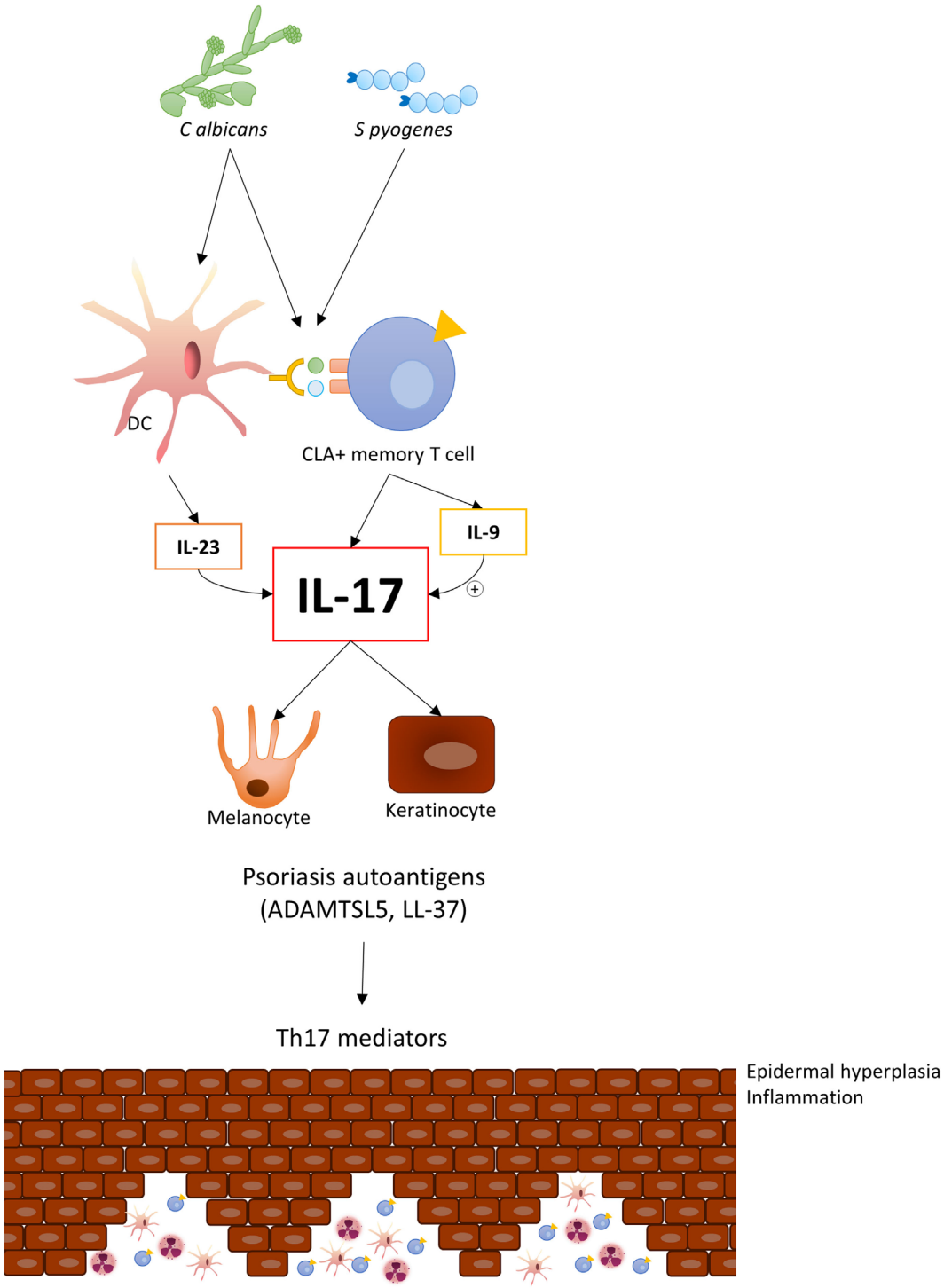

FIGURE 1 | *Candida albicans* and *Streptococcus pyogenes* induce IL-17 response in circulating CLA+ T cells in psoriasis, thereby indicating an established adaptive immune response to these microorganisms in this disease. Upon migration to cutaneous lesions, these cells react with those microbes and locally trigger IL-17 and IL-9 production, which will contribute to inducing psoriasis autoantigens.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

LFSB conceived the ideas and together drafted the manuscript. All authors revised and approved the final version of the manuscript.

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T Cell Hierarchy in the Pathogenesis of Psoriasis and Associated Cardiovascular Comorbidities

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The key role of T cells in the pathogenesis of cutaneous psoriasis has been well described in the last decade and the knowledge of the relative role of the different subsets of T cells in psoriasis pathogenesis has considerably evolved. Now, it is clear that IL-17A-producing T cells, including Th17/Tc17, have a central role in the pathogenesis of cutaneous psoriasis and therapies blocking the IL-17A pathway show high clinical efficacy. By contrast, the contribution of IFNγ-producing T cells has progressively become less clear because of the lack of efficacy of anti-IFNγ antibodies in clinical studies. In parallel, the role of $CD8^+$ T cells specific for self-antigens has been revived and increasing evidence now indicates that in psoriatic skin the majority $CD8^+$ T cells are present in the form of epidermal tissue-resident memory T cells. In the last years it also emerged the possibility of a contribution of T cell recirculation in the pathogenesis of psoriasis and its systemic manifestations. The aim of this review is to define a hierarchy for the different subsets of T cells in the T cell-mediated inflammatory cascade in psoriatic skin. This analysis will possibly help to distinguish the subsets that initiate the disease, those involved in the establishment of the self-sustaining amplification loop that leads to the cutaneous clinical manifestations and finally the subsets that act as downstream players in established lesions. Specific T cell subpopulations finally will be considered for their possible role in propagating inflammation at distant sites and for representing a link with systemic inflammation and cardiovascular comorbidities.

Keywords: psoriasis, skin, inflammation, psoriatic arthritis, TCR repertoire, comorbidities

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INTRODUCTION

Psoriasis is a chronically relapsing inflammatory disease of the skin affecting about 2% of the population worldwide.

Histologically, psoriasis is characterized by three principal features: epidermal hyperplasia, leukocyte infiltrate, and an increased number of tortuous and leaky vessels in the dermis (1–3). In recent studies, the presence of lymphoid aggregates/memory T cell clusters in the dermis of psoriatic plaques has been reported (4, 5).

20–30% of patients with psoriasis also develops psoriatic arthritis (PsA) and there is evidence that psoriasis is associated with systemic inflammation and with comorbidities, such as cardiovascular disease (6–12).

With regards to the pathogenesis, it is now emerging that psoriasis is an immune-mediated disease with a central autoimmune component mediated by T cells. Specifically, the disease pathogenesis

involves a dynamic interplay between dermal dendritic cells, T cells ($CD8^+$ autoreactive T cells, Th1, and Th17) and keratinocytes giving rise to a self-sustaining inflammatory cycle that develops around the TNF/IL-23/IL-17A axis (13–17). Despite this evidence, the hierarchical sequence of T cell-mediated events in the psoriatic inflammatory cascade is not completely defined. On the basis of the current literature and results of cytokine-blocking therapies it is possible to distinguish, in the T cell-mediated psoriatic inflammatory cascade, (i) an initial skin T cell activation phase, (ii) the establishment of chronic inflammation, (iii) the maintenance of clinically established lesions, and (iv) the egress from the skin of specific subsets of T cells that could possibly take part in the development of extra-cutaneous manifestations of psoriasis, including joint inflammation and cardiovascular comorbidities (18, 19).

T CELL HIERARCHY IN THE FORMATION OF PSORIATIC PLAQUES

T cell responses against self-antigens in psoriasis are initiated by dendritic cells in the dermis of pre-psoriatic skin (20). Mature dermal dendritic cells can produce TNFα and IL-23, present self-antigens, and stimulate the activation of autoreactive $CD8^+$ T cells together with a fraction of $CD4^+$ T cells polarized toward Th17 phenotype or the $IL\text{-}17^+IFN\gamma^+$ pathogenic Th1/Th17 as described by Annunziato and co-workers and Eisdmo and co-workers (20–23).

Activated T cells can migrate to the epidermis and recognize epidermal autoantigens and possibly progress toward differentiation to tissue-resident memory T cells (T_{RM}) characterized by $CD69^+CD103^+CCR7^-CD45RA^-CD62L^-$ phenotype (24, 25). Recognition of epidermal autoantigens by Tc1/Tc17 induces the secretion of cytokines, including IL-22, that mediate the initial phase of epidermal hyperproliferation and activation of keratinocytes (**Figure 1A**). These will produce chemokines and antimicrobial peptides, which lead to the progression of the inflammatory process (26–29).

Epidermal autoantigens include LL-37 antimicrobial peptide expressed by keratinocytes, keratin 17, and melanocyte-derived antigen ADAMTS-like protein 5 (ADAMTSL5) recognized by IL-17A-producing $CD8^+$ T cells, restricted by HLA-C*06:02 (26, 30–35).

This evidence together with early studies strongly supports that $CD8^+$ T cells represent the autoimmune core of the disease. In psoriatic skin lesions, $CD8^+$ T cells accumulate in the form of T_{RM} with a pathogenic IFNγ–IL-17A cytokine profile that were detected also in resolved psoriatic lesions and identified as potential mechanisms of site-specific disease memory (20, 25, 36–42).

The major role of $CD8^+$ T cells is also strongly supported by the fact that the main psoriasis risk gene is the HLA-class I allele HLA-C*06:02 on psoriasis susceptibility locus 1 on chomosome 6 and additional HLA-class I alleles are associated with psoriasis (43, 44).

Among tissue-resident T cells in human psoriatic skin, Clark and co-workers have recently shown that the vast majority has an αβ TCR (42), despite γδ T cells have been reported to play a role in the production of IL-17A and maintenance of inflammation (45, 46).

As regards to the TCR repertoire, studies of TCRBV chain sequencing showed mono or oligoclonal expansions of T cells mainly of $CD8^+$ lineage in psoriatic skin (47–49). TCRBV expansion indicates antigen-driven $CD8^+$ T cell responses, whereas the $CD4^+$ T cell fraction was reported to be polyclonal (50). In patients with cutaneous psoriasis, however, the presence of oligoclonal TCRBV expansion in peripheral blood is debated (51, 52). Two different studies of high-throughput sequencing of the entire TCR repertoire have shown that alongside with a limited oligoclonally expanded T cell subpopulation in psoriatic skin that was in part retained in resolved lesions, there was a vast majority of polyclonal T cells (42, 53). This data suggests that similarly to what has been described in PsA by Winchester and co-workers, antigen-driven mono and oligoclonally expanded T cell populations represent a limited component of the T cell reactions in psoriatic skin, whereas the vast majority is represented by polyclonal $CD4^+$ T cells that are not present in resolved lesions (54).

This evidence together confers to autoreactive $CD8^+$ T cells a dominant role in the plaque formation and in psoriasis recurrence and possibly a common role in psoriasis and PsA. It also underlines the importance of mechanisms of T cell plasticity and polarization toward pathogenic phenotypes.

With respect to CD8 T cell targetability, a recent study on a AGR mouse model of psoriasis has reported the accumulation of epidermal $CD8^+$ T cells during psoriasis development which was associated with IL-17A production and increased keratinocyte proliferation. Importantly, in this study injection of anti-CD8 antibody completely prevented psoriasis development (29).

Nevertheless, it is likely that Tc1/Tc17 autoreactive $CD8^+$ T_{RM} cells overlap their features and functions with the cells that under physiological conditions work as immune sentinels at barrier tissue. For this reason in a clinical setting, it could be difficult to target epidermal $CD8^+$ T_{RM} cells by the available immunotherapeutic tools without incurring in major adverse effects (20, 28, 29, 37).

As regards T helper cells, they have been reported to be more abundant in psoriatic skin lesions. While initially they were described mainly as Th1 cells, more recently the attention has been focused on IL-17-producing cells and the IL-23/Th17 axis clearly emerged as central in the control of the pro-inflammatory cycle in psoriatic plaques (14, 15). The high efficacy and fast outcome of IL-17A blocking therapies in resolving cutaneous clinical symptoms has strongly supported this concept (28, 55, 56). In addition, at skin level an expanded subset of T cells that produces IL-22 but not IL-17A (Th22) with a main role in the induction of acanthosis was identified. Importantly, these cells were found to be present also in resolved psoriatic lesions (57, 58).

In line with the key role of IL-17A, the main gene variants associated with psoriasis outside the MHC locus are single nucleotide polymorphisms, belonging to the IL-23/IL-17A axis (*IL23R*, *IL12B*, and *IL23A*) and the NF-κB pathway (*TNFAIP3*) (44, 59). It is, however, possible to hypothesize that Th17 or pathogenic Th1/Th17 cells could be expanded in the dermis as an event downstream of the epidermal autoimmune T_{RM} and occurs through bystander activation mechanisms like the one described by Winchester and co-workers in psoriatic synovial tissues (54).

In this view, the cycle that develops around the IL-23/IL-17A axis could involve some antigen-driven populations and a

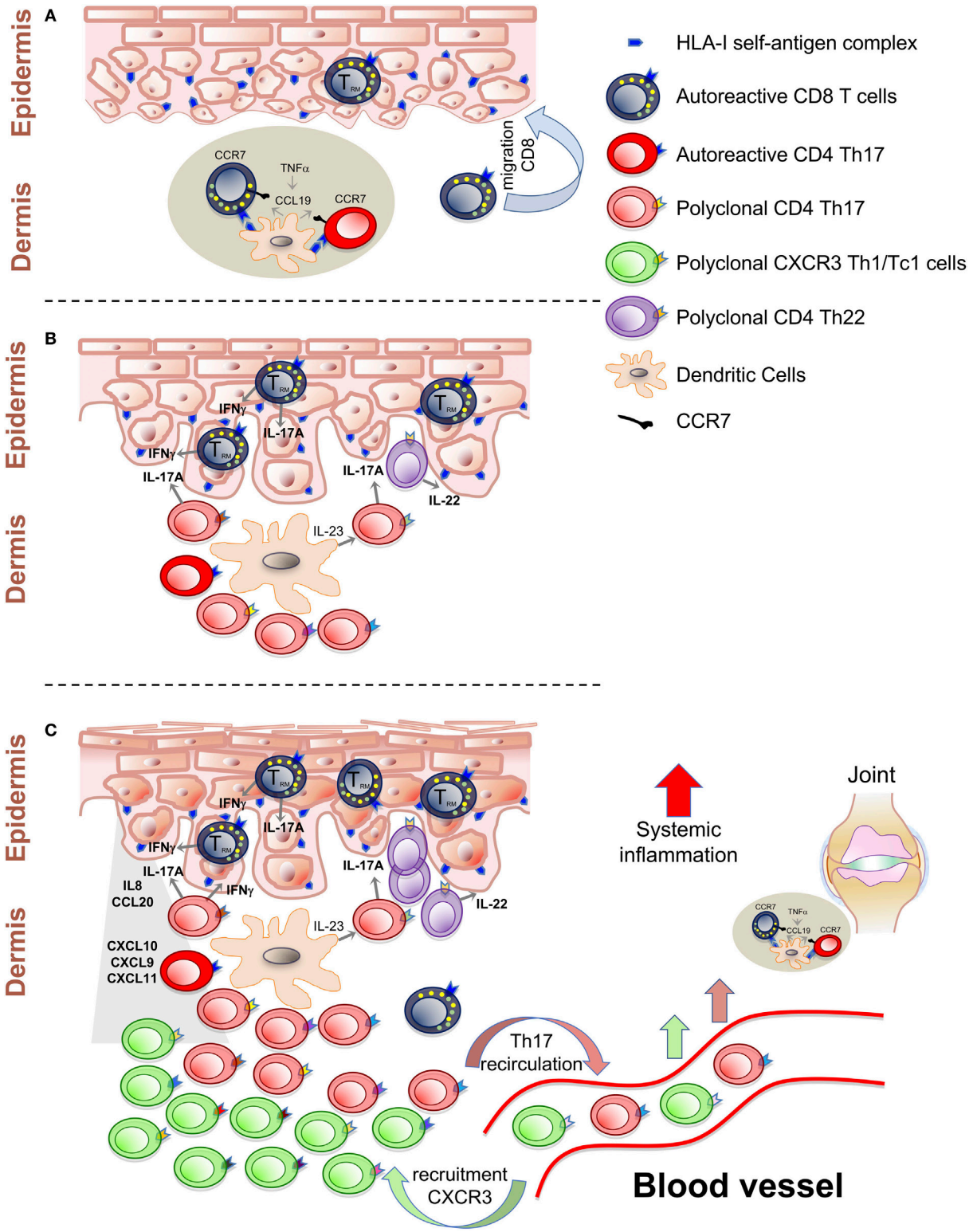

FIGURE 1 | T cell-mediated events in the psoriatic inflammatory cascade. **(A)** Activation of autoreactive T cells by self-antigens presented in the dermal lymphoid aggregates. Establishment of $CD8^+$ T_{RM} cells as central autoimmune component of disease pathogenesis and potential mechanisms of site-specific disease memory. **(B)** Polyclonal T cells proliferation and Th17/Tc17-mediated inflammation around the IL-23/IL-17A axis. **(C)** Recruitment of Th1/Tc1 cells, with multiple specificities, from the blood stream, induced by CXCL10 chemokines. Recirculation of T cells from the skin to the blood can spread inflammation at systemic level and at distant sites.

considerable fraction of polyclonally expanded T cells that can represent the second step of the pro-inflammatory cascade, responsible for the amplification of inflammation and for the exacerbation of clinical symptoms (**Figure 1B**).

As a consequence of the strong evidence of the central role of IL-17A-producing cells, the relevance of Th1 cells and IFNγ has become less clear. In lesional skin of psoriasis patients, Th1 cells and IFNγ levels are clearly increased (60, 61). However, in a small pilot study in patients with psoriasis, treatment with a humanized anti-IFNγ antibody induced improvement of histological and some clinical parameters but only minor therapeutic effects (62).

These controversial findings focus on the complexity of the interplay between the Th1/Tc1 and Th17/Tc17 cells in the pathogenesis of psoriasis and it is not clear how the marked increase of IFNγ and IFNγ-producing cells in psoriatic skin can actually link with the failure of IFNγ-blockade to show therapeutic efficacy.

Results from a study by Kryczek et al. have suggested that IFNγ exerts one of its effects by programming myeloid dendritic cells to produce CCL20, ligand of CCR6, and to secrete IL-23. This in turn would favor the recruitment and expansion of IL-17A-producing cells (63, 64). On the other hand, IFNγ mRNA is markedly upregulated in psoriatic plaques and noticeably IFNγ-induced genes, such as *CXCL9*, *CXCL10*, and *CXCL11* are strongly increased in psoriatic lesions (65).

In line with this evidence, we have previously reported gene expression data in psoriatic skin showing significant enhancement of *CXCR3* and *CXCL10* expression with an inverse correlation between the circulating fraction of $CXCR3^+$ $CD4^+$ effector memory T cells and the severity of cutaneous psoriasis (66, 67). Therefore, we can postulate an ultimate downstream phase in the psoriatic cascade, driven by the CXCL10/CXCR3 axis which induces the recruitment of Th1/Tc1 from the blood stream (**Figure 1C**).

T CELLS IN THE PATHOGENESIS OF PsA

Psoriatic arthritis develops in a fraction of patients with psoriasis and in the majority of cases it follows the development of the cutaneous disease by a mean of 10 years (68).

In addition to the skin, PsA targets the attachment sites of ligament to bone (entheses), the peripheral joints, and the spine (12, 69).

Enthesitis is indeed a distinctive feature of PsA and it has been hypothesized that, in PsA joints, inflammation can start from the entheses. The disease progression, in patients with PsA, can finally lead to destructive bone loss and 67% of patients exhibit signs of erosive bone disease (70).

Similarly to psoriasis, T cells are involved in the pathogenesis of PsA and reduction of $CD3^+$ cells in PsA synovium correlated with the clinical response to biological therapies (71).

In PsA patients, Canete and co-workers have evidenced the presence of lymphoid aggregates in synovial tissues that was significantly reduced by TNF-blocking agents. This result could be paralleled by the observation of lymphoid aggregates in psoriatic skin and the role of CCR7/CCL19 axis, modulated by TNF in the initial clustering of dendritic cells and T cells in the dermis (4, 5, 72–75).

In line with the concept of shared pathogenic mechanisms between psoriasis and PsA, Belasco and colleagues provided the evidence that gene expression in PsA synovium was more closely related to gene expression in the PsA patient skin than to gene expression in the synovium of patients with other forms of arthritis. *IL17* gene, however, was upregulated more in skin than in the synovium, whereas *TNF* and *IFN*γ were similarly upregulated in both tissues (76).

As regards the TCR repertoire analysis in PsA joints, Tassiulas et al. (47) showed oligoclonal and monoclonal T cell expansions in the synovial tissue, some of which were shared with the skin. In a subsequent study, Curran et al. performed TCR β-chain nucleotide sequencing in peripheral blood and synovial tissues/fluid showing that 76% of the clones in inflamed tissues were polyclonal, whereas 12% of the expanded clones had structurally homologous CDR3 β-chain sequence and were only $CD8^+$ in lineage (54). Interestingly, some of the expanded $CD8^+$ clones identified in the synovial tissue were present also in peripheral blood and joint fluid.

A second population of moderately expanded inflammation-related clones, that were either of $CD4^+$ or $CD8^+$ lineage, was identified only in inflamed synovial tissue and joint fluid. These cells could represent a secondary consequence of the inflammation induced by other proliferating clones.

Finally, one major population consisted of unexpanded polyclonal $CD4^+$ T cells that did not persist in the tissue during methotrexate treatment, were most likely effector memory $CD4^+$ T cells recruited by inflammatory chemokines released by other cell populations. The lack of clonal expansion in $CD4^+$ T cells suggests a lower hierarchical role in driving inflammation.

These findings together suggest a role for cognate T cell responses in the pathogenesis of PsA and further suggest that T cell clones specific for identical or homologous antigens in skin and synovium may represent central elements in promoting inflammation in both tissues (47).

It remains to be established how self-reactive T cell responses in the skin, can be mechanistically linked to the one found in the synovial fluid and how these events can occur with years of time-lapse.

To this end, genetic association studies can provide some interpretation keys. In addition to the HLA-C*06:02 which is common with psoriatic plaque, additional HLA-alleles are associated with PsA. These HLA-alleles include B*08:01:01, B*27:05:02, B*38:01:01, and B*39:01:01 (77).

This underlines the importance of $CD8^+$ T cells recognizing HLA-class I associated self-antigens in the pathogenesis of PsA and suggests that the autoimmune basis of PsA may be even broader than the one of cutaneous psoriasis. In this view, enthesis that anatomically links mechanical stress to immunologically active tissue (synovium), could be central for the pathogenesis (78).

Entheses are commonly subject to microdamage associated with local cytokine release, which may evolve into subsequent inflammation (79, 80). Inflammation in turn can favor cross-presentation of self-antigens.

It is, therefore, possible that recirculation of pathogenic skin T cells recognizing common self-antigens can start subclinical inflammation by localizing to the synovioenthesial complex (**Figure 1C**).

Patients with psoriatic disease have shown an increased level of circulating $CCR6^+$ $CD4^+$ T_{EM} and T_{EFF} cells that correlated positively with systemic inflammation (66). Therefore, it is possible to hypothesize that a fraction of IL-17-producing T cells recirculates from the skin and relocalize to the entheses.

This is reinforced by the findings of Sherlock et al. reporting that IL-17A$^+$ cells locate mainly at the entheses and synovial tissues and by the evidence that among the genetic risk factors outside the MHC locus there are gene variants falling into the IL-23/IL-17A axis (77, 80–85). Importantly, it has been reported that IL-23 is expressed in synovial membrane with ectopic lymphoid tissue (78).

The fraction of IL-17A-producing cells recirculating from the skin could, therefore, determines subclinical inflammation and favor, in the long-term, cross-presentation of self-antigens and epitope spreading phenomena (73, 78).

In established PsA, it has also been reported an increased percentage of IL-17A-producing CD8$^+$ T cells in synovial fluid which correlate with bone erosion and disease severity (86–88).

In the light of a hierarchical T cell clonality in psoriatic tissue and the evidence of the multiple-specific clones, including EBV-specific clones, observed in synovial fluid, we can postulate that massive recruitment of T cells with Th1/Tc1 phenotype from peripheral blood occurs as a consequences of inflammatory chemokine release (54). By analogy with the observation in psoriatic skin and by evidence from the data provided by Gladman and co-workers it is possible that CXCL10/CXCR3 axis can act as a downstream cell recruitment mechanism of tissue inflammation common to cutaneous psoriasis and PsA (89–91).

T CELLS IN PSORIASIS-ASSOCIATED CARDIOVASCULAR COMORBIDITIES

Increasing epidemiological and clinical evidence indicates that psoriasis is an independent risk factor for cardiovascular disease (92, 93).

As a consequence of poorly controlled tissue inflammation, psoriasis patients can develop systemic inflammation and atherosclerosis. In this process, it has been postulated that inflamed tissue-derived factors or cells may directly affect distant vessels for the development of athero-thrombosis (7, 94, 95). Nevertheless, the cellular mechanisms that link the cutaneous manifestations of plaque psoriasis with the initiation and progression of atherosclerosis in psoriasis patients are largely unknown.

A recent study on human tissues showed that psoriasis and atherosclerosis exhibit significant overlap of their transcriptomes and in particular that *TNF*, *IFNg*, and IFNγ-induced genes, which are common between psoriasis and atherosclerosis may provide the link between the two diseases (96). By contrast, *IL-17A* and *CCL20* were higher in psoriasis than in atherosclerotic tissue, whereas *IL17R* gene was expressed at similar levels.

Because of the link between IL-17A and neutrophil infiltration in atherosclerotic plaques and its key role in the pathogenesis of psoriasis it has been suggested that the IL-17A/neutrophil axis could take part to atherogenesis associated with psoriatic disease (97). Nevertheless, the role of IL-17A in psoriasis-associated atherosclerosis is still controversial. Indeed, Usui et al. reported that IL-17A deficiency protected against atherosclerosis in apoE$^{-/-}$ mice due to reduced macrophage infiltration and inflammatory cytokine secretion in the lesions (98).

Other mouse studies have indicated that IL-17A may promote plaque stability by contributing to fibrous cap formation (99). Collectively, the results indicate that IL-17A may exert both anti- and pro-atherogenic effects, depending on the inflammatory context. However, further studies will be necessary to clarify the contribution of T cells recirculating from the psoriatic plaque in the development of atherosclerosis.

IMPLICATIONS FOR THE DEVELOPMENT OF THERAPEUTIC PROTOCOLS

From this analysis it emerges a differential contribution of the individual subsets of T cells in the pathogenesis of psoriasis, PsA, and associated cardiovascular comorbidities namely, atherosclerosis. In particular, TNFα has a relevant role in inducing the CCL19/CCR7-mediated formation of clusters of dendritic cells and T cells in both psoriasis and PsA. It also emphasizes the role of IL-23/IL-17 axis in the amplification loop critical for the clinical manifestations of cutaneous psoriasis and possibly in the initial phase of join inflammation.

On the other hand, the possibility of a third step of CXCL10/CXCR3-mediated recruitment of Th1/Tc1 cells from the blood stream may explain the apparent controversy between the high amount of IFNγ-producing cells and the low therapeutic efficacy of anti-IFNγ antibody treatment.

CONCLUSION

By defining the hierarchy of the T cell-mediated events of the psoriatic cascade in psoriatic plaques it is possible to distinguish a core antigen-driven oligo or monoclonally expanded autoreactive CD8$^+$ T cell component, a secondary self-reactive CD4$^+$ T cell component, a polyclonal CD4$^+$ Th17 and pathogenic Th1/Th17 population amplified by the IL-23/IL-17A axis, and a downstream recruitment of CXCR3$^+$ T cells with different specificities induced by the upregulation of CXCL10 chemokine. Similarly in PsA the analysis of the TCR repertoire has evidenced central antigen-driven expansion of mainly CD8$^+$ T cells and broad polyclonal CD4$^+$ T cells expansion. In addition, a group of common clones were also expanded in peripheral blood of patients with PsA and T cell clones specific for identical or homologous antigens were present in both skin and in synovial tissues. These may represent central elements in promoting inflammation in both tissues. In addition, oligoclonally expanded clones in peripheral blood of patients with PsA may also suggest that T cell recirculation can represent a mechanistic link between skin and joints inflammation.

AUTHOR CONTRIBUTIONS

FC wrote parts of the manuscript and prepared the figures. PP collaborated to the writing. PS and RG discussed the content and critically revised the manuscript. ER supervised the work and wrote the final version of the manuscript.

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REVIEW
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The Interleukin-23/Interleukin-17 Axis Links Adaptive and Innate Immunity in Psoriasis

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Research into the pathophysiology of psoriasis has shed light onto many fascinating immunological interactions and underlying genetic constellations. Most prominent among these is the crosstalk between components of the innate and the adaptive immune system and the crucial role of interleukins (IL)-23 and -17 within this network. While it is clear that IL-23 drives and maintains the differentiation of Th17 lymphocytes, many aspects of the regulation of IL-23 and IL-17 are not quite as straightforward and have been unraveled only recently. For example, we know now that Th17 cells are not the only source of IL-17 but that cells of the innate immune system also produce considerable amounts of this central effector cytokine. In addition, there is IL-23-independent production of IL-17. Besides other innate immune cells, neutrophilic granulocytes prominently contribute to IL-17-related immune regulations in psoriasis, and it appears that they employ several mechanisms including the formation of neutrophil extracellular traps. Here, we strive to put the central role of the IL-23/IL-17 axis into perspective within the crosstalk between components of the innate and the adaptive immune system. Our aim is to better understand the complex immune regulation in psoriasis, a disorder that has become a model disease for chronic inflammation.

Keywords: psoriasis, interleukin-17, interleukin-23, innate immunity, adaptive immunity

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INTRODUCTION

Psoriasis has evolved into an instructive model disease for many immune-mediated disorders. Numerous different types of immune cells are involved in the disease process (**Figures 1A–E**). Our increasing understanding of pathophysiological principles has facilitated the development of effective therapies. Perhaps equally important, such therapies have taught us a lot about disease mechanisms (1, 2). In consequence, both research into the pathophysiology and targeted treatments of psoriasis have been and still are progressing hand-in-hand. Psoriasis-directed precision medicine illuminates vividly how pieces of the immunological mosaic fall into place to ultimately improve our patients' lives. In this light, we here discuss immunological mechanisms governing the pathogenesis of psoriasis with a certain emphasis on links between the adaptive and the innate immune system. We believe that such interactive and dynamic links are of paramount importance for complex immune regulations in general and are key to successful therapeutic interventions.

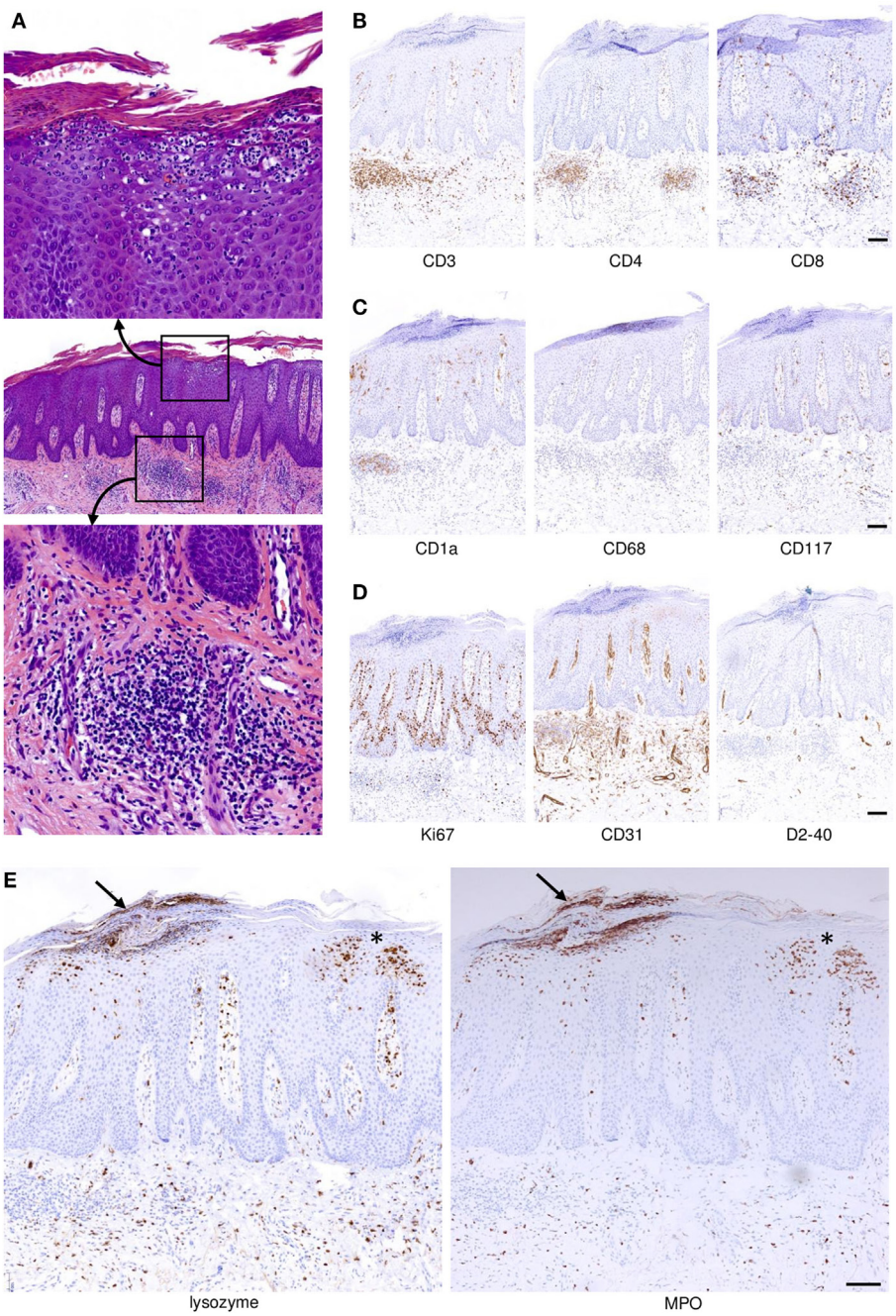

FIGURE 1 | Spatial distribution and compartmentalization of cells of the immune system in psoriatic skin. **(A)** A full-fledged psoriasis lesion was biopsied and stained with hematoxylin and eosin. Within the profoundly altered epithelial and mesenchymal compartments there are abundant cells of the adaptive and innate immune system (middle panel). The indicated magnified insets depict focal accumulations of neutrophilic granulocytes underneath and within the epidermal *stratum corneum* (upper image) and focal dermal aggregations of lymphocytes (admixed with other immunocytes; bottom image). **(B)** T cells indicated by expression of CD3 (left photomicrograph) reside within both the dermal compartment and, albeit to a lesser extent, the epidermis. CD4+ T cells are more abundant compared to CD8+ T cells, but epidermal T cells are almost exclusively CD8+. **(C)** Langerhans cells expressing CD1a are not only found in the epidermis but also within the dermal inflammatory infiltrate of psoriatic skin. The majority of macrophages expressing CD68 reside within the dermis, and a smaller proportion migrates up into higher layers of the epidermis. Mast cells expressing CD117 are present in the perivascular area and directly underneath the hyperplastic epidermis. **(D)** Highly increased proliferation of keratinocytes with some suprabasal proliferative activity is indicated by staining with Ki67, and dermal blood vessels are vastly increased in number and size as visualized by staining for CD31. By contrast, lymphatics identified by the D2–40 antibody are not significantly increased. **(E)** Neutrophilic granulocytes expressing lysozyme (left image; lysozyme is also expressed by some macrophages) and myeloperoxidase (MPO, right) migrate upward through the epidermis forming the telltale spongiform pustules of Kogoj within the *stratum spinosum* (asterisk near the right-hand margins of the images) and microabscesses of Munro directly underneath and within the *stratum corneum* (arrow near the left-hand-margin of the images). All images represent sequential sections of the same biopsy specimen. Scale bars = 100 μm.

ON THE BRINK OF UNDERSTANDING: THE LINK BETWEEN GENETICS AND IMMUNITY IN THE PATHOGENESIS OF PSORIASIS

Psoriasis is a systemic chronic inflammatory disease with primary manifestations on the skin and joints, and associations with a number of systemic comorbid diseases. The disorder has an immunogenetic basis and can be provoked by extrinsic or intrinsic stimuli. The familial occurrence of psoriasis evinces the relevance of genes for its pathogenesis (3). Several dozens of gene loci have been associated with psoriasis (so-called psoriasis susceptibility loci) (4, 5). Genome-wide association studies (GWAS), which also take into account single-nucleotide polymorphisms, associate the risk of psoriasis with genes that encode factors of antigen presentation and the innate and adaptive immune system. Psoriasis is associated with several human leukocyte antigens [HLA, also termed major histocompatibility complex (MHC)] class I genotypes. This applies to both skin psoriasis (HLA-C*06 and HLA-B*57) and psoriatic arthritis (PsA; HLA-B*27 and HLA-B*39). Patients with given HLA genotypes can be assigned to certain clinical characteristics of psoriasis as well as functional immunological parameters. Likewise, the detection of certain autoantigens depends on HLA genotypes such as HLA-C*06:02 (6). Potential autoantigens in psoriasis include peptide fragments of keratin 17 with sequence homologies to streptococcal M-proteins (7, 8), the antimicrobial peptide LL37 (9), and the melanocytic autoantigen ADAMTSL5 (10). While LL37 can activate both CD4+ T helper cells (Ths) and CD8+ cytotoxic T cells, ADAMTSL5 only activates CD8+ T cells. Interestingly, both peptides are recognized by the immune system after binding to HLA-C*06:02. This underlines the importance of certain HLA genotypes for the development of psoriasis.

A group of psoriasis-associated polymorphisms were found in genes encoding transcription factors such as REL, TYK2, STAT3, or RUNX3 (3). The transcription factor REL belongs to the NF-κB-family and is involved not only in the regulation of different inflammatory factors, but also in the regulation of keratinocyte proliferation (3, 11, 12). The Janus kinase (JAK) TYK2 is involved in the signal transduction of interferons and cytokines such as interleukin (IL)-12 and IL-23. The association with the transcription factor STAT3 is of particular interest, since STAT3 is essential for the differentiation of Th17 cells on the one hand and regulates the expression of IL-23R on the other (13). Furthermore, STAT3 activation in keratinocytes has a proliferation-promoting effect. The transcription factor RUNX3 is involved in the pathogenicity of autoreactive Th17 cells (14). Another important genetic association to psoriasis is the gene TRAF3IP2, which encodes the protein Act1, which is part of the signal cascade of IL-17.

Genome-wide association studies analyses also revealed psoriasis-associated genes encoding cytokines and cytokine receptors (3). These include the IL12B, IL23A, IL23R, and IL4/IL13 gene loci. The heterodimeric cytokine IL-23, one of the most important mediators in the immunopathogenesis of psoriasis, is composed of the gene products of IL12B (p40) and IL23A (p19).

Pustular psoriasis may represent a distinct entity, at least in a considerable proportion of cases. Recent studies have revealed associations of generalized pustular psoriasis with mutations in the genes of CARD14 and IL36RN (15–17) and, as a consequence, several studies to block either IL-36 or the IL-36 receptor (IL-36R) are underway (18). Furthermore, mutations in the AP1S3 gene, encoding the AP-1 complex subunit σ1C, which lead to a disruption of the endosomal translocation of toll-like receptor 3 (TLR3), are associated with pustular psoriasis (19).

Palmoplantar pustulosis is associated with missense mutations in CARD14, but not IL36RN (20). CARD14 is expressed by keratinocytes and endothelial cells, and mutations in this gene lead to increased activation of NF-κB. IL-36RN is a natural antagonist of the IL-1 family cytokine IL-36 (21). The consequence of mutations within the IL36RN gene is an increased production of NF-κB-regulated messengers (3, 22). IL-36 is also relevant for clonal responses of Th17 cells in patients with generalized pustular psoriasis (23). However, many patients with generalized pustular psoriasis and the vast majority of localized pustular psoriasis do not share mutations in the IL-36RN gene (24).

ADAPTIVE IMMUNITY AND THE IL-23/IL-17 AXIS IN THE PATHOGENESIS OF PSORIASIS

The pathogenesis of psoriasis is thought to be based on tight interactions between components of the innate and adaptive immune system (1, 3, 22, 25, 26). Several classical studies underscores the importance of T cells for the pathogenesis of psoriasis: the disease can be improved by cyclosporin A (27) or other drugs that inhibit the function (e.g., CD2 blockade) or recruitment (e.g., LFA-1 blockade) of T cells (28, 29). A similar effect can be achieved by IL-4, which shifts the cytokine environment toward a Th two-weighted immune response (30), with a likely attenuation of the Th17 function due to decreased IL-23 production in antigen-presenting cells (31). IL-10 can also reduce psoriatic symptoms by influencing T cell functions (32). Psoriasis can be triggered by bone marrow transplantation (33) and, like other autoinflammatory diseases, it shows the above-mentioned association with certain HLA expression patterns (34–36). Finally, psoriatic skin inflammation in animal models without pre-existing epithelial changes can be induced by certain CD4+ T cells alone (37, 38), and T cells induce psoriatic lesions in transplanted human skin (39–41). These older studies have culminated in the more recent and above-mentioned discovery of potential autoantigens in psoriasis (8–10).

In recent years, few areas in immunological science have attracted as much attention as the research on Th17 cells, a group of CD4+ T lymphocytes that differ from the "classical" Th1 and Th2 cells (42) and which were named after their production of IL-17 (**Figure 2**). Th17 cells are prominently involved in the pathogenesis of psoriasis (43–45), palmoplantar pustulosis (46), as well as other chronic inflammatory diseases (47, 48). Even healthy human skin contains some IL-17-producing T lymphocytes (49), which suggests their involvement in immune surveillance. Changes in the number and activation state of Th17 cells lead to

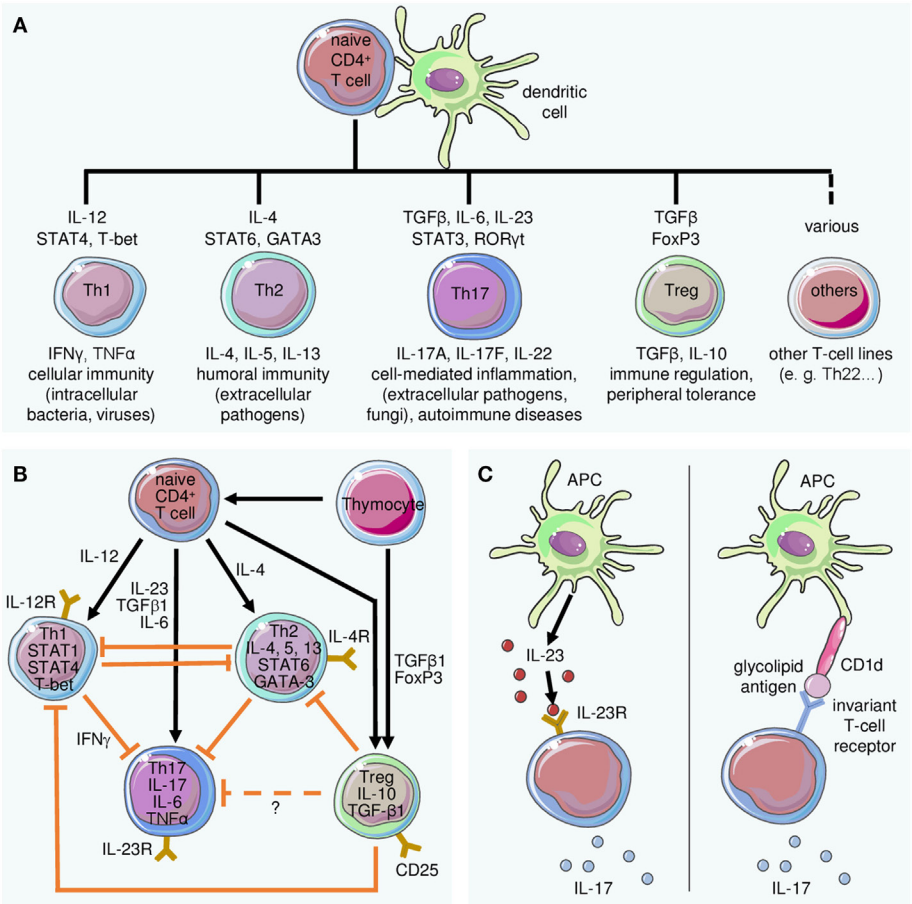

FIGURE 2 | Differentiation of T cell subsets and lineage-defining role of interleukin (IL)-23 for Th17 cells. **(A)** The differentiation of T cell subsets from naive T cells requires stimulation by dendritic cells and specific mediators. Key cytokines and transcription factors driving differentiation of the indicated populations are depicted above the respective T cell type, while their primary function is indicated below. **(B)** The differentiation of Th17 cells is embedded in a complex regulatory network. Antigen presentation by dendritic cells and cytokine stimulation lead to differentiation of effector cells including (but not limited to) Th1, Th2, or Th17, the latter induced by IL-23 in conjunction with other mediators. Regulatory T cells (Treg) inhibit differentiation and effector functions of Th1 and Th2 cells. Their effect on Th17 cells is not exactly known. **(C)** Besides the classical IL-23-dependent stimulation of IL-17 secretion (left panel), IL-23-independent induction of IL-17A may occur in response to presentation of glycolipid antigens by CD1d (right panel).

a dysbalance between these and regulatory T cells, resulting in inflammation (43, 50, 51). Circulating effector memory T cells can be stimulated by streptococcal extracts to produce Th17 cytokines and stimulate keratinocyte proliferation (50). In turn, activated keratinocytes stimulate the IL-17 production of T cells, which culminates in a positive feedback loop (52) (**Figure 3**). In psoriatic skin, IL-17 is not only produced by CD4+ Th17 cells but also by CD8+ T cells (53) and, as alluded to below, by cells of the innate immune system. IL-17A is the most important of the six known IL-17 isoforms for the pathophysiology of psoriasis (45).

Although the initiation of development from naive precursor cells has not yet been fully clarified, a robust body of evidence supports the notion that IL-23 produced by myeloid cells is essential for terminal differentiation and the preservation of Th17 cells (54–56) (**Figure 2**). This activity is conveyed *via* the IL-23 receptor expressed on naive T cells, together with other cytokines and their receptors such as TNFα, IL-1, and IL-6. This differentiation in combination and balance with Th1 cells is of central importance for the pathogenesis of psoriasis (57) and other chronic inflammatory diseases (58). The Th17-mediated inflammation can be modulated by exogenous factors such as vitamin D3 or UV radiation (59, 60), or by other cytokines such as IL-9 (61).

In addition to IL-23-dependent production of IL-17A, a series of studies has demonstrated an alternative route which is independent of IL-23 and has been described, for example, for γδ-T cells (a subset of the so-called "unconventional" T-cells) or invariant natural killer cells (62–65) (**Figure 2C**). However, implications of this alternative pathway on the course of inflammatory diseases or potential side effects following blockade of either IL-23 or IL-17 are not entirely clear yet.

In general, unconventional T-cells appear to merit further studies in the context (66). They include CD1-restricted T cells, MR1-restricted mucosal-associated invariant T cells (MAIT cells), MHC class Ib-reactive T cells, and the above-mentioned γδ

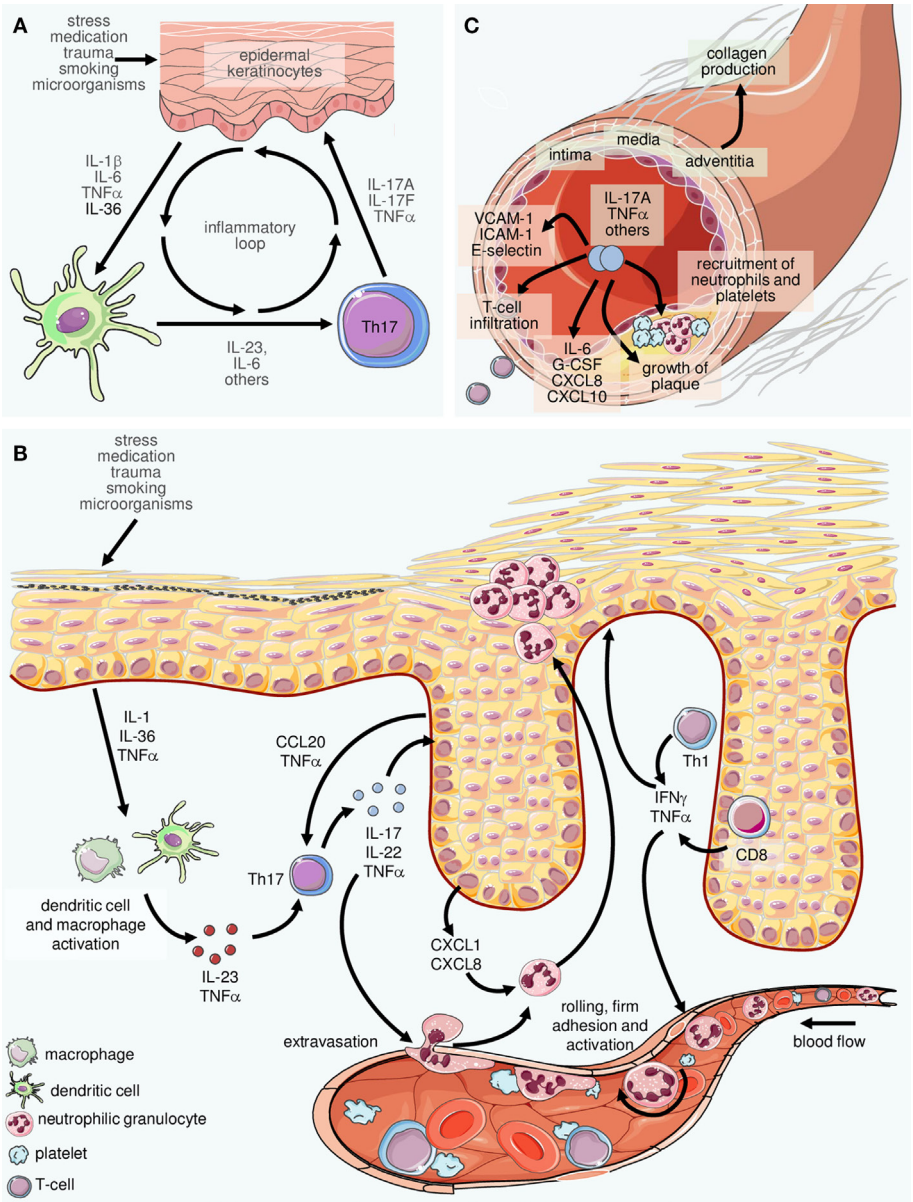

FIGURE 3 | Inflammatory loops in psoriasis involving innate and adaptive immune cells. **(A)** Inflammatory cytokines including interleukin (IL)-23 and IL-6 produced by cells of the innate immune system such as dendritic cells and macrophages facilitate the differentiation of Th17 cells. The latter secrete IL-17 and other mediators which stimulate epidermal cells to produce cytokines and chemokines that attract and activate cells of the innate immune system. The result is an inflammatory loop or "vicious circle" in which IL-23 and IL-17 play central roles. **(B)** On a larger scale, a complex network of inflammatory mediators connects virtually all resident and immigrating cells within the skin. In fact, this machinery can be considered the core of psoriasis pathophysiology. The examples depicted here (in reality, there are considerably more players orchestrating the pathophysiology of psoriasis) highlight the intertwined crosstalk of cells of the innate and the adaptive immune system with activated resident cells such as vascular endothelial cells and epidermal keratinocytes. Such interactions can explain most, if not all, hallmark features of psoriasis such as, on the one hand, recruitment, activation, spatial compartmentalization, and disease-promoting differentiation of cells of the immune system, as well as, on the other hand, pathological changes of resident tissues such as the epidermis and the cutaneous vasculature. The alterations extend to additional skin components not depicted here such as cutaneous nerves and the connective tissue. **(C)** Inflammatory mediators such as IL-17 and TNFα are present at elevated levels in the serum of psoriasis patients. Their systemic activity also facilitates vascular changes, thus contributing to the accrual and course of comorbid diseases, in particular cardiovascular disorders (depicted here is atherosclerosis).

T cells. A role of these cells in chronic inflammatory disorders is currently emerging, although actual data in psoriasis are still scant (66). For example, MAIT cells seem to be altered or activated in patients with inflammatory bowel disease, psoriasis, or autoimmune diseases (67–69). However, further studies are needed to assess the role of the other populations in psoriasis.

Innate lymphoid cells (ILCs) also appear to be promising new candidates to reveal novel aspects in the pathogenesis of

psoriasis. ILCs are a heterogeneous group of innate immune cells, characterized by their lack of somatic rearrangement of antigen-specific receptors. They are divided into subsets according to their function, cytokine profile, and transcription factors (NK, ILC1, ieILC1, ILC2, ILC3, LTi, and ILCP) (70). Recent studies show considerable diversity of ILCs between and within these major subsets. Interestingly, the group 3 ILCs are characterized by the ability to produce Th17-like cytokines and express the transcription factor RORγt, traits which are reminiscent of Th17 T-cells. Even more strikingly, as a subset of group 3 ILCs is able to generate both IL-17 and IL-22 (70), it is reasonable to assume that these cells play a role in psoriasis and other chronic inflammatory diseases. Nevertheless, more research is needed to solidify these initial findings.

The still young field of IL-17 research has recently experienced a paradigm shift due to the observation that not only Th17 cells but also cells of the innate immune system and resident skin cells can secrete IL-17 (71–73). Among the cells that produce IL-17 are mast cells and neutrophilic granulocytes. It appears that the presentation of IL-17 through so-called neutrophil extracellular traps (NETs) may also play a role (74). The production of IL-17 by different cell types in psoriatic skin and its effect on different target cells could explain why targeted blocking of IL-17 by new drugs works so quickly and effectively.

The IL-23/IL-17 axis clearly illustrates the close interaction of different components of the innate immune system (in this case IL-23-producing myeloid cells, granulocytes, macrophages, and mast cells) with cells of the adaptive immune system (Th17- and IL17-producing CD8+ T cells) in psoriasis. Translational research into the immunology of this "model disease" has given us fascinating insights into the complex pathogenesis of chronic inflammation including comorbid diseases (**Figure 3**).

NOT LOST IN TRANSLATION: THERAPIES TARGETING THE IL-23/IL-17 AXIS

The discovery that the IL-23/IL-17 axis is of major importance for the pathogenesis of psoriasis has been confirmed by the efficacy of new therapeutics (1): in 2009, Ustekinumab (Stelara®), a monoclonal antibody that inhibits the p40 subunit found in both IL-12 and IL-23, was approved for the treatment of psoriasis (75). The development of this compound began when it was assumed that IL-12 was significantly involved in the development of psoriasis (76). It was somewhat fortunate that Ustekinumab also inhibited IL-23, which is now considered to be more pathogenetically relevant than IL-12 (77). Guselkumab (Tremfya®), which specifically neutralizes human IL-23 by blocking the p19 subunit, has been approved recently and shows very good efficacy against psoriasis (78). In fact, circumventing a potential unwanted effect of concomitant blocking of IL-12 (**Figure 2B**) by specific blockade of IL-23 may account, at least in part, for the seemingly higher efficacy of guselkumab as compared to ustekinumab. Several further anti-p19 antibodies, in addition to a large number of other anti-psoriatic agents, are currently in phase 3 clinical trials. The direct blockade of IL-17A by Secukinumab (Cosentyx®) and Ixekizumab (Taltz®) also leads to a convincing improvement in psoriasis (79, 80). Similarly, the blockade of the IL-17 receptor by brodalumab (Siliq®, Kyntheum®) was very effective (81, 82).

In addition to specifically targeting the IL-23/IL-17 axis, this pathway is also modulated by more broadly acting classical compounds. Two examples of orally available pharmaceuticals highlight this notion: fumaric acid esters and apremilast are registered for the treatment of psoriasis. The main component of the fumaric acid ester preparation Fumaderm® is dimethyl fumarate (DMF), which was recently approved as a single-substance medication (Skilarence®). DMF reduces the production of IL-23 and IL-12 in DC and promotes the production of the anti-inflammatory messenger IL-10 (83). It shifts the Th17/Th1 dominated immune response—similar to IL-4—toward an IL-4+ Th2 phenotype. In patients treated with DMF-containing preparations, fewer IL-17+ and IFNγ+ T cells are found, and IL-4+ Th2 cells increase (83). DMF can also reduce the endothelial recruitment of immune cells (84, 85). The phosphodiesterase 4 inhibitor apremilast (Otezla®) has a somewhat similar immunomodulating effect with respect to the IL-23/IL-17 axis. This inhibitor also diminishes the production of IL-23, IL-12, TNFα, and IFNγ and, like DMF, it increases the formation of the anti-inflammatory cytokine IL-10 (86).

New classes of immune modulators are JAK inhibitors and other tyrosine kinase inhibitors, which we know from the treatment of malignant diseases (87). These kinases are associated with cytokine receptors and are therefore also important for immune regulation, e.g., several cytokine receptors require the activation of JAKs for their signal transmission (88). In the pathogenesis of psoriasis, receptors of the cytokines IL-6, IL-12, IL-21, IL-22, IL-23, IFNα, and IFNγ are of particular importance in this respect (87). Selective inhibitors have been developed, and a number of JAK inhibitors for psoriasis are in phase 2 and 3 clinical trials (89). However, it remains to be seen whether the potential risks of infections under this treatment will limit the broad systemic use of JAK Inhibitors.

Given the importance of IL-23/IL-17 signaling in psoriasis and the expression of the transcription factor RORγt in Th17 cells, a blockade of RORγt with orally administered drugs is also aimed at (90). Pre-clinical studies showed positive results. A tangible goal are personalized therapies and prediction of individual therapeutic success of selected drugs, also known as "Precision Medicine" (91, 92).

BRIDGING THE GAP: COMMUNICATION BETWEEN INNATE AND ADAPTIVE IMMUNE SYSTEM IN PSORIASIS

While the puzzle of the multifactorial immunogenetic pathology of psoriasis is emerging ever more clearly (93), the mechanisms of its first manifestation are quite far from being understood. Infections with streptococcal bacteria and medications such as lithium or β-blockers have been described as triggers. Mechanical stress eliciting an isomorphic irritant effect (Köbner's phenomenon) may explain the symmetrical localization of psoriasis, for example, on the elbows and knees. Minimal trauma may lead to responses with rapid immigration and activation of immune cells

such as neutrophilic granulocytes and T cells (71), some of which seem to react in the above-mentioned autoimmune fashion (9, 10). Amplifying feedback loops between T cells as representatives of the adaptive immune system and neutrophilic granulocytes and keratinocytes of the innate immune system finally lead to an amplification and chronification of the immune response (**Figure 3**). In addition, ILC with traits of both innate and adaptive immunocytes and the capacity to produce IL-17 and IL-22 have entered the stage very recently (94, 95).

Characteristic for keratinocytes in psoriatic plaques are their increased proliferation rate, altered differentiation and production of antimicrobial peptides and proteins (AMP). AMPs are the first line of innate immune defense. Due to their specific properties (positive charge, hydrophobicity, and amphiphilic properties) they can form pores and thus exert their antimicrobial functions. Owing to their strong pro-inflammatory properties, these peptides have also been called alarmins. Many studies have been carried out on cathelicidin (LL37), which is expressed at elevated levels in psoriatic skin (96) and has a direct stimulatory effect on keratinocytes (97). In addition, the positively charged LL37 is able to form immunostimulatory complexes with negatively charged DNA and RNA. These complexes are taken up by myeloid dendritic cells (mDC) and plasmacytoid DC (pDC), where RNA motifs stimulate TLR7 and 8 and DNA leads to the stimulation of TLR9 (98–100). TLR7/8-stimulated myeloid DC secrete the messenger substances TNFα, IL-23, and IL-12, while pDC produce large amounts of interferon α <χιτ>(93).

In addition to LL37, S100 proteins are important for the pathogenesis of psoriasis. An important stimulus for the production of S100A7 (psoriasin) and S100A15 (koebnerisin) by keratinocytes is IL-17A (101). Both AMPs have pro-inflammatory properties (102). The calgranulins, S100A8 and S100A9, are produced by myeloid cells and keratinocytes. They stimulate the proliferation and cytokine production of keratinocytes (103) and are able to facilitate a T cell-dependent autoimmune response in murine models (104). Defensins are also alarmins produced by keratinocytes in psoriasis plaques and, similar to LL37, human β-defensin 2 and 4 are known to bind DNA and stimulate pDC in a TLR9-dependent manner (105). DC performs important regulatory functions in psoriasis. Activated by alarmins of keratinocytes and neutrophils, they stimulate pathogenetically important T cells (93). Primary activation and programming of relevant Th17/Th1 and Th22 cells takes place in the lymph node. Activated DC facilitates the differentiation of naive T cells through IL-1β, IL-6, and IL-23 into Th17 cells (9). IL-12 assumes these functions for Th1 cells (which dampen Th17 activity), and TNFα and IL-6 lead to the programming of Th22 cells (**Figures 2** and **3**).

In skin lesions of psoriasis patients, CD11c+ inflammatory DC can be detected more frequently, expressing TNFα, IL-23, and iNOS (so-called TNFα- and iNOS-expressing TIP-DC) (100). Attempts to define these cells more precisely have suggested that they are not classical myeloid CD1c+ DC1 or CD141+ DC2 (100). A portion of the TIP-DC corresponds to the so-called slanDC (106). In addition, CD163+ macrophages with phenotypic properties of TIP-DC could also be detected (107). These slanDC were first detected in the blood using the specific marker slan and the expression of CD16 (108–110). They are now believed to be of monocytic origin and produce large amounts of pro-inflammatory cytokines such as IL-12, IL-23, IL-1β, and TNFα. Numerous pDCs perform stimulatory functions in psoriatic skin and are characterized by high production of interferon α (111).

NEUTROPHILS, NETs, AND MECHANISMS OF DISEASE

In addition to DC, macrophages, and T cells, neutrophilic granulocytes are a hallmark feature of psoriatic skin lesions (**Figures 1E** and **4A**). They are key effector cells of the innate immune system and they target invading microbes by phagocytosis, the generation of reactive oxygen species (ROS), as well as the release of AMPs and inflammatory mediators. While these "classical" strategies are well known for many years, the recent discovery of NETs has catapulted neutrophils back into the focus of immunological science including psoriasis research (112).

The mechanisms leading to the formation of NETs are only partially understood. It is clear that upon contact with various stimuli including bacteria, fungi, activated platelets, antigen-antibody complexes or CXCL8 (IL-8), calcium ionophores, phorbol 12-myristate 13-acetate, or lipopolysaccharide neutrophils enter a cell-death pathway that is different from apoptosis and necrosis (113–115). To this end, the neutrophils go through a series of dramatic alterations of their morphology and behavior. In the course of several hours, they stop migrating and rearrange their cytoskeleton. The nuclear and granular membranes dissolve and a mixing of granular content with chromatin occurs before NETs are finally released. On the molecular level, this pathway generally requires the production of ROS and is therefore dependent on the NADPH oxidase complex (114, 116), although ROS-independent mechanisms have also been proposed (117). In addition, there is evidence for an involvement of protein kinase C and the Raf–MEK–ERK pathway (118). NETosis relies strongly on myeloperoxidase (MPO) and neutrophil elastase (NE). NE is released from azurophilic granules into the cytosol in an MPO-dependent manner (119) and subsequently translocates to the nucleus, where it cleaves histones to decondense chromatin (120). MPO also travels to the nucleus where it synergizes with NE in promoting chromatin decondensation independent of its enzymatic activity (120). Finally, a crucial player is peptidylarginine deiminase 4, an enzyme that, after translocation to the nucleus (121), leads to (global) histone hypercitrullination and enables histone decondensation (122), a prerequisite for expelling the chromatin content of the cell in the form of NETs (**Figure 4B**).

While originally described as a method to entrap and kill bacteria, we now know that NETs play a broader role in the immune system. Free DNA of host origin, as released during NET formation, indicates a disruption of cellular integrity and therefore constitutes a potent "danger" signal. Interestingly, DNA complexed with LL-37, is much more stable and activates DCs more effectively than "naked" DNA, triggering them to produce pro-inflammatory interferons (98). In addition, LL-37 (as well as other AMPs such as human beta defensin-3 and human neutrophil peptide-1) protects neutrophil-derived DNA against nuclease degradation (123). At the same time LL37 appears to

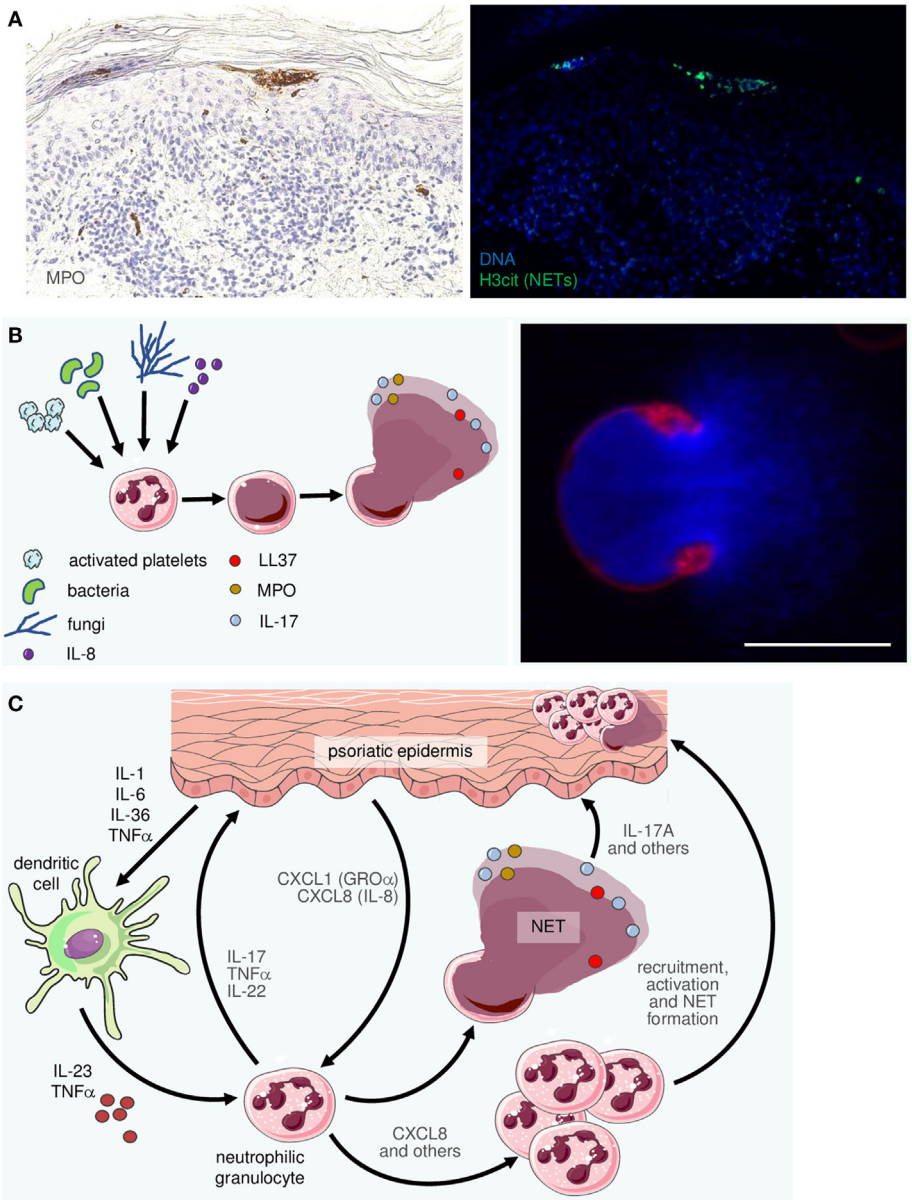

FIGURE 4 | Neutrophilic granulocytes become recruited, activated, and form neutrophil extracellular traps (NETs) in psoriatic skin. **(A)** Two consecutive sections of an early psoriatic lesion were stained by immunohistochemistry for myeloperoxidase (MPO; left photomicrograph) and by immunofluorescence for citrullinated histone-3 (H3cit; right). Within the typical subcorneal neutrophil accumulations (Munro's microabscesses) there are numerous H3cit-positive cells indicating NETosis. **(B)** Schematic of NET formation: a variety of stimuli can activate neutrophils. Consequently, their chromatin decondenses until it fills the entire cell. Finally, the cell membrane ruptures to release the NET, consisting of DNA, histones and a plethora of antimicrobial peptides, chemokines, etc. The right-hand image depicts a characteristic confocal image of a neutrophil directly after NET release (DNA staining by Hoechst in blue, membrane staining by the PHK26 dye in red). Scale bar = 10 µm. **(C)** The complex interactions between neutrophils, which are among the most prominent representatives of the innate immune system in psoriatic skin, include recruitment and activation by CXCL8 [interleukin (IL)-8], CXCL-1 (GROα), and other mediators as well as activation by cytokines such as IL-23 and TNFα. In turn, neutrophils undergo NETosis and they are thought to produce and release pro-inflammatory factors including IL-17, IL-22, CXCL8, and TNFα.

lose its antimicrobial activity when bound to DNA, implying that antimicrobial peptides may have different, mutually exclusive roles in the immune system. The high presence of AMPs within NETs indicates that most likely NETs and AMPs act in unison to either directly kill invading pathogens or to modulate the immune system.

The clinical relevance of these mechanisms becomes apparent when studying their impact in diseases like systemic lupus erythematosus (124) or autoimmune diabetes (125). In addition, NETs can directly prime T cells by reducing their activation threshold. Thus, NET-mediated priming increases T cell responses to antigens and even to suboptimal stimuli, thus

providing an additional link between the innate and the adaptive immune system (126).

In autoimmune diseases featuring anti-neutrophilic cytoplasmic antibodies (ANCAs) like lupus erythematosus or ANCA-associated small-vessel vasculitis, tolerance against nuclear components of neutrophils is disrupted. In order for these ANCAs to form, neutrophil proteins such as MPO or proteinase-3 must be processed by professional antigen-presenting cells and presented to T- and B-cells. It has been shown that the structure of NETs favors the upload of neutrophilic antigens into mDCs, leading to their activation and presentation of these antigens to the effector cells of the adaptive immune system (127). The formation of antigen-antibody complexes that ensues from this process may then lead to a vicious circle as antigen-antibody complexes in turn effectively trigger NETosis. Deregulated NETosis may therefore be important for the pathophysiology of inflammatory diseases.

So what does all this mean in the context of psoriasis? While here the pathogenic importance of NETs is less well-established than in other autoimmune diseases, NETs are prominently present in both psoriatic plaques (**Figures 4A,C**) and psoriatic pustules (112). Research into the contribution of NETs and neutrophils in general to the pathogenesis of psoriasis is hampered by the fact that the currently most popular mouse model, namely that of imiquimod-induced dermatitis, does not reflect the importance of neutrophils in human psoriasis (128). In this model, γδ-T cells are the primary source of IL-17, limiting its suitability to study neutrophils or NETs in murine skin (129). So far only neutrophil depletion in flaky skin mice has provided evidence for the involvement of neutrophils in a psoriasis-like phenotype in an animal model (129). The use of other animal models, for example, IL-23-induced psoriasis-like skin inflammation will hopefully help to overcome this limitation (130).

NEUTROPHILS, IL-17, AND OTHER CYTOKINES

While it was long assumed that Th17 cells were the most important sources of IL-17 in psoriasis, there is accumulating evidence that cells of the innate immune system like neutrophils, mast cells, γδ T cells, and ILCs are major sources of IL-17 (74, 131). Similar to Th17 cells, neutrophils possess the machinery to produce IL-17. In humans, two models of psoriasis-like inflammation (leukotriene B4 application or repeated tape stripping) have shown the coexpression of the IL-17-associated transcription factor RORγt and IL-17 (71, 131). In neutrophils, incubation with keratinocytes (72) or IL-23 induced IL-17 and IL-22 in an mTOR-dependent manner (126). However, to what extent neutrophil-produced IL-17 stimulates inflammatory reactions and the mechanism of IL-17-release, in particular within the context of NET formation, still remains enigmatic (74). It appears likely that IL-17 is released alongside NETs and may even be displayed on them, as IL-17 co-localizes with indirect immunohistochemical markers of NETs in psoriatic tissue and Munro's microabscesses (74).

Interleukin-17 stimulates many pro-inflammatory and immunomodulatory functions including production of IL-6 and IL-8 (CXCL8) but also TNFα, IL-1, CXCL10, and CCL20 (112, 132). In turn, IL-8 promotes the recruitment and activation of neutrophils and has long been known to trigger NETosis (133, 134). In addition, interaction of CCL20 with its corresponding receptor CCR6 is enhanced in chronic inflammatory diseases such as inflammatory bowel disease and may augment the recruitment of Il-17-producing cells such as Th17 T-cells (135, 136).

Neutrophils themselves produce a number of cytokines such as TNFα, IL-12 or, again, IL-8 (CXCL8), in addition to the aforementioned IL-17, which may add to the overall pro-inflammatory environment, recruitment of additional leukocytes, and NET production (137), creating a vicious circle that can be efficiently intercepted by modern therapies directed against the key cytokines IL-23 and IL-17 as well as TNFα in psoriasis (**Figure 4C**). In fact, IL-17 and TNFα have been shown to synergistically regulate cytokine levels, for example, upregulating beta-defensins and, yet again, IL-8, and to exert synergistic effects both on keratinocytes (138) and on melanocytes (139). One may hypothesize that the reason IL-17 blockade and TNFα-inhibition have similarly strong effects in patients is the abolition of this synergism.

WHAT CAN WE EXPECT?

Psoriasis remains a fascinating entity, and while we have been able to solve some of the mysteries surrounding this disease, many aspects still remain enigmatic. Among the most mesmerizing novel concepts in the disease mechanisms are the emerging roles of "young" immune cells such as ILCs and nonconventional T-cells, whose role in immunology we are only just beginning to understand. Just as importantly, we have learned that cells such as neutrophils and mast cells may have a central role in psoriasis that reaches beyond the originally described functions of innate immune cells and may in fact bridge the innate and the adaptive immune system. Last but not least, "classical" psoriasis-associated cells such as certain Ths keep presenting us with surprising findings when it comes to the complex, often synergistic effects of chemokines.

These exciting developments show potential for novel therapeutic approaches. While we have already come very far in applying our knowledge and generating new therapies that make a vast difference in patients' quality of life, the inhibition of all or some of the above-mentioned mediators theoretically present therapeutic opportunities. For example, inhibition of IL-9 could be explored in the context of psoriasis (61). In general, medicine is moving toward more personalized therapeutic approaches. For psoriasis, this could mean targeting the cytokines most relevant for the given subtype of psoriasis. For example, while chronic plaque-type psoriasis is dominated by the IL-17/INFγ axis, pustular forms of psoriasis feature an IL-17/IL-36/IL-1 signature (140). The development of highly effective (targeted) therapies with mild to moderate side effects also allows the exploration of "early intervention" to prevent the psoriatic march which may lead to cardiovascular diseases resulting from systemic inflammation. Similar to approaches used in rheumatoid arthritis, it can be speculated that blocking inflammatory mediators early on in the disease process could intercept the evolution of psoriasis toward

a more detrimental systemic disease. Such approaches may also include targeting of important resident cell types such as vascular endothelial cells (141).

The above-mentioned "novel players" in psoriasis, including ILCs, unconventional T-cells but also "old" new candidates like neutrophils (including NETs released by them) and mast cells also present interesting new targets in psoriasis. It is conceivable that targeting these cells or other factors in psoriasis may require an approach adapted to the disease stage or activity, as for example, NETs might only be present in acute inflammatory exacerbations while other cells and cytokines may dominate more chronic phases of the disease.

Whatever the future holds in stock for us and patients suffering from psoriasis, looking back on the last couple of years of psoriasis research certainly justifies optimism.

AUTHOR CONTRIBUTIONS

MS and LE jointly wrote the manuscript. They contributed equally.

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REVIEW
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The IL-17 Family of Cytokines in Psoriasis: IL-17A and Beyond

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Psoriasis is a frequent chronic inflammatory skin disease, nowadays considered a major global health problem. Several new drugs, targeting the IL-23/IL-17A pathway, have been recently licensed or are in clinical development. These therapies represent a major improvement of the way in which psoriasis is managed, since they show an unprecedented efficacy on skin symptoms of psoriasis. This has been made possible, thanks to an increasingly more accurate pathogenic view of psoriasis. Today, the belief that Th17 cells mediate psoriasis is moving to the concept of psoriasis as an IL-17A-driven disease. New questions arise at the horizon, given that IL-17A is part of a newly described family of cytokines, which has five distinct homologous: IL-17B, IL-17C, IL-17D, IL-17E, also known as IL-25 and IL-17F. IL-17 family cytokines elicit similar effects in target cells, but simultaneously trigger different and sometimes opposite functions in a tissue-specific manner. This is complicated by the fact that IL-17 cytokines show a high capacity of synergisms with other inflammatory stimuli. In this review, we will summarize the current knowledge around the cytokines belonging to the IL-17 family in relation to skin inflammation in general and psoriasis in particular, and discuss possible clinical implications. A comprehensive understanding of the different roles played by the IL-17 cytokines is crucial to appreciate current and developing therapies and to allow an effective pathogenesis- and mechanisms-driven drug design.

Keywords: psoriasis, IL-17 family, IL-25, IL-17E, interleukin, comorbidities, Th17 cells, IL-17A

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INTRODUCTION

Psoriasis is a frequent, chronic, non-communicable inflammatory skin disease, for which there is no clear cause or cure. Psoriasis affects people of all countries and of all ages. The disease manifests as well-defined, red, scaly plaques, appearing with a chronic-recurrent course at preferential sites such as elbows, knees, and scalp. Individuals with psoriasis are at an increased risk of developing other chronic and serious diseases, including psoriatic arthritis, metabolic syndrome, cardiovascular diseases, and depression (1–3). The negative impact of psoriasis on people's lives can be immense. Disfiguration and disability are common aspects experienced by patients, who are in addition challenged by emotional and social burden caused by psoriasis stigmatization. In 2014, the Executive Board of the Word Health Organization became spokesman of more than 100 million people affected worldwide, approving a resolution to raise awareness of psoriasis as a major global health problem (4).

Psoriatic hallmark features include profound modification of the epidermis, such as hyperproliferation and altered differentiation of keratinocytes, concurrent with the presence of a prominent inflammatory infiltrate and neo-angiogenesis. T cells, along with innate immune cells, are thought

to produce a key effector cytokine, IL-17A, which then triggers the epidermal modification (1). The emergence of a detailed pathogenic concept in the last decade has fueled the development of targeted therapies. Thus, treatments have moved from broad immunosuppression to interference with T cells and the IL-23/IL-17 pathway, and nowadays to downstream effector molecules such as IL-17A and its cell targets (5).

IL-17A was initially thought to be a "unique" cytokine, exclusively produced by T cells in psoriasis. To date, it is evident that many other cells contribute to the bulk of IL-17A found in the diseased skin, and that many isoforms of IL-17 may participate to psoriasis. These data open a new scenario for innovative therapeutic interventions, as our knowledge of the pathophysiology of psoriasis becomes more precise. This review will briefly debate the role of IL-17A as key effector molecule, retracing the key discoveries that led to the current understanding of psoriasis as an IL-17A driven disease. This will lead to an in-depth discussion of the IL-17 family of cytokines and the contribution of IL-17 isoforms other than IL-17A to psoriasis manifestations in the skin and its comorbidities. Finally, the clinical implications will be addressed.

PSORIASIS: AN IL-17A-DRIVEN DISEASE

In the 1960, psoriasis was thought to be a primary disease of the epidermis caused by hyperproliferative keratinocytes. The involvement of the immune system became apparent only in the 1980, when lymphocyte-targeted therapies were proven to be an effective way to treat the disease (6, 7). The pathogenesis of psoriasis was initially proposed to rely on Th1 responses, based on the identification of elevated expression of Th1 cytokines, such as IFNγ, TNFα, and IL-12, in the lesion (8). In the wake of these results, a monoclonal antibody (ustekinumab) designed to block the p40 subunit of IL-12, key factor in Th1 cell commitment, was developed. This antibody showed the highest therapeutical efficacy ever observed at that time. Concomitant to ustekinumab generation, a second cytokine, named IL-23, was found to contain the identical p40 subunit (9). The "unwanted" blockade of this latter cytokine turned out to be the more relevant mechanism of action in the context of treating psoriasis.

In the mid 2000s, IL-23 was shown to induce the production of IL-17 by activated T lymphocytes, later named Th17 cells (10, 11). These cells, which express RORγt as master transcription factor, have limited inherent pathogenicity and promote mucosal defense, whereas exposure to IL-23 turns them into autoimmune-associated inflammatory cells (12). The involvement of IL-23 in psoriasis was supported by its ability to induce psoriasiform characteristics in a preclinical model of intradermal administration (13); a phenotype linked to the infiltration of IL-22- and IL-17A-producing T cells (14). Th17 cells became thus the center of extensive research, and the hallmark cytokine IL-17A was identified as a novel key effector pathogenic factor in psoriasis.

Genome-wide association studies also confirm the role of the immune system in the pathogenesis of psoriasis, with the HLA-Cw6 allele accounting for almost 50% of the disease heritability. Variations in loci containing genes involved in the IL-23/Th17 signaling are frequently observed and suggest the particular involvement of Th17 cells: these include genes upstream of the IL-17 expression, such as IL-23R and IL-12B, or downstream the IL-17 receptor, such as STAT3 and Act1 (15). No variants in the IL-17A gene itself was shown to predispose to psoriasis so far, whereas the IL-17RA allele rs4819554 was recently associated with risk of developing psoriasis in a Spanish cohort (16).

The central role of IL-17A in the pathophysiology of psoriasis has recently been reviewed elsewhere (17) and will only be briefly described here. IL-17A mainly acts on non-hematopoietic cells, particularly epithelial cells, and consistently participates in protective immunity at boundary tissues. With regard to the skin, IL-17A leads to increased proliferation and aberrant differentiation of keratinocytes (18) and contributes to skin barrier disruption by downregulating the expression of molecules involved in keratinocyte differentiation, such as fillagrin (19). In addition, IL-17A participates in generating and amplifying the inflammatory network by promoting the release of antimicrobial peptides and proinflammatory cytokines/chemokines (20, 21). The factors induced by IL-17A are poised toward the activation of a neutrophil/Th17 cell-dependent immune response. These include IL-8, a potent neutrophil chemoattractant; G-CSF, a survival factor for neutrophils; CCL20 that favors Th17 cell recruitment; and the key Th17 polarizing cytokines IL-1β and IL-6. In addition, IL-17A directly contributes to leukocyte migration and tissue remodeling by promoting the secretion of metalloproteases. To note, IL-17A synergizes with and potentiates the effects of many other inflammatory mediators, possibly *via* stabilization of target mRNA. IL-10 and IL-1 family members, as well as type-I cytokines, such as TNFα, are the most relevant factors in this regard (22–24). The genes synergistically upregulated by TNFα and IL-17A in keratinocytes were shown to mimic the gene signature observed in the lesional skin, underling the importance of these integrative responses (23). Similarly, IL-17A, together with TNF and IL-22, were reported to upregulate the expression of the IL-1 like family member IL-36, which in turn was found to augment the function of Th17 cytokines, revealing the existence of a feedback loop between Th17 and IL-36 cytokines (24). These cytokine networks may also be of particular importance to distinguish different forms of psoriasis: inactivating mutation of the IL36RN gene, which encodes the IL-36 receptor antagonist, have been particularly associated with generalized pustular psoriasis (25). The importance of IL-17A and its interaction with other cytokines has also extensively been proved in murine models of psoriasiform inflammation, through the use of deficient mice and in neutralizing experiments. Finally, the first biologics following ustekinumab that entered the market of anti-psoriatics were specific anti IL-17A antibodies, namely secukinumab and ixekizumab (26, 27). Stressing the importance of IL-17A, these therapies represent the most effective approach to treat psoriasis so far.

The effects of IL-17A are not limited to keratinocytes and encompass several other cells, including endothelial cells, fibroblasts, chondrocytes, and synovial cells. IL-17A is clearly of major importance also in the context of psoriasis-associated comorbidity, namely, psoriatic arthritis and cardiovascular disease/atherosclerosis, as highlighted elsewhere (28, 29) and summarized in **Table 1**.

TABLE 1 | Overview of the role exerted by IL-17A in inflammation.

Skin inflammation – *Human/patient data*: IL-17A is increased in several skin disorders, including psoriasis, atopic dermatitis as well as neutrophilic, granulomatous, and bullous skin diseases (30) – *Animal models*: IL-17A contributes to skin inflammation in multiple models of cutaneous inflammation, including IMQ application and K5hTGFβ1 transgenic mice (17).
Joint inflammation – *Human/patient data*: IL-17A is expressed in the synovium of RA and PsA patients and promotes bone-destructive cytokine production and bone resorption *in vitro* (28) – *Animal models*: IL-17A contributes to the immune-inflammatory events in CIA and other models of arthritis (28)
Gut inflammation – *Human/patient data*: IL17A expression is increased in inflammatory bowel disease, while neutralization of IL-17A has no effect or rather exacerbate CD (31). – *Animal models*: IL-17A neutralization exacerbates symptoms in DSS and $CD4^+$ t cell transfer model of colitis, while reduced pathology in an IL-10-deficient colitogenic model. IL-17A has important roles in preserving the intestinal epithelial barrier in DSS mice (32, 33)
CNS – *Human/patient data:* IL-17A levels are increased in MS lesions and peripheral blood (34) – *Animal models:* IL-17A plays an important role in EAE (35)
Cardiovascular disease – *Human/patient data*: IL-17A/Th17 cells are increased in patients with acute coronary syndrome and correlate with systemic inflammation markers (36) – *Animal models*: IL-17A inhibition results in the reduction of the size of atherosclerotic plaques in apoE deficient mice (37)

CNS, central nervous system; IMQ, imiquimod; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CD, Crohn's disease; DSS, dextran-sulfate sodium; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis.

The current view of the pathogenesis of psoriasis relies thus on pathogenic Th17 cells, which arise following an unknown trigger in genetically predisposed individuals as result of the production of Th17 polarizing cytokines by myeloid cells. The antimicrobial peptide LL37, in complex with nucleic acids released by dying cells, has been proposed as a possible autoantigen driving the activation of cutaneous plasmacytoid and myeloid DCs (38). Th17 cells would travel back to the skin, where they directly activate keratinocytes *via* the release of effector cytokines, among which IL-17A is the most important. Activated keratinocytes proliferate in an abnormal manner and release further inflammatory mediators and chemokines amplifying the inflammatory response (1).

Recent findings provide new evidence that is slightly but definitely changing the paradigmatic view of the pathogenesis of psoriasis: from Th17- to IL-17A-driven disease (**Figure 1**). Reich and colleagues demonstrated that a single dose of the anti-IL-17A antibody secukinumab resulted in skin normalization as soon as 2 weeks after injection, a finding paralleled by disappearance of IL-17A + neutrophils but not T cells (39). Meanwhile, many immune cells other than Th17 lymphocytes, globally called "Type 17" cells, were reported to release IL-17A. Many of them are thymus dependent, including adaptive and natural Th17 cells, T CD8 cells, γδ T cells, and invariant NKT (iNKT) cells; others are rather thymus independent, such as group 3 innate lymphoid cells (ILC), mast cells, and neutrophils (12, 40, 41). Th17 cells, with the exception of tissue-resident memory cells, reside in lymphoid organs in steady state and drain peripheral tissues only in inflammatory situations. Conversely, the other cells are found at the periphery, particularly at mucosal and skin tissues, thus representing a potential immediate source of IL-17A. Of interest, in lesional psoriatic skin, at least from a histological point of view, IL-17A + T cells are sparse, while the bulk of IL-17A-expressing cells is represented by neutrophils and mast cells (42, 43). Whether being still debated, neutrophils and mast cells appear to actively synthetize IL-17A in the skin, and release IL-17A, at least in part, *via* extracellular trap formation (40, 42). The abovementioned subsets express RORγt and the IL-23R, and require IL-23 for their effective activation (12). This might explain why targeting specifically IL-23 through blockade of the p19 subunits represents a promising therapeutic option, even in a scenario dominated by anti-IL-17A treatments (44). IL-17A production can, however, also occur in both γδ and iNKT cells independently of IL-23 (45, 46).

The idea that psoriasis is purely a Th17 cell-dependent disease is thus replaced by the concept of psoriasis as an IL-17A-driven disease (**Figure 1**). This further evolution of the pathogenic concept opens new questions, which will likely allow a better understanding of the disease and a rational drug design. One of these questions is whether IL-17A has "homologous" cytokines, which would be simultaneously produced, and which might substitute for or synergize with IL-17A, or affect sites other than the skin such as the joints. In the next sections, we will thus discuss the IL-17 family of cytokines and its implication in psoriatic skin inflammation.

AN OVERVIEW OF THE IL-17 FAMILY OF CYTOKINES

IL-17A, originally termed CTLA-8, was cloned in 1993 from a rodent-activated T cell hybridoma (47). Its amino acid sequence is unusual for a cytokine, being 58% identical to the open reading frame of the T cell-tropic gammaherpesvirus *Herpesvirus samiri* (48). In the early 2000s, genomic sequencing led to the identification of several proteins structurally related to IL-17A: IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25) and IL-17F. Together, these cytokines are known as the IL-17 family. IL-17F shares the highest homology with IL-17A (55%) and is often co-expressed with IL-17A (49). IL-17B, IL-17D, and IL-17C sequences overlap from 29 to 23% with IL-17A, while IL-17E appears to be the most divergent member of the family, sharing only 16% sequence homology. The members of the IL-17 family exert their functions as disulfide-linked homodimers, with a molecular weight of the monomer ranging from 17 to 21 kDa. As an exception to the rule, IL-17A and IL-17F can also form heterodimers.

History repeated itself for the IL-17 receptor. Discovered in 1995, the IL-17R did not fall into any previously known class of receptors (48). Later, it was discovered the existence of five homologous subunits, namely IL-17RA to IL-17RE, which

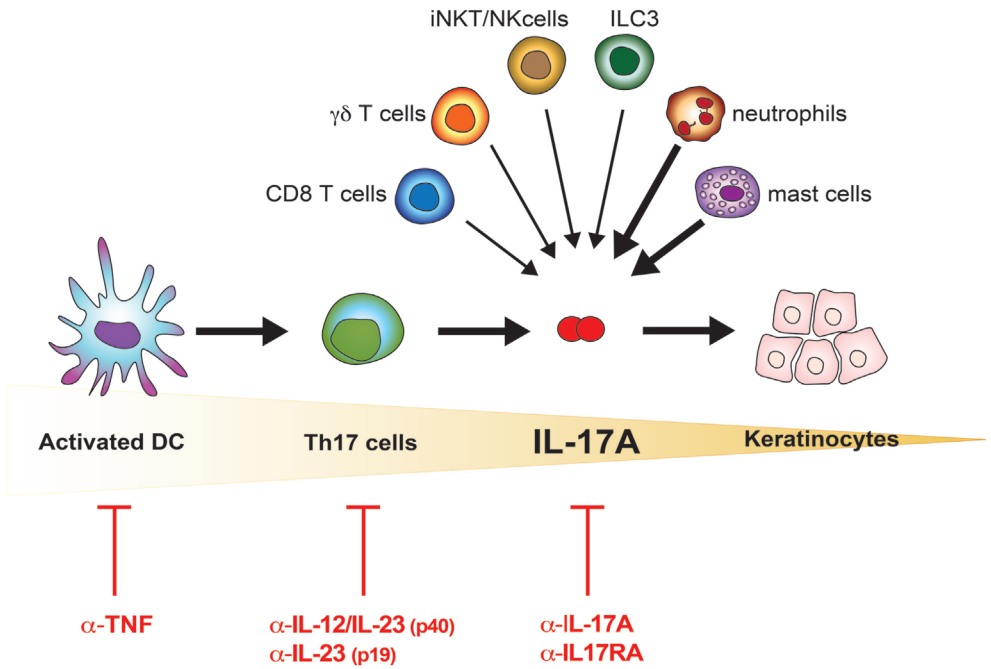

FIGURE 1 | IL-17A in the pathogenesis of psoriasis. Schematic representation of the increasingly accurate selection of therapeutic targets in psoriasis. The cells possibly contributing to the bulk of IL-17A in lesional psoriatic skin are shown. Bolded arrows indicate higher contribution to IL-17A production in psoriatic lesional skin.

together are now classified as a new class of receptors: the IL-17R family. All IL-17 cytokines signal *via* a heterodimeric receptor composed by a different combination of these subunits (**Figure 2**). IL-17A homodimers, IL-17F homodimers, and IL-17A/F heterodimers bind to the same receptor complex, comprising IL-17RA and IL-17RC subunits. IL-17RA is also the co-receptor used by two additional IL-17 family members; associated to IL-17RB mediates IL-17E signaling, bound to IL-17RE transduces signals by IL-17C. All subunits of the IL-17R family exhibit a broad expression pattern, with IL-17RA being ubiquitous (50).

All receptor subunits are single transmembrane chains sharing unique properties. The extracellular region of the IL-17R family members contains two fibronectin III-like (FN) domains, which mediate protein–protein interactions and ligand binding. The cytoplasmic region, at the C-terminus, contains a conserved sequence known as SEFIR (similar expression of fibroblast growth factor genes and IL-17Rs) domain. This region, which helped defining the IL-17 family as a new receptor class, is related to the TIR (toll/interleukin-1 receptor) domain found in IL-1 and toll-like receptor family. While having many features in common with the signaling induced by these innate receptors, the IL-17R-induced pathway has notable differences (12, 51). The study of the interaction of IL-17A with its receptor has generated many insights in that regard, as briefly described below and extensively discussed elsewhere (51, 52). The most striking difference is the unique usage of a cytoplasmic protein, called Act1, which also contains a SEFIR domain. Upon ligand binding, this molecule is engaged to the IL-17R complex through homotypic SEFIR interactions to mediate downstream events (53). Recruitment of Act1 represents a hallmark of IL-17 signaling, an event not shared by any other known class of receptors. Act-1 activates several independent signaling pathways operating as a docking station for different TRAF proteins. Upon recruitment of TRAF6 and its ubiquitination (Act1 may indeed function as E3 ubiquitin ligase), a cascade of molecular interactions is turned on, leading to the phosphorylation and consequent proteasomal degradation of IκB, ultimately allowing the nuclear translocation of NFκB and the activation of NFκB targeted genes (54–56). Although less clear mechanistically, TRAF6 has been also linked to the activation of MAPK pathways, including ERK, p38, and JNK, and C/EBP molecules (51). By recruiting TRAF2/5, Act-1 favors the sequestration of RNA decay factor, such as SF2 (57), as well as the activation of RNA-binding proteins as, such HuR (58), which results in increased mRNA stability of target genes. Thus, the signaling pathways downstream the IL-17A receptor may induce at least two distinct events: *de novo* inflammatory gene transcription and stabilization of target mRNA (**Figure 2**). Whether the other members of the IL-17 family similarly activate these pathways is unknown, but highly probable.

One peculiarity of IL-17s is that they are not strong inducers of signaling when acting in an isolated manner. Early reports have shown the implication of NFκB in mediating the pro-inflammatory role of IL-17A in fibroblasts and synoviocytes. However, IL-17A resulted to be far less potent than other inflammatory molecules, such as TNF. Nevertheless, IL-17A is an extremely powerful inducer of inflammation due to its capacity to

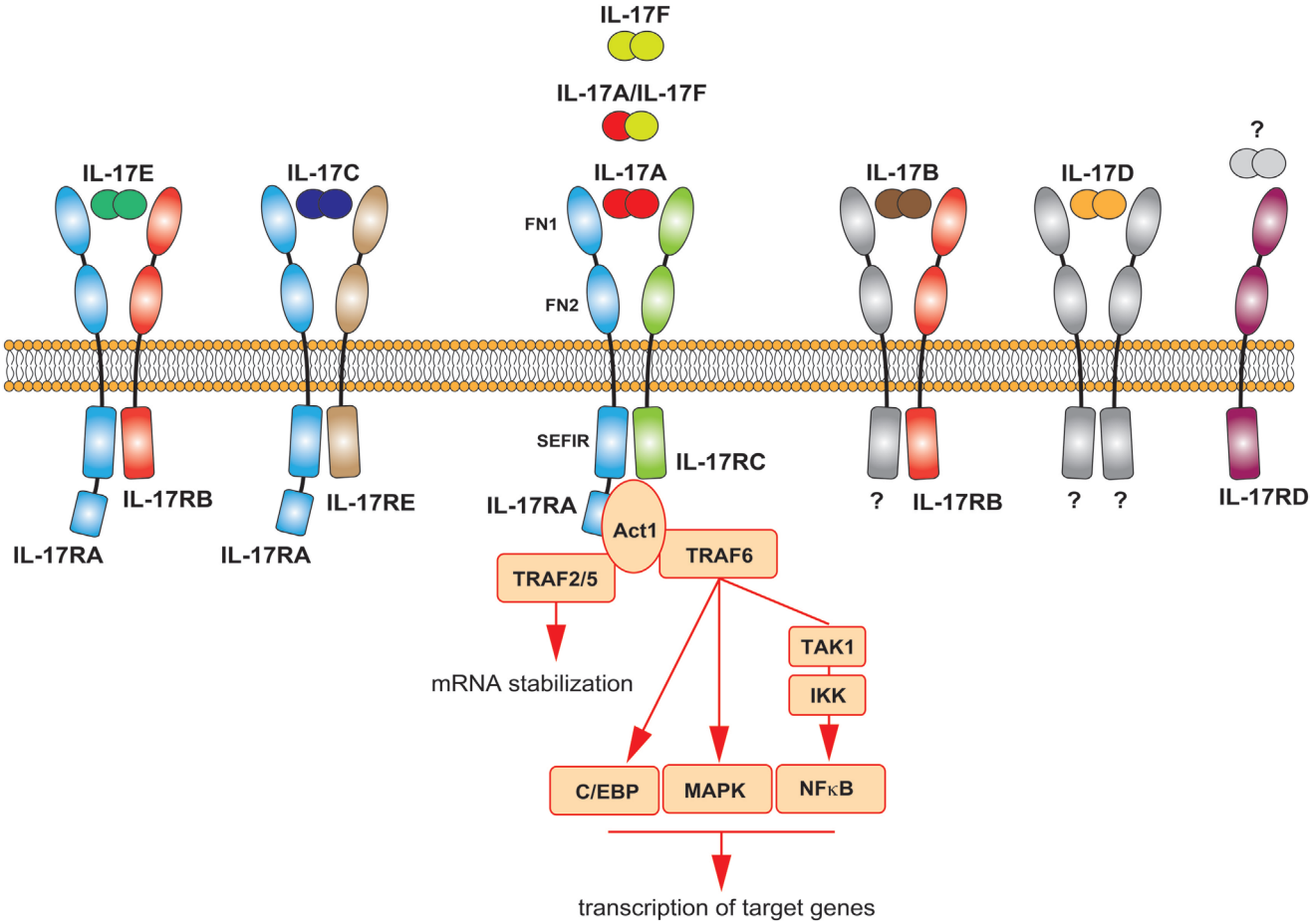

FIGURE 2 | The IL-17 family of cytokines. Schematic representation of the different IL-17 cytokines and their receptors. The main signaling events downstream the activation of the IL-17A receptor are summarized.

synergistically act with other stimuli. In the case of TNF, IL-17A helps stabilizing the mRNA of the TNF-activated genes, which are inherently unstable, leading to a great amplification of TNF effects (59, 60). Therefore, even though in isolated experimental conditions, IL-17A does not appear to be potent, its physiological impact *in vivo* could be profound. An additional feature of the IL-17 family is that IL-17 cytokines might regulate several genes in a tissue-specific fashion. For instance, IL-17A was shown to induce the production of occludin specifically in the gut, where it helps maintaining the intestinal barrier integrity (46), or favor the release of kallikrein 1 by renal epithelial cells, conferring protection against candidiasis (61).

High capacity of synergy and tissue-specific functions are features shared by several members of the IL-17 family. As shown below, a considerable functional overlap, as well as main differences, characterizes the members of the IL-17 family. The following section will describe the contribution of each IL-17 family member to psoriasis, while especially focusing on isoforms other than IL-17A, as this latter has recently extensively been reviewed elsewhere (17).

IL-17s IN PSORIASIS: BEYOND IL-17A

IL-17F

IL-17F, discovered in 2001 on chromosome 6p12 (the same locus as IL-17A!), is the most homologous cytokine to IL-17A and signals *via* a receptor composed by the IL-17RA and IL-17RC subunits (again, the same used by IL-17A). IL-17F levels are elevated in sera and lesional psoriatic skin compared to non-lesional tissue (62, 63). Despite that, no specific polymorphisms in the IL-17F gene have so far been associated with psoriasis susceptibility, although the IL-17F polymorphism rs763780 was linked to a better response to anti-TNF therapy (64). IL-17F is also increased in sera of atopic dermatitis patients and positively correlates with higher clinical score (65). Additional evidence of the involvement of IL-17F in psoriatic inflammation comes from experiments in mice models. Indeed, IL-17F together with IL-17A and IL-22, are rapidly induced upon imiquimod application, as result of infiltration of γδ T cells and RORγt + innate lymphocytes. Of interest, IL-17F$^{-/-}$ mice show a higher disease resistance than IL-17A$^{-/-}$ mice (66, 67).

Both IL-17A and IL-17F are expressed by the same immune cell types, including Th17 cells, γδ T cells and ILC3. Human *IL17A* and *IL17F* genes are found in the same locus and are genetically co-regulated, thus it is not surprising that these cytokines are often co-expressed (49). IL-23 participates in IL-17A and IL-17F co-production in Th17 cells (10). As mentioned earlier, IL-17A and IL-17F can be secreted as homodimers or IL-17A/IL-17F heterodimers. IL-17F has been shown to stimulate *in vitro* a qualitative similar pattern of genes than IL-17A, although being generally weaker, with IL-17A/IL-17F heterodimers having an intermediate potency (52). However, this is not always true, either in cell- and target-specific situations or because of synergism with other inflammatory mediators. Thus, IL-17F was shown to be more potent than IL-17A, or even TNF, to induce IL-8 and IL-6 production in normal human epidermal keratinocytes (68, 69). Or, though less potent in absolute terms, IL-17F was shown to be almost as potent as IL-17A when combined with TNF in RA synoviocytes (60, 70). Moreover, IL-17A and IL-17F have been shown to synergistically act, since their dual neutralization leads to greater downregulation of inflammatory mediators than IL-17A blockade alone in skin and joint fibroblasts (71).

With respect to their similar functions, both cytokines are needed for effective responses against mucoepithelial bacterial infections and synergistically cooperate to protect the host from fungal infections (72, 73). Consistently, inborn errors of IL-17F, as well as of IL-17RA or ACT1, display chronic mucocutaneous candidiasis (74). Candida infections are also a common adverse event observed upon anti-IL-17A or anti-IL-17RA-targeted therapies. Whether bi-specific antibodies blocking both IL-17A and IL-17F have an increased risk of Candidiasis is not yet known. Despite having similar, and sometimes even synergistic actions *in vitro*, knock-out experiments in mice revealed also diverse roles of IL-17A and IL-17F in complex *in vivo* inflammatory settings. This might well reflect tissue-specific functions and the capacity of IL-17 cytokines to synergize with other inflammatory mediators. IL-17F, at difference to IL-17A, is not required in several T-cell-dependent autoimmune diseases in mice, while being pathogenic in DSS colitis model and in acute allergic responses in the lung (72, 75–78). Nevertheless, IL-17F gene expression is increased in human active Crohn's disease (CD) and multiple sclerosis (79, 80). Finally, IL-17F was associated with increased susceptibility in many forms of human cancer, while playing rather a protective role in colon tumorigenesis in mice (81). **Table 2** shows an overview of the role of IL-17F in inflammation.

In summary, IL-17F acts in a similar manner as IL-17A, although being generally weaker. IL-17F might contribute to skin manifestations and comorbidities of psoriasis in a tissue-specific fashion. The function of IL-17F may become prominent in complex cytokine milieus, and preclinical data suggest that it might be pathogenic in both joint and gut inflammation.

TABLE 2 | Overview of the role exerted by IL-17F in inflammation.

Skin inflammation
– *Human/patient data*: IL-17F is increased in psoriatic lesional skin. Elevated levels of IL-17F are found in sera from psoriatic and atopic dermatitis patients (62, 63, 65).
– *Animal models*: IL-17F contributes to skin inflammation induced by IMQ application (66, 67).
Joint inflammation
– *Human/patient data*: IL-17F is expressed in the synovial tissue from RA patients and contributes to human chronic joint inflammation *in vitro* and *in vivo* (62, 70, 71)
– *Animal models*: IL-17F is increased in CIA (78), while playing only marginal roles on CIA and arthritis in Il1rn$^{-/-}$ mice (72, 74).
Gut inflammation
– *Human/patient data*: IL17F gene expression is increased in active CD (80)
– *Animal models*: IL-17F contributes to experimental induced colitis (76, 82)
CNS
– *Human/patient data:* IL-17F mRNA is increased in mononuclear cells from MS patients (79)
– *Animal models*: Neutralization of IL-17F (alone) does not prevent the development of EAE (77)
Cardiovascular disease
– Not investigated yet

CNS, central nervous system; IMQ, imiquimod; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CD, Crohn's disease; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis.

TABLE 3 | Overview of the role exerted by IL-17B in inflammation.

Skin inflammation
– *Human/patient data*: IL-17B mRNA levels are decreased in lesional psoriatic skin (62)
Joint inflammation
– *Human/patient data*: IL-17B is expressed in RA and OA synovia, and RA pannus (83, 84).
– *Animal models*: IL-17B contributes to the exacerbation of inflammatory arthritis in CIA model (78)
Gut inflammation
– *Human/patient data*: IL-17B is expressed in the intestine and stomach, unknown function (85, 86)
CNS
– *Human/patient data*: IL-17B is localized to the neuronal cell bodies and axons in humans. IL-17B gene maps to a region associated with a rare autosomal recessive form of Charcot–Marie–Tooth demyelinating disease (86).
– *Animal models*: IL-17B is expressed by neurons of the cerebral cortex and the adjacent hippocampal (H) layer (86).
Cardiovascular disease
– Not yet investigated

CNS, central nervous system; RA, rheumatoid arthritis; OA, osteoarthritis; CIA, collagen-induced arthritis.

IL-17B and IL-17D

Transcripts of IL-17B and IL-17D are decreased in psoriatic lesional skin when compared to non-lesional tissue (62). For that reason, they have not been studied in detail in the context of psoriasis. It is, however, relevant to briefly review their reported functions, as clinical targeting of IL-17 signaling molecules might also affect the functions of these isoforms. The roles exerted by IL-17B and IL-17D in inflammation are summarized in **Tables 3** and **4**, respectively.

IL-17B, discovered in 2000 on chromosome 5q32, is secreted as non-covalent dimer, and signals *via* a still unknown receptor

TABLE 4 | Overview of the role exerted by IL-17D in inflammation.

Skin inflammation
– *Human/patient data*: IL-17D mRNA levels are decreased in lesional psoriatic skin, unknown function (62)
Joint inflammation
– *Human/patient data*: IL-17D is expressed in the rheumatoid nodule, unknown function (83)
Gut inflammation
– Not yet investigated
CNS
– *Human/patient data*: IL-17D is expressed in the brain, unknown function (87)
Cardiovascular disease
– *Human/patient data*: IL-17D is expressed in the heart, unknown function (87)

CNS, central nervous system.

containing the subunit IL-17RB. This latter is shared with the IL-17E receptor (12). Regarding the cells producing IL-17B, information is punctual. Activated T cells do not produce IL-17B, while IL-17B was found to be expressed in neutrophils, germinal center B cells, neurons and stromal cells, and gut epithelium (88). In terms of function, high expression of IL-17B was mainly linked to poor prognosis in cancer; such as breast and gastric cancer (88) Besides that, IL-17B could play a pathogenic role in the joint (78, 83, 84). IL-17B is overexpressed in the inflammatory cartilage of the collagen-induced arthritis mouse model, where it induces the production of IL-8 and the recruitment of neutrophils. Consistently, neutralizing antibodies targeting IL-17B ameliorates signs and symptoms in this model (78). Thus, while not being involved in skin manifestation of psoriasis, IL-17B was shown to play an important role in the pathogenesis of inflammatory arthritis in preclinical models (78, 84, 89).

IL-17D, cloned in 2002 and mapping to chromosome 13q12, is the cytokine most recently added to the IL-17 family. IL-17D is most homologous to IL-17B, with 27% identity. It is secreted as disulfide-linked homodimer and signals *via* a still unknown receptor. IL-17D is found in a variety of tissues, including skeletal muscle, brain, adipose, heart, lung, and pancreas, while is poorly expressed by activated immune cells, such as lymphocytes and monocytes (87). IL-17D levels have been reported increased in rheumatoid nodules (83). Although not directly stimulating immune cells, IL-17D has been shown to modulate the production of cytokines by endothelial cells. Similar to other members of the family, these include pro-inflammatory cytokines such as IL-6, IL-8, and GM-CSF. Despite that, IL-17D demonstrates an inhibitory effect on hematopoiesis of myeloid progenitors cells *in vitro* (87). In addition, IL-17D plays a role in the control of viral infections and cancer, as IL-17D deficiency predisposes animals to these conditions (90).

Taken together, the knowledge around IL-17B and IL-17D is still limited. Overall, these isoforms induce pro-inflammatory responses in non-immune cells, leading to regulation of tumor or joint immunity, at least in the case of IL-17B. The reason of their reduced expression in psoriasis remains unknown and awaits further investigation.

IL-17C

IL-17C was cloned at the same time as IL-17B in 2000. It maps to chromosome 16q24 and signals *via* a receptor composed by IL-17RA/IL-17RE subunits (85). IL17C shows only 23% homology with IL-17A, and unlike IL-17A, is expressed mainly by epithelial cells rather than immune cells. Much evidence links IL-17C to skin inflammation. Importantly, IL-17C is overexpressed in lesional skin of psoriatic (91, 92) and atopic dermatitis patients (93). IL-17C is secreted by epithelial cells *in vitro* in response to inflammatory stimuli, such as TNF and TLRs (91, 94, 95). Epithelial cells of the skin and the gut are also main targets of IL-17C, which acts in an autocrine manner to induce a pattern of genes similar to those induced by IL-17A, including pro-inflammatory cytokines, chemokines, and antimicrobial peptides (92, 96, 97). Similar to IL-17A, IL-17C was shown to synergize with TNF in this respect (92).

Experiments in mice confirmed that IL-17C participates in skin inflammation. Intradermal injection of IL-17C leads to epidermal thickening (96). In addition, IL-17C is upregulated in murine psoriasiform dermatitis (62, 92, 93), and IL-17C-deficient mice develop milder skin inflammation upon imiquimod application (96, 98). Conversely, selective overexpression of IL-17C in murine epidermis results in marked psoriasiform dermatitis (92). Finally, antibody-dependent blockade of IL-17C inhibited cutaneous inflammation in the IL-23-induced psoriasis model and in AD-like inflammation in mice (93).

Studies in mice also corroborate some clinical evidence obtained with targeted antibody therapies. In this respect, TNF-alpha blocking therapies result in early decrease of IL-17C levels in patients (91). The same neutralizing strategy led to an amelioration of the psoriasiform phenotype in IL-17C transgenic mice (92), pointing toward a possible role of the TNF/IL-17C axis in psoriasis. Despite this evidence, IL-17C was shown to be upregulated in paradoxical psoriasis upon anti-TNF therapy in patients presenting inflammatory bowel disease (IBD), in a mechanisms depended on IL-36γ (99). Paradoxical psoriasis onset in RA patients has also been reported after IL-6 inhibition. Similarly, genetic ablation of IL-6 leads to an increased psoriasiform phenotype in an IL-17C transgenic mouse model. This suggests that in absence of IL-6, compensatory mechanisms may occur resulting in exacerbation of disease (100).

Outside the skin, IL-17C promotes protective antimicrobial responses in the gut (96, 97, 101) and participates in mucosal responses to *Citrobacter rodentium* (97). Mice lacking IL-17C exhibited exacerbated DSS-induced colitis and IL-17C was shown to induce the production of occludin, participating in the establishment of intestinal barrier functions (101). On the other hand, IL-17C is found overexpressed in tissue from IBD patients (102, 103). In addition, IL-17C was implicated in pathogenic responses in the joints, leading to the exacerbation of arthritis induced by collagen in the mouse (78). Of interest, IL-17C was found upregulated in rheumatoid nodules and in extract from synovial fluid mononuclear cells of RA patients (83, 104). Despite its action at epithelial surface, IL-17C was shown to potentiate Th17 cell responses in EAE model (105) and to act in a pro-atherogenic manner in transgenic IL-17C mice (106, 107). With regards to cancer, IL-17C is upregulated in several forms of

cancer, including colorectal and lung cancer, and was consistently shown to contribute to enhanced tumorigenesis in mice models (108). An overview of the role of IL-17C in inflammation is reported in **Table 5**.

Taken together, IL-17C appears to have much in common with the most widely studied members of the family: IL-17A and IL-17F. Synergizing with TNF, IL-17C potentiates protective antibacterial immune responses at epithelial surfaces, including the gut and the skin. Its overexpression is linked to skin conditions such as psoriasis. Similar to IL-17F, preclinical data in mice suggest that IL-17C might be pathogenic in joint disease while being protective in gut inflammation.

IL-17E (Also Known as IL-25)

Cloned in 2001, IL-17E maps to chromosome 14q11 and signals via a heterodimeric receptor complex composed of IL-17RB (also known as IL-25R) and IL-17RA (52) (**Table 6**). IL-17E is more commonly known as IL-25. As for other members of the IL-17 family, IL-17E is secreted as a disulfide-linked homodimer (50), while sharing only 16% sequence homology with IL-17A. This makes IL-17E the most divergent cytokine of the family. IL-17E is produced by many cell types: including epithelial cells, endothelial cells and several immune cells, such as T cells, macrophages, type-2 myeloid cells, DC, eosinophils and ILC2s (109, 110).

Several observations argue for a possible role of IL-17E in the skin. IL-17E is upregulated in the lesional tissue of several skin inflammatory disorders: atopic dermatitis (111–113), psoriasis (43, 111, 114), and, recently, contact dermatitis (115). The precise role of IL-17E in skin inflammation may well be disease-specific.

In atopic dermatitis, IL-17E is reported to negatively affect the level of the barrier protein fillagrin and to favor the loss of epidermal barrier function (111, 112). In addition, IL-17E-stimulated ILC2 cells were reported to play important roles in the regulation of skin inflammation in a mouse atopic dermatitis model (123). In psoriasis, we have found that IL-17E, produced by epidermal keratinocytes in the lesional skin, activates dermal macrophages to produce inflammatory cytokines, including TNF, and neutrophil chemokines, such as IL-8. Of note, IL-17E expression in lesional psoriatic skin correlated with the number of neutrophils, while negatively correlating with the number of T cells, suggesting that IL-17E may play a role in the chemoattraction of innate immune cells in the skin (43). These data are consistent with experiments *in vitro*, in which IL-17E has been shown to promote the expression of pro-inflammatory cytokines such as IL-8, CCL-5, and GM-CS, by human dermal and lung fibroblasts, and kidney cells (32, 78, 124, 125). The fact that a single nucleotide polymorphism (rs79877597) in the IL-17E gene associates with more severe disease and the presence of psoriatic arthritis further suggests that IL-17E may be pathogenic in psoriasis (16). In addition, improved symptoms after phototherapy was shown to correlate with a decrease in IL-17E serum levels in one patient presenting high steady-state levels of this cytokine (126). In contact dermatitis, IL-17E was shown to stimulate IL-1β production by DC, leading to enhanced Th17-, but not Th2 cell-, mediated inflammation (115).

The abovementioned results are puzzling, because the current belief depicts IL-17E as a Th2 cytokine, or a cytokine favoring Type 2 responses. IL-17E was originally reported to be expressed by Th2-polarized $CD4^+$ T cells (127). Later, other immune cells were found to respond to IL-17E by producing Th2 cytokines, and transgenic overexpression or systemic administration of IL-17E in mice results in eosinophilia in addition to neutrophilia, increased production of Th2 cytokines and pathological changes in the lungs and digestive tract. These included the presence of immune infiltrates, increased mucus production, and epithelial cell hyperplasia (128, 129). IL-17E was found to induce Th2 cell

TABLE 5 | Overview of the role exerted by IL-17C in inflammation.

Skin inflammation
– *Human/patient data*: IL-17C is increased in lesional psoriatic and atopic dermatitis skin (62, 91–93)
– *Animal models*: IL-17C contributes to skin inflammation induced by IMQ application and IL-23-injection (93, 96, 98). IL-17C overexpression in keratinoctes induces psoriasiform dermatitis (92).
Joint inflammation
– *Human/patient data*: IL-17C is expressed in the rheumatoid nodule (83) and by synovial fluid mononuclear cells of RA patients (104)
– *Animal models*: IL-17C contributes to the exacerbation of inflammatory arthritis in CIA model (78)
Gut inflammation
– *Human/patient data*: IL-17C expression is enhanced in the intestinal tissues from active IBD patients (102, 103)
– *Animal models*: IL-17C participates in mucosal responses to *Citrobacter rodentium*, promotes intestinal barrier functions, and protects from DSS-induced colitis (96, 97, 101)
CNS
– *Animal models*: IL-17C potentiates Th17 cell responses in EAE (105)
Cardiovascular disease
– *Animal models*: IL-17C plays a pro-atherogenic role (106). IL-17C induced skin inflammation (K5-IL17C model) is associated with faster arterial thrombotic occlusion (107)

CNS, central nervous system; IMQ, imiquimod; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; IBD, inflammatory bowel disease; DSS, dextran-sulfate sodium; EAE, experimental autoimmune encephalomyelitis.

TABLE 6 | Overview of the role exerted by IL-17E in inflammation.

Skin inflammation
– *Human/patient data*: IL-17E is increased in the lesional skin of psoriasis, atopic dermatitis, and contact dermatitis (43, 111–115)
Joint inflammation
– *Human/patient data*: IL-17E is increased in the serum and synovial fluid of RA patients (116, 117)
– *Animal models*: IL-17E attenuates CIA development (117)
Gut inflammation
– *Human/patient data*: IL-17E is downregulated in patients with IBD (118)
– *Animal models:* 17E was found to either ameliorate or aggravate colitis in mice depending on model (118, 119)
CNS
– *Animal models*: IL-17E suppresses Th17 immune responses in EAE (120)
Cardiovascular disease
– *Animal models*: IL-17E inhibits atherosclerosis development (121, 122)

CNS, central nervous system; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; IBD, inflammatory bowel disease; EAE, experimental autoimmune encephalomyelitis.

differentiation and activation (110), being thus crucial in host immune responses to nematode infections (130) and the development of allergic airway inflammation (131). Furthermore, IL-17E was shown to suppress Th17-mediated autoimmune diseases in mice, such as EAE and rheumatoid arthritis, mainly by skewing the immune system toward a Th2 response (120). In the gut, IL-17E may play both anti- and pro-inflammatory roles depending on the type of inflammation that is present (118). Moreover, IL-17E has been shown to inhibit atherosclerosis development in mice (121).

In summary, IL-17E is essential for protection against parasites and plays critical roles in Th2-mediated diseases (such as allergic asthma). In addition, it may limit Th17 cell responses, at least in mouse models of EAE and colitis. On the other hand, IL-17E is pathogenic in skin diseases, where it may function in « opposite » fashion and rather favor Th17 responses and recruitment of neutrophils. Thus, IL-17E effects appear to be highly tissue-specific.

CLINICAL IMPLICATIONS

Neutralization of IL-17A (secukinumab and ixekizumab) or the receptor subunit IL17RA (brodalumab) *via* monoclonal antibodies represents a highly effective approach to treat psoriasis (26, 27, 132). Blockade of IL-17RA also results in the inhibition of an array of members of the IL-17 family: namely IL-17A, IL-17F, IL-17C, and IL-17E. In addition to these licensed drugs, several other molecules targeting multiple IL-17 family members [IL-17A and IL-17F in the case of bimekizumab (71)], or the IL-17 pathway (either upstream, i.e., IL-23, or downstream signaling molecules) are in clinical development (133). Thus, a better knowledge of the structure of the IL-17 family and the function of their members with respect to inflammation is critical.

Although direct comparative trials have not been performed yet, indirect evidence suggests that IL-17RA inhibition may be superior to IL-17A inhibition, at least with respect to a greater likelihood of achieving PASI 100 and PASI 90 (134). Consistently, IL-17A, IL-17F, IL-17C, and IL-17E (all signaling *via* IL-17RA) have similar pro-inflammatory functions in the skin and have shown to play a pathogenic role in psoriatic skin manifestations. However, these same cytokines have also been reported to have non-redundant functions outside the skin, at least in the mouse. Thus, IL-17E has a protective role in CNS inflammation (120) and participates in protective responses against parasites in the intestine (130, 135, 136); and IL-17F and IL-17C are important for antibacterial immunity at epithelial surfaces (72, 73, 96, 97, 101). It remains to be addressed whether these tissue-specific functions exist in man too, and whether neutralization of several IL-17 cytokines might generate unwanted side effects outside the skin.

The possibility of inhibiting more than one IL-17 family member at a time could, however, reveal to be promising, as shown in the case of bimekizumab, a bi-specific anti-IL-17A and anti-IL-17F antibody (71, 137). While having a similar efficacy in the skin compared to IL-17A inhibition, this approach seems to perform better at the level of the joints, though larger trials have still to confirm this initial observation. This is consistent with a pathogenic role of IL-17F in joint inflammation, as observed in the mouse. Since IL-17C also participates in joint damage in mouse models of arthritis (133), it remains to be addressed whether its inhibition could be advantageous. On the other hand, both IL-17F and IL-17C are protective in murine gut inflammation (76, 96, 97, 101, 138). Whether a higher risk of intestinal inflammation due to IL-17F or IL-17C inhibition exists is unknown.

In summary, IL-17 family cytokines may elicit similar effects in target cells, but simultaneously may have very different (and sometimes opposite) functions in a tissue-specific manner. In addition, IL-17 cytokines have a great capacity of synergism, and their potency could be highly augmented by the cytokine milieu found at the inflamed site. These properties of the IL-17 family have direct clinical implications, as blocking more than one cytokine is a strategy currently under evaluation. A more comprehensive understanding of the mechanisms orchestrating the tissue-specific functions of the IL-17 family members in humans, and the relationship (causal or effector) existing among the different members of the family is required for a more rational drug design.

AUTHOR CONTRIBUTIONS

NB and W-HB defined the content of the manuscript, contributed to literature search and manuscript writing. LS contributed to literature search and manuscript writing. NB created graphical illustrations. All authors approved the final version of the manuscript.

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IL-36γ Is a Strong Inducer of IL-23 in Psoriatic Cells and Activates Angiogenesis

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The IL-1 family member cytokine IL-36γ is recognised as key mediator in the immunopathology of psoriasis, hallmarks of which involve the activation of both resident and infiltrating inflammatory myeloid cells and aberrant angiogenesis. This research demonstrates a role for IL-36γ in both myeloid activation and angiogenesis. We show that IL-36γ induces the production of psoriasis-associated cytokines from macrophages (IL-23 and TNFα) and that this response is enhanced in macrophages from psoriasis patients. This effect is specific for IL-36γ and could not be mimicked by other IL-1 family cytokines such as IL-1α. IL-36γ was also demonstrated to induce endothelial tube formation and branching, in a VEGF-A-dependent manner. Furthermore, IL-36γ-stimulated macrophages potently activated endothelial cells and led to increased adherence of monocytes, effects that were markedly more pronounced for psoriatic macrophages. Interestingly, regardless of stimulus, psoriasis monocytes showed increased adherence to both the stimulated and unstimulated endothelium when compared with monocytes from healthy individuals. Collectively, these findings show that IL-36γ has the potential to enhance endothelium directed leucocyte infiltration into the skin and strengthen the IL-23/IL-17 pathway adding to the growing evidence of pathogenetic roles for IL-36γ in psoriatic responses. Our findings also point to a cellular response, which could potentially explain cardiovascular comorbidities in psoriasis in the form of endothelial activation and increased monocyte adherence.

Keywords: psoriasis, IL-36γ, IL-23, macrophages, monocytes, angiogenesis, endothelial, inflammation

INTRODUCTION

Psoriasis is an immune mediated inflammatory disease which affects 2–3% of the world's population (1). Psoriatic lesions manifest as hyperkeratotic plaques, dermo-epidermal inflammation, and aberrant blood vessel formation caused by the complex interplay between tissue resident cells, dendritic cells, macrophages, and T cells and resultant enhanced expression of the IL-23–Th17/Th22 and IL-12–IFNγ/TNFα pathways (2).

IL-36α, IL-36β, and IL-36γ are members of the IL-1 family of cytokines that signal through a common receptor composed of IL-36 receptor (IL-36R) and IL-1R/AcP to activate NF-κB and MAPKs, such as p38 and JNK, and promote inflammatory responses.

IL-36α, IL-36β, and IL-36γ are members of the wider IL-1 family of cytokines. These cytokines mediate inflammatory events through the IL-36R and activate NF-κB and MAPKs, such as p38 and JNK in susceptible cells. The significance of IL-36γ for psoriatic inflammation is increasingly being recognised (3–6). IL-36 cytokines, in particular IL-36γ is dramatically upregulated in lesional psoriasis when compared with healthy controls (5). As well as acting as a psoriatic biomarker, loss-of-function mutations in the IL-36R antagonist (IL-36RA) in multiple cohorts of generalised pustular psoriasis (GPP) patients provide evidence that IL-36 plays a causative role in the pathology of psoriasis (7–9). IL-36 has recently also been implicated in other skin inflammatory diseases including acne and hidradenitis suppurativa, and allergic contact dermatitis (10, 11). IL-36γ, which is highly expressed by epithelial cells, is thought to be released in the context of cell damage or *via* non-conventional secretory pathways (12–14). Following release, it has been shown that IL-36γ is processed into its bioactive form by cathepsin S and results in the subsequent stimulation of surrounding tissues (15). IL-36R-mediated signal transduction has been shown to induce the release of pro-inflammatory cytokines (e.g., IL-8, TNFα, and IL-6), upregulate antimicrobial peptides and proliferative mediators such as defensins and HB-EGF, as well as T cell attracting or polarising cytokines such as CCL20 and IL-12, respectively (16–19).

Angiogenesis is the formation of new blood vessels from the preexisting vasculature and is a hallmark of psoriasis lesions (20). Microvascular changes within psoriasis lesions include pronounced dilation, increased permeability and endothelial cell proliferation. Immature permeable blood vessels may enhance dermal inflammation through immune cell recruitment (21, 22). A recent study confirmed a positive correlation between hypervascularisation and disease severity (23). Excessive capillary-venular dilatation precedes development of psoriatic inflammation, and resolution of these vascular changes is associated with remission of psoriasis lesions (24). VEGF-A is thought to be the driving force behind angiogenesis observed in psoriatic lesions. Mice that overexpress VEGF-A show an inflammatory response that histologically resembles psoriasis (25, 26). The *VEGFA* gene is located on chromosome 6 at 6p21, close to PSORS 1, which is a known chromosomal locus for psoriasis susceptibility (27, 28). The +405 CC *VEGFA* genotype, also known as the "high VEGF-A-producing genotype," is associated with early onset psoriasis, whereas the "low VEGF-A-producing genotype" has no association with psoriasis (29–31). This suggests that the pro-angiogenic potential of an individual may influence disease progression.

Treatment of human psoriasis with biologics has unequivocally shown that activation of the IL-23/IL-17 pathway is key for clinical symptom development (32). IL-23 induces and maintains the differentiation of IL-17- and IL-22-producing lymphocytes, which serve as the primary source of IL-17 and IL-22, both of which orchestrate epidermal hyperplasia and tissue inflammation in psoriasis (2). In murine induced psoriasis models, infiltrating macrophages, monocytes, and monocyte-derived dendritic cells and their subsequent T cell activating cytokines such as IL-23 have been shown to drive inflammation (33–37). A mechanistic link between IL-36 and the IL-23/IL-17 axis is becoming increasingly clear (6, 38–40). Work on other inflammatory skin diseases has also highlighted a correlation between IL-36 and IL-17 (41, 42).

Whilst previous reports have shown that IL-36γ induces inflammatory mediators from macrophages, little is known about its ability to induce psoriasis relevant cytokines such as TNFα and IL-23 (16). The ability of IL-36γ to induce such inflammatory mediators from infiltrating macrophages could escalate the inflammatory cascade by activating surrounding fibroblasts, endothelial cells (18), and keratinocytes and ultimately lead to further immune cell recruitment. In recent studies, GPP patients with DITRA (Deficiency of IL-36R Antagonist) showed significant disease improvement after receiving monocyte apheresis therapy, highlighting the potential importance of an IL-36-macrophage axis in the pathology of psoriasis (43, 44).

In this study, we highlight the role of IL-36γ in both macrophage and vascular activation in the context of psoriatic lesions. Our data demonstrate that IL-36γ induces the secretion of a key driver of psoriasis, IL-23, by macrophages and that this induction is enhanced in macrophages of psoriasis patients. IL-36γ also induces angiogenesis and branching of endothelial cells in a VEGF-A-dependent manner. Supernatant from IL-36γ treated macrophages potently activate endothelial cells and increased ICAM-1 expression. Psoriasis monocytes show an increased adhesion to both stimulated and untreated endothelial cells. Overall, the presented findings add to the growing body of evidence for IL-36γ as highly relevant molecule in psoriasis immunopathology.

MATERIALS AND METHODS

Cell Isolations and Cell Culture

Blood was collected in sodium citrate tubes. PBMCs were separated using Lymphoprep density gradient centrifugation. Monocytes were isolated from PBMCs using magnetic separation CD14+ beads (Miltenyi Biotech) using the Dynal MPC column (Invitrogen, CA, USA). Monocytes were resuspended in RPMI (ThermoFisher Scientific, MA, USA) containing 10% FCS and penicillin/streptomycin (100 U/100 mg/ml; both Life Technologies, Carlsbad, CA, USA). CD14+ purity was tested by FACs analysis with mouse antihuman CD14 FITC conjugated or mouse IgG isotype control (both 1:100; both ImmunoTools, Friesoythe, Germany). Purity for healthy patients was >90% (Figure S2 in Supplementary Material). Umbilical cords were supplied by Bradford Royal Infirmary under the approval and processing of Ethical Tissue Bradford. Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords in a previously described method (45). Monocytes were seeded onto plates (dependent on application) in RPMI overnight to generate day 1 macrophages.

Macrophage Purity and IL-36R Confirmation

Isolated macrophages were seeded onto coverslips overnight. Cells were washed in PBS and fixed in 4% formaldehyde for 20 min. Cells were then blocked for 1 h in 5% BSA in PBS. Cells were incubated overnight with rabbit anti-human IL-36R 1:500 (Novus Biologics, Littleton, CO, USA) or rabbit IgG isotype control (1:500; Abcam, Cambridge, UK). Cells were then washed with PBS and incubated with donkey anti-rabbit Alexa 594 conjugated and mouse anti-human CD14 FITC conjugated or mouse IgG isotype control (both 1:100; both ImmunoTools, Friesoythe, Germany). Cells were visualised using the EVOS XL microscope (Thermo Fisher Scientific).

Patient Demographics

Details on patients who gave blood for the study are listed below in (**Table 1**). All patients included are under care in the dermatology department and have a diagnosis of plaque psoriasis; one patient presented mainly with palmoplantar pustular psoriasis at the time point blood was taken. Patients receiving conventional systemic treatment known to change the biological response of leucocytes, in particular methotrexate, cyclosporine A, or leflunomide were excluded from the study. For this experimental setup, where cells were isolated involving multiple washing steps, cell culture and *ex vivo* stimulation, biologics treatment was not an exclusion criteria. We carefully checked the dataset, and there was no tendency for a difference in our outcome measured between cells derived from patients with or without biologics treatment.

As for the healthy controls, none were known to suffer from psoriasis, eczema or any active inflammatory disease under systemic treatment. Healthy controls were matched regarding gender distribution; the age range was between 28 and 52.

Macrophage Cytokine Stimulation

Monocytes were seeded at 1×10^5 in 96-well plates (Greiner Bio-One, Stonehouse, UK) in RPMI overnight to generate day 1 macrophages. Where relevant, macrophages were primed for 24 h with IFNγ 20 ng/ml. Macrophages were stimulated with IL-36γ protein, which was generated as previously described (15, 46), IL-17, TNFα, and IL-1α (PeproTech, Rocky Hill, NJ, USA). Following 48 h stimulation, supernatant was stored at −80°C. Concentrations of IL-23 and TNFα were measured using ELISA kits from eBioscience/ThermoFisher (Waltham, MA, USA). ELISAs were carried out according to the manufacturer's protocols. Reproducibility of the supernatants was confirmed by triplicate testing, with <10% error.

Tubulogenesis Assay

Primary human foreskin fibroblasts (PromoCell, Heidelberg, Germany) were cultured in 48-well plates in complete DMEM [containing 10% (v/v) FCS, 1% (v/v) non-essential amino acids and 1% (v/v) sodium pyruvate] until confluent. 6,500 HUVECs were seeded onto the fibroblasts monolayer in a 1 ml 1:1 mixture of complete DMEM and ECGM (PromoCell). Cells were left to acclimatise for 24 h. Media were aspirated and replaced with fresh ECGM ± growth factors (VEGF-A, 10 ng/ml) or IL-36 (50 ng/ml) or inhibitors (IL-36RA, 50 ng/ml), Sutent (Sigma, 1 nM) or anti-VEGF-A neutralising antibody (R&D Systems, 50 or 100 ng/ml) as indicated; media were replaced every 2–3 days for 9 days. Cocultures were fixed in 200 µl 10% (v/v) formalin for 20 min and blocked in 5% (w/v) BSA for 30 min at RT. Cocultures were then incubated with 1 µg/ml mouse anti-human PECAM-1 (CD31) (Santa Cruz, Dallas, TX, USA) overnight at 4°C. Cells were washed three times with PBS before incubation with anti-mouse Alexa Fluor 594 conjugate (Invitrogen) for 3 h at RT. Wells were washed three times with PBS. Endothelial tubules were visualised *via* immunofluorescence microscopy using an EVOS-fl inverted digital microscope (Thermo Fisher Scientific). Three random fields were imaged per well. Total tubule length and number of branch points were quantified from each photographic field using the open source software AngioQuant (www.cs.tut.fi/sgn/csb/angioquant) and values were averaged. For a more detailed method, see Ref. (47).

VEGF-A Induction Quantification by Immunoblot and ELISA

Endothelial or fibroblast cells were seeded into 6-well plates and cultured in ECGM or complete DMEM until ~80%

TABLE 1 | Patient demographics.

Diagnosis	Age	Gender	Disease duration (years)	PsA	PASI	Current systemic treatment
Plaque psoriasis	48	Female	42	Yes	Minimal disease activity	Secukinumab
Plaque psoriasis	38	Male	10	No	14.4	None
Plaque psoriasis	43	Female	Unknown	No	Minimal disease activity	Ustekinumab
Plaque psoriasis	55	Male	10	No	Minimal disease activity	None
Plaque psoriasis	41	Male	10	Yes	Minimal disease activity	Adalimumab
Plaque psoriasis	24	Female	13	No	5.1	None
Plaque psoriasis	52	Male	13	No	6	Ustekinumab
Plaque psoriasis	48	Male	10	No	8.1	None
Plaque psoriasis	29	Female	22	No	7.7	None
Plaque psoriasis	41	Female	28	Yes	1.2	Ustekinumab
Plaque psoriasis	35	Male	15	No	4	Ustekinumab
Plaque psoriasis	37	Male	10	No	8	None
Plaque psoriasis	48	Female	30	Yes	13	None
Pustular palmoplantar and plaque psoriasis	70	Female	Unknown	No	Minimal disease activity	Ustekinumab

PsA, psoriatic arthritis; PASI, Psoriasis Area and Severity Index.

confluent. Cells were then washed twice with PBS and starved in MCDB131 + 0.2% (w/v) BSA for 2 h before stimulation with IL-36 (50 ng/ml) for 24 h. Cells were then washed twice with ice-cold PBS and lysed in 2% (w/v) SDS, TBS, 1 mM PMSF and protease inhibitor cocktail (Sigma-Aldrich). Protein concentration was determined using the bicinchoninic acid assay (ThermoFisher). 20 µg of protein lysate was subjected to SDS-PAGE before transfer onto nitrocellulose membrane and analysis *via* immunoblotting using antibodies against VEGF-A, VEGFR1, and VEGFR2 (R&D Systems). For a detailed immunoblot protocol, see Ref. (48). The relative expression of the non-stimulated control was set to 1, and all other results expressed as a ratio of this. To measure VEGF-A secretion from both cell types, the supernatant was tested using VEGF-A ELISA kit (eBioscience/ThermoFisher).

Macrophage Supernatant-Endothelial Activation Assay

Following 48 h stimulation, supernatant was removed and stored at −80°C. HUVEC was cultured on black TC grade fluorescence plates (PerkinElmer, Waltham, MA, USA), in PromoCell endothelial cell media containing penicillin/streptomycin (100 U/100 mg/ml) (Life Technologies, Carlsbad, CA, USA). Supernatant was cultured with HUVEC at ratio of 1:10 for 24 h. Recombinant IL-36γ was added to control wells to serve as a blank. After 24 h, the cells were fixed for 15 min with 4% formaldehyde in PBS. Mouse anti-human ICAM-1 FITC or mouse IgG isotype control was added (1:500) (BioLegend, San Diego, CA, USA). The fluorescence intensity of each well was measured using the Promega GloMax plate reader (Madison, WI, USA). For immunocytochemistry, the cells were visualised using the EVOS XL microscope.

Monocyte Adherence Assays

Monolayers of HUVEC were grown to confluence in 24-well plates (Greiner Bio-One). HUVECs were stimulated with macrophage supernatant as above, or with TNFα (10 ng/ml) for 24 h and then suspended in fresh media before experiments. 1×10^5 monocytes were added per chamber for 30 min. After 30 min, non-adherent cells were washed away, and the cells were fixed using 4% formaldehyde in PBS and blocked in 5% BSA in PBS for 1 h. The cells were the stained with mouse anti-human CD14 FITC conjugated or mouse IgG isotype control (both 1:100) (both ImmunoTools). The cells were visualised using the EVOS XL microscope, and the number of adhered cells counted using ImageJ software.

Statistical Analysis

This was performed using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test, two-way ANOVA followed by Bonferroni multiple comparison or single unpaired *t*-test using GraphPad Prism software (La Jolla, CA, USA). Significant differences between control and test groups were evaluated with *p* values less than $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ indicated on the graphs. Error bars represent the SEM.

RESULTS

IL-36γ Induces Increased IL-23 and TNFα from Psoriasis Macrophages

Psoriasis is driven by aberrant type-3 immune responses, characterised by high levels of IL-17 and IL-22 (32, 49). A key inducer of type-3 responses is IL-23, which is expressed by antigen-presenting cells including macrophages upon stimulation with TLRs agonists such as LPS and flagellin (50, 51). IL-36γ is an abundant and prominent mediator in skin psoriasis, and we were interested in its ability to induce IL-23 expression by macrophages.

In support of previous mRNA data (52) IL-36R protein was found to be expressed by blood derived CD14+ monocyte/macrophages (**Figure 1A**) ($n = 3$, healthy). To assess the functional significance of IL-36γ interactions with macrophages, IL-36γ stimulation was performed for 48 h before analysis of TNFα and IL-23 secretion *via* ELISA (**Figure 1B**). As macrophages are known to be sensitive to LPS stimulation, boiled IL-36γ was included as a control for potential endotoxin contamination of the protein preparation. TNFα induction was measured at 24 and 48 h (Figure S1 in Supplementary Material). Differences between treatment groups became more apparent when more time was allowed for the secreted mediator to accumulate. Both 10 and 50 ng/ml IL-36γ induced a significant increase in IL-23 secretion when compared with unstimulated cells, which was further amplified when the macrophages were primed with IFNγ 20 ng/ml. For both doses of IL-36γ, macrophages from psoriatic donors secreted significantly more IL-23 compared with cells from healthy individuals. Other psoriasis relevant mediators such as IL-17, TNFα, and IL-1 did not induce a significant increase in IL-23 secretion, regardless of IFNγ priming. IL-36γ also induced significant TNFα secretion from macrophages, as did both IL-1 and IL-17 when compared with untreated controls. However, following IFNγ priming, IL-36γ induced secretion exceeded both IL-1 and IL-17.

IL-36-Stimulated Endothelial Cell Tubulogenesis

IL-36's relationship with angiogenesis in the context of inflammation is presently unknown. To close this knowledge gap, we investigated the role of IL-36 in blood vessel formation. Endothelial cell tubulogenesis was assessed using an endothelial-fibroblast coculture assay. Here, human endothelial cells were cocultured on a monolayer of primary human fibroblasts, before IL-36 or VEGF-A (positive control) stimulation, fixation, and visualisation of PECAM-1 positive endothelial cells (**Figure 2A**). Quantification revealed that both IL-36γ and IL-36α (50 ng/ml) stimulation produced a significant increase in both tubule length (**Figure 2B**) and branch point number (**Figure 2C**). Such effects were dependent on IL-36/IL-36R interactions, as treatment with an IL-36RA impaired IL-36-stimulated tubulogenesis (**Figures 2A–C**). Endothelial cell tubulogenesis was also enhanced in response to VEGF-A (10 ng/ml; **Figures 2A,D,E**); however, as expected, this was unaffected by co-treatment with IL-36RA (**Figures 2A,D,E**). Thus, these data show that IL-36R-mediated signal transduction promotes endothelial cell tubulogenesis.

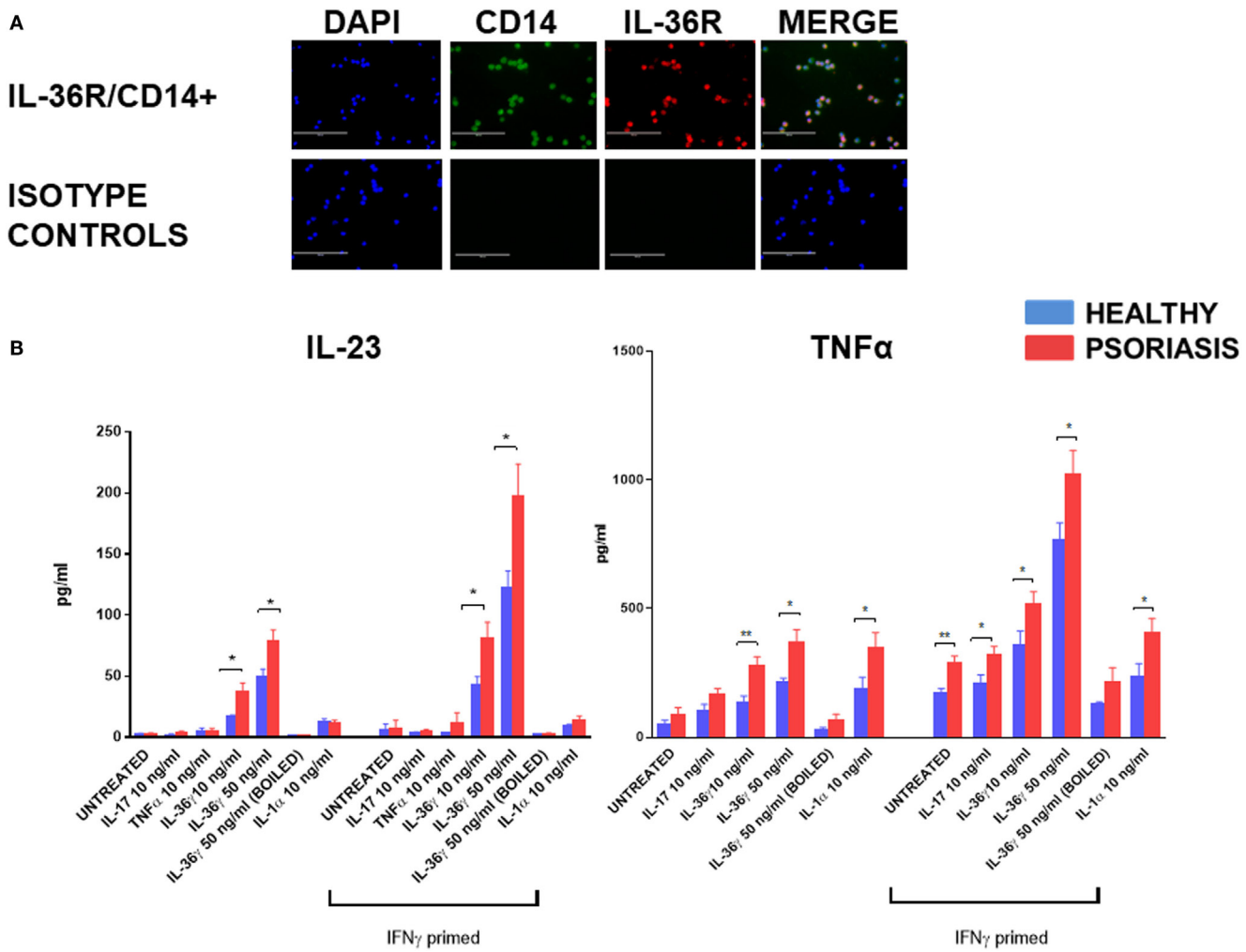

FIGURE 1 | **(A)** IL-36 receptor (IL-36R) (red) on macrophages; CD14+ (green) (magnification 20×). **(B)** Following 48 h stimulation with IL-36γ, IL-1α, IL-17A, TNFα, and IFNγ (24 h priming), supernatant was analysed by ELISA for TNFα and IL-23. Unpaired *t*-test $^{*}p < 0.05$, $^{**}p < 0.01$ psoriasis versus healthy (sample size: psoriasis = 9, healthy = 9, and boiled control $n = 3$).

IL-36-Stimulated Endothelial Cell Tubulogenesis Is VEGF-A Dependent

VEGF-A is a strong promoter of angiogenesis (53) and endothelial cell tube formation (**Figure 2**). Pro-angiogenic molecules such as IL-6 have been shown to induce the expression and secretion of VEGF-A; therefore, one possibility was that IL-36-mediated endothelial cell tube formation could be dependent on increased VEGF-A signalling. To test this, human endothelial cells were cocultured on a bed of primary human fibroblasts and stimulated with IL-36 or VEGF-A (positive control) in the presence or absence of an anti-VEGF-A neutralising antibody or the VEGFR inhibitor, Sutent (**Figure 3A**). Here, quantification revealed that IL-36-stimulated (50 ng/ml) endothelial cell tubulogenesis was significantly impaired in response to either the anti-VEGF-A neutralising antibody (50 and 100 ng/μl) or Sutent (1 nM; **Figures 3A–C**). As expected, VEGF-A-stimulated endothelial cell tubulogeneis was also impaired in response to either the anti-VEGF-A neutralising antibody or Sutent (**Figures 3A,D,E**). Therefore, these data show that IL-36-stimulated endothelial cell tube formation is dependent on VEGF-A-mediated signal transduction.

IL-36 Stimulation Upregulates VEGF-A Protein Levels in Fibroblast Cells

After concluding IL-36-induced angiogenesis is dependent on VEGF-A-mediated signal transduction (**Figure 3**), we assessed the effect of IL-36 stimulation on the protein levels of VEGF-A and its receptors VEGFR1 and VEGFR2. Here, endothelial or fibroblast cells were serum-starved before IL-36 stimulation (50 ng/ml; 24 h); cells were then lysed and subjected to immunoblotting (**Figure 3A**). Here, IL-36 stimulation significantly increased VEGF-A protein levels (~2-fold) in primary fibroblasts (**Figures 4A,B**), but not in endothelial cells (**Figures 4A,B**). IL-36 also induced significant VEGF secretion by fibroblasts, but not endothelial cells, as detected by ELISA (**Figure 4C**). However, IL-36

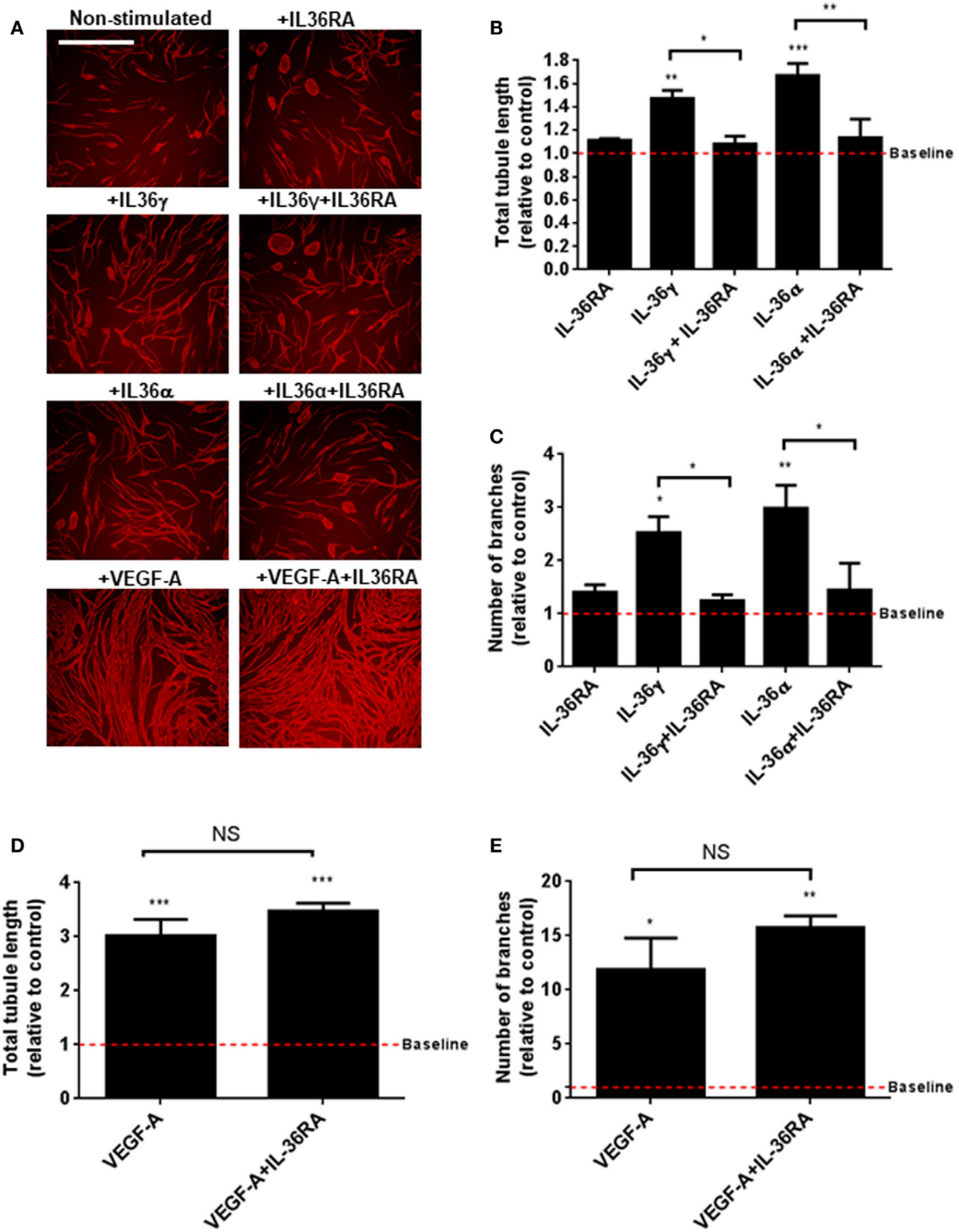

FIGURE 2 | IL-36 stimulates endothelial cell tubulogenesis. **(A)** Human umbilical vein endothelial cells were cocultured on a bed of primary human fibroblasts for 9 days and stimulated with either IL-36 (50 ng/ml) or VEGF-A (10 ng/ml). Cocultures were then fixed and stained for PECAM-1, before visualisation using immunofluorescence microscopy. **(B,C)** Quantification of **(B)** total tubule length or **(C)** number of branches upon IL-36 stimulation. **(D,E)** Quantification of **(D)** total tubule length or **(E)** number of branches upon VEGF-A stimulation. Scale bar represents 1,000 μm ($n = 3$). One-way analysis of variance was performed (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

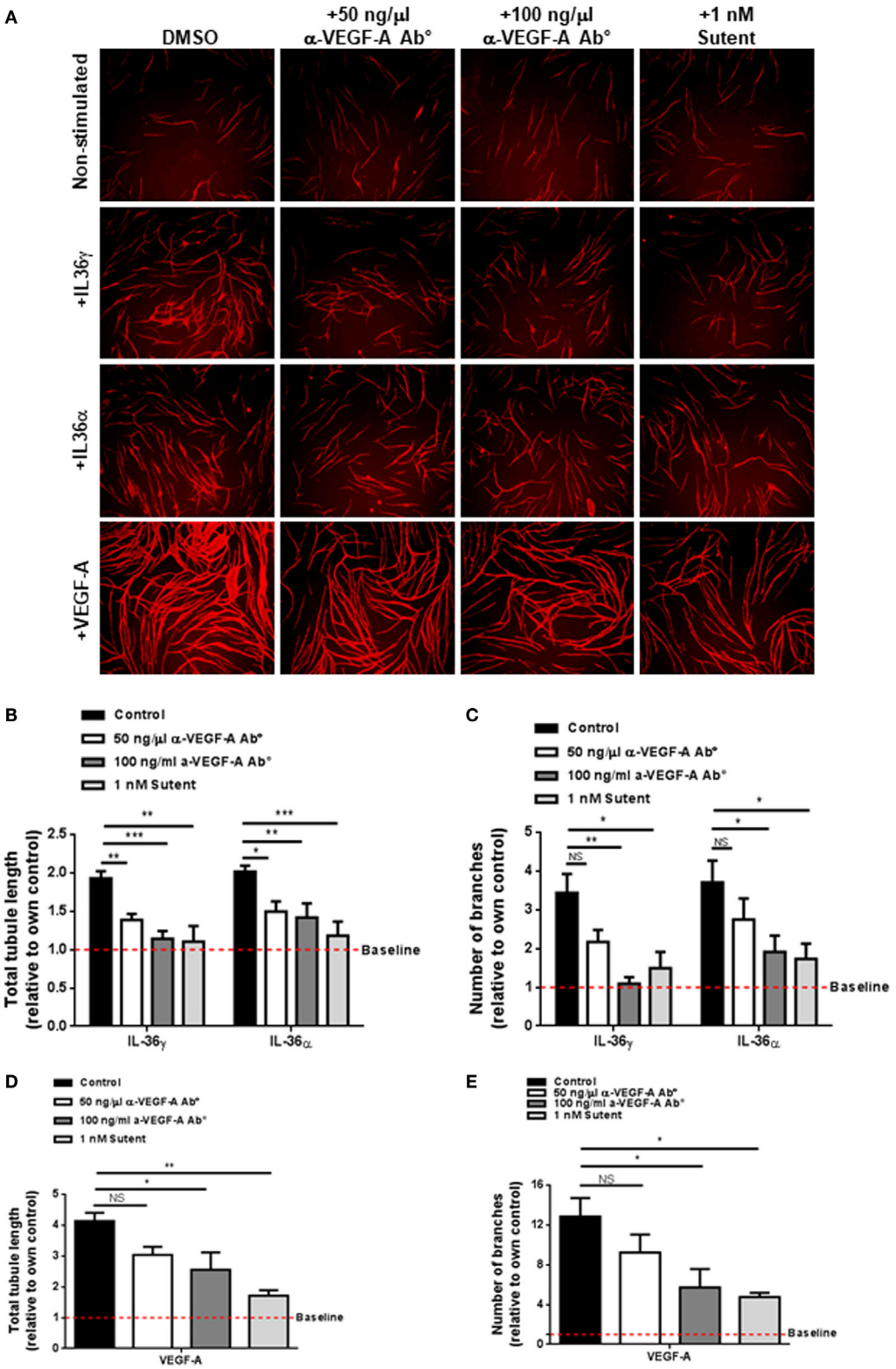

FIGURE 3 | IL-36-stimulated endothelial cell tubulogenesis is VEGF-A dependent. **(A)** Human umbilical vein endothelial cells were cocultured on a bed of primary human fibroblasts for 9 days and stimulated with either IL-36 (50 ng/ml) or VEGF-A (10 ng/ml) ± 50 ng/ml anti-VEGF-A antibody, 100 ng/ml anti-VEGF-A antibody, or 1 nM Sutent. Cocultures were then fixed and stained for PECAM-1, before visualisation using immunofluorescence microscopy. **(B,C)** Quantification of **(B)** total tubule length or **(C)** number of branches upon IL-36 stimulation. **(D,E)** Quantification of **(D)** total tubule length or **(E)** number of branches upon VEGF-A stimulation. Scale bar represents 1,000 µm ($n = 3$). Two-way analysis of variance (ANOVA) **(C,D)** and one-way ANOVA **(D,E)** were applied (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

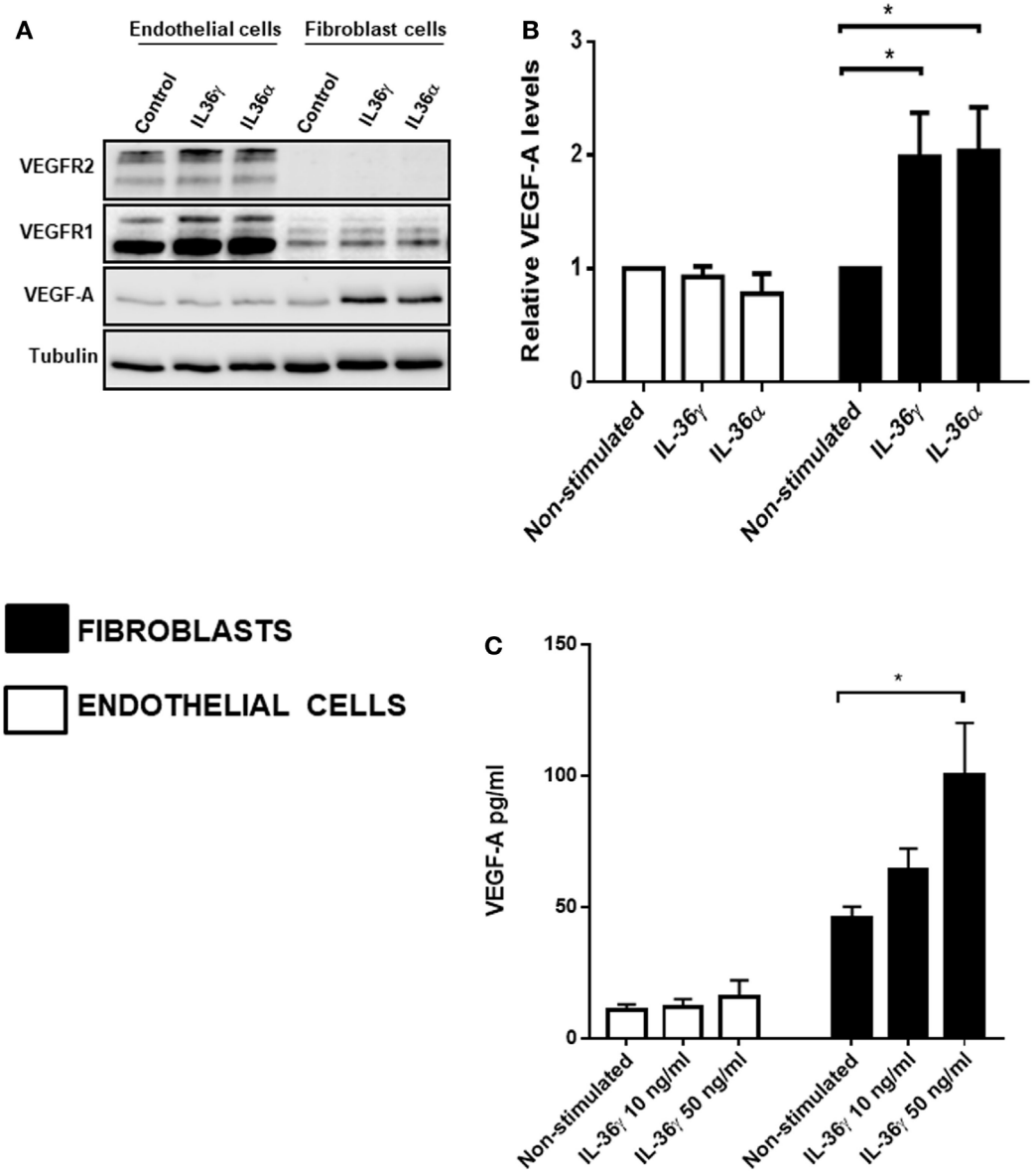

FIGURE 4 | IL-36 stimulates VEGF-A expression and secretion by primary human fibroblasts. **(A)** Human umbilical vein endothelial cells or foreskin-derived fibroblasts were stimulated with IL-36 (50 ng/ml) for 24 h, before cell lysis. Endothelial cell or fibroblast lysates were processed for detection of VEGF-A protein levels *via* immunoblot analysis. **(B)** Quantification of VEGF-A protein levels upon IL-36 stimulation by 2D-densitometry ($n = 3$). **(C)** The supernatant from both the stimulated cell types was also tested for VEGF-A protein concentration. Two-way analysis of variance was performed (*$p < 0.05$).

stimulation had no significant effect on VEGFR1 or VEGFR2 protein levels in either cell type (**Figure 4A**). These data suggest that IL-36-induced VEGF-A secretion from surrounding fibroblasts cell is capable of stimulating endothelial cell tubulogenesis.

Psoriasis Monocytes Show Increased Adhesion

As monocyte recruitment is crucial for psoriasis lesion development, but also comorbidities associated with psoriasis such as atherosclerosis, we decided to investigate the adhesive properties of psoriasis patients' monocytes to HUVECs. To fully visualise potential differences in monocyte adhesion we worked with non-stimulated HUVEC monolayers but also used the best described stimulus, TNFα, to reliably upregulate adhesion molecules on endothelial cells (54). Psoriasis and healthy monocytes (healthy $n = 8$, psoriasis $n = 8$) were allowed to adhere for 30 min to HUVECs. Psoriasis patients' monocytes showed increased adherence to both untreated and TNFα activated HUVECs (**Figures 5A,B**).

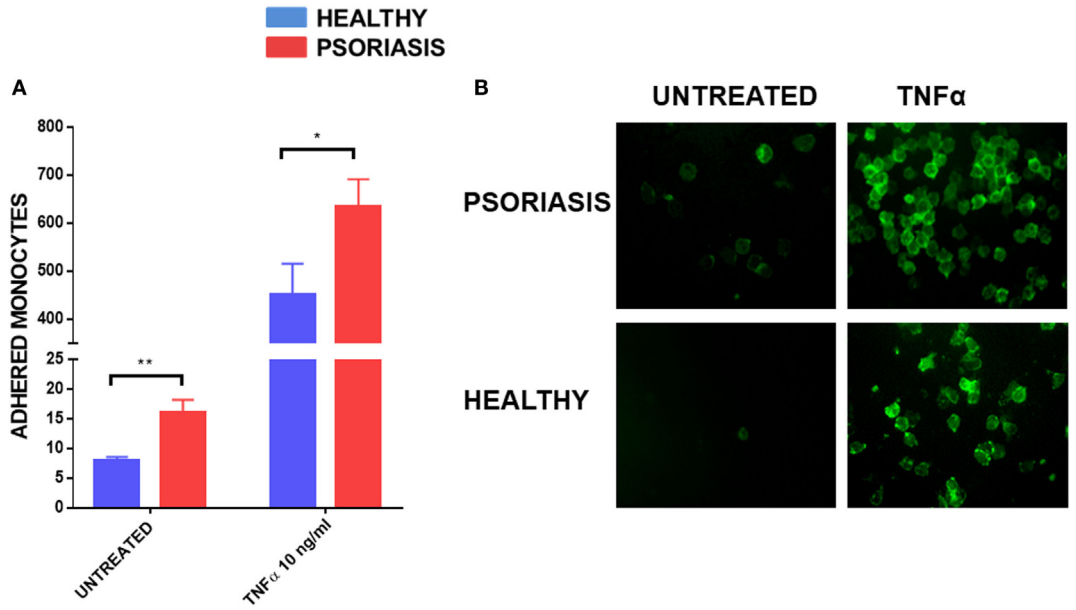

FIGURE 5 | Human umbilical vein endothelial cell monolayer was stimulated with or without TNFα 10 ng for 24 h. 1 × 10^5 monocytes were allowed to adhere to the monolayer for 30 min. Cells were visualised by immunofluorescence microscopy following CD14+ staining **(B)** and counted **(A)** (patient monocytes: psoriasis = 8; healthy = 8). Magnification 40×. Unpaired *t*-test *$p < 0.05$ and **$p < 0.01$.

IL-36γ-Stimulated Macrophage Supernatant Activates Endothelial Cells

To further understand the functional role of IL-36γ beyond the epidermal compartment we examined the aspect of immune cell recruitment into the skin using endothelial cells and monocytes/macrophages. Supernatants from IL-36γ-stimulated macrophages were incubated with endothelial cells (HUVECs) for 24 h at a ratio of 1:10. As IL-36 alone has pro-inflammatory effects on HUVEC, a recombinant control was also added. IL-36γ-stimulated supernatant markedly increased expression of the adhesion molecule ICAM-1 (**Figures 6A,B**). Supernatant derived from IL-36γ-stimulated, psoriasis macrophages induced significantly more ICAM-1 expression when compared with healthy macrophages (**Figures 6A,B**). Stimulation of HUVECs with psoriasis macrophage supernatant resulted in increased adhesion of both healthy and psoriasis monocytes (**Figure 6C**) (healthy $n = 8$, psoriasis $n = 8$). However, regardless of HUVEC stimulation (untreated or treated, supernatant from healthy or psoriasis macrophages), psoriasis monocytes showed increased endothelial adhesion.

DISCUSSION

IL-36γ is an IL-1 family cytokine with an increasingly recognised importance in the pathology of psoriasis (5). Various myeloid cells are thought to contribute to the pathology of psoriasis, including macrophages (33). Myeloid cells are capable of secreting IL-23 and thus contributing to the IL-23/IL-17 axis, prominent in psoriasis (36). The central role of a type-3 immune response shift in psoriatic inflammation is convincingly demonstrated by the overwhelmingly positive therapeutic response of biologics interfering with the IL-23/IL-17 axis, which leads to complete or almost complete clearing of psoriatic symptoms in a large number of patients receiving these treatments. Within psoriasis lesions, monocytes, macrophages and dendritic cells all show positive staining for IL-23 (55).

With IL-36γ being released by keratinocytes, probably in the context of cellular stress/environmental challenges, its downstream actions on dermal cells including fibroblasts, endothelial cells but also resident and infiltrating myeloid cells such as macrophages could represent a key step in both early and chronic lesion pathology. While IL-36γ has previously been shown to induce IL-23 mRNA in murine bone marrow derived dendritic cells, we here report secretion of IL-23 protein by human macrophages (56). Interestingly, other inflammatory cytokines prominent in psoriatic lesions, TNFα, IL-17, and IL-1, had little or no ability to induce IL-23 when compared with IL-36γ. Psoriasis macrophages secreted significantly more IL-23 following IL-36γ stimulation than healthy macrophages. Our findings also complement findings from an imiquimod-induced mouse model of psoriasis which has shown to be dependent on MyD88 signalling in macrophages (57). Whilst macrophage derived IL-23 is thought to be crucial to the immunopathological development of psoriasis lesions, we are the first to report a viable cytokine agonist for this induction, in IL-36γ. Clinical case reports also support our findings and the idea of a potential IL-36-macrophage pathway within psoriasis pathology. Two case reports show patients with DITRA, who suffer from a lack of function mutation in the endogenous IL-36RA, benefit from monocyte apheresis treatment (44, 58).

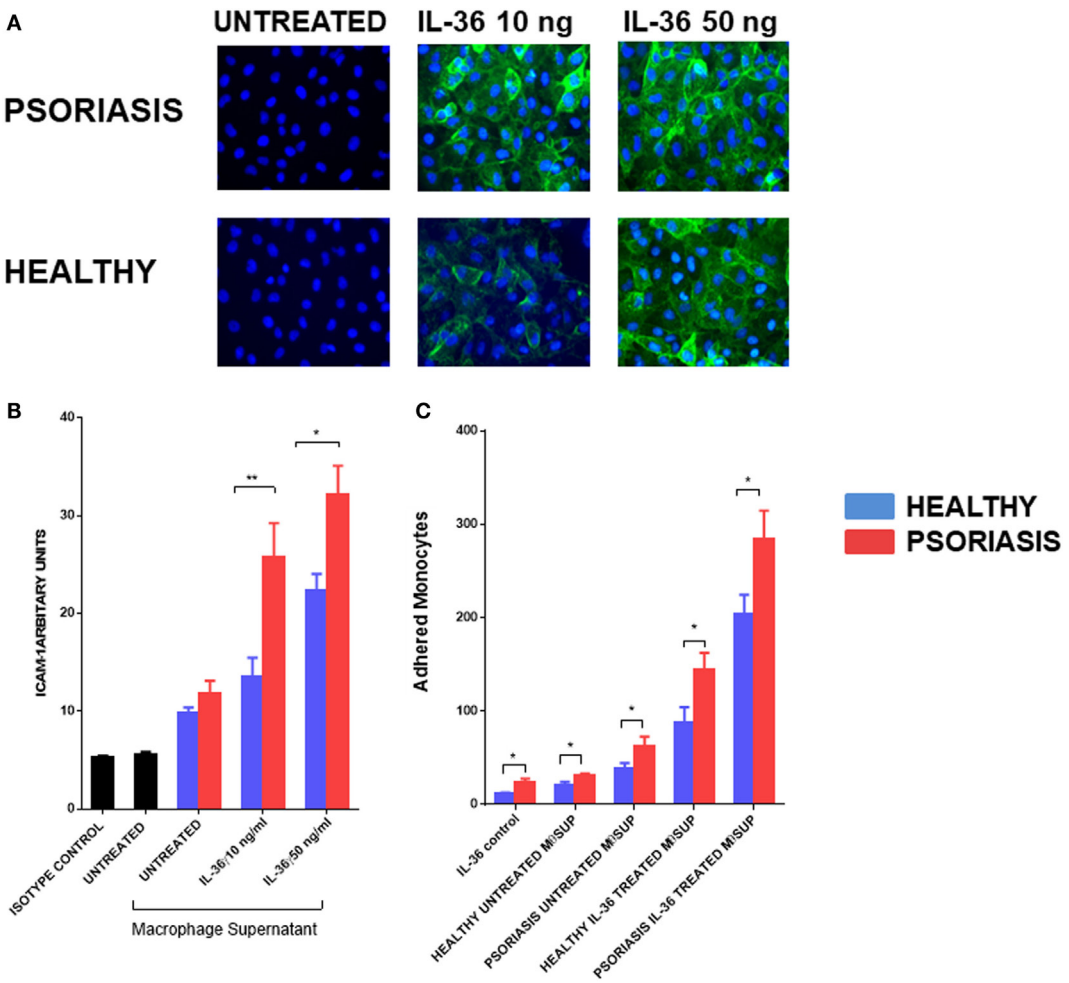

FIGURE 6 | **(A,B)** Supernatants from IL-36γ-stimulated psoriasis or healthy macrophages were used to stimulate human umbilical vein endothelial cell (HUVECs) for 24 h, and ICAM-1 (green) and DAPI (blue) expression as visualised by immunofluorescence microscopy is depicted in panel **(A)** and intensity of staining measured by fluorescence absorbance summarised in panel [**(B)**; psoriasis $n = 8$, healthy $n = 8$]. **(C)** Healthy and psoriasis monocytes (1×10^5) were allowed to adhere to a HUVEC monolayer for 30 min. The monolayer was stimulated with supernatant derived from IL-36-stimulated/non-stimulated psoriasis or healthy derived macrophage supernatant. Patient monocytes: psoriasis $n = 8$, healthy $n = 8$. Unpaired t-test $^*p < 0.05$ and $^{**}p < 0.01$.

IL-36γ induced secretion of IL-23 was enhanced when macrophages were primed and activated with IFNγ. IFNγ enhancement of/priming for IL-23 secretion from macrophages has previously also been shown with other TIR agonists such as TLRs (50, 59). IFNγ has also been shown to induce an inflammatory phenotype characteristic of psoriasis when injected into the skin and serum levels correlate with disease severity (60, 61). In this context it is noteworthy that IL-36, similar to other IL-1 family members has been shown to enhance IFNγ production in CD4+ T cells (62, 63). We found in our experimental setting that synergy with IFNγ is a prominent feature for IL-36 induced responses but not for IL-17, TNFα, or the IL-1 family member and TIR agonist IL-1. Previous reports have also shown that IL-1 induced TNFα secretion from macrophages is not enhanced by IFNγ (64).

TNFα was prominently induced in macrophages by IL-36γ. Similar to IL-23, psoriasis macrophages had higher basal expression levels and secreted significantly more TNFα when stimulated with IL-36γ. Consequently, IL-36γ induced TNFα from infiltrating macrophages would be well placed to potently stimulate the surrounding tissues to further orchestrate the immune response, activate the endothelium, and increase leucocyte migration.

TNFα is known to be a prominent inflammatory activator of the endothelium and we show that IL-36 induced macrophage supernatant is a potent activator of the endothelium, with the adhesion molecule ICAM-1 showing upregulation (65). In accordance with enhanced cytokine secretion seen from psoriasis macrophages, their supernatant was able to achieve increased endothelial activation when compared with healthy supernatant. Deciphering the most important activator of the endothelium within the supernatant will require further study. Whilst, TNFα is a known activator of the endothelium and several biologic treatments targeting TNFα have proved successful in psoriasis

conditions (66), IL-36γ is known to induce other cytokines from macrophages (including a positive autocrine feedback on its own production) which could induce similar effects. The key information from this set of experiments is the striking difference in the endothelium activating potential of IL-36-stimulated macrophages between healthy and psoriasis individuals. IL-36 is expressed in high abundance in all lesional psoriasis epidermis (5).

Enhanced cytokine secretion from psoriasis macrophages is characteristic of the exaggerated immune response associated with psoriasis (67). Previous studies have also shown psoriasis macrophages to secrete increased IL-8, IL-1α/β, and TNFα when untreated and this is in agreement with our findings (68, 69). Numerous genetic variations are thought to exist in psoriasis cells which lead to dysregulated immune responses (67). IL-36γ signals through NF-κB, and various variants—including TNFAIP3 (A20) and CARD14—are thought to exist within psoriasis cells that lead to increased activity of NF-κB (67, 70). Recent studies also suggest IL-36 may play a role in macrophage polarisation (40), and thereby affect subsequent cytokine secretion. However, whether IL-36 has a direct or indirect role in macrophage polarisation within psoriasis is as yet unknown.

Whilst damaged keratinocytes may be a potential source of IL-36γ, macrophages within lesions also show positive IL-36 staining (6). Interestingly, lung macrophages secrete IL-36γ in microparticles following LPS stimulation (71). Potential autocrine actions of IL-36 on macrophages thus require further study.

We report that following stimulation of endothelial cells with IL-36 induced macrophage supernatant, monocytes show significantly increased adherence. When compared with healthy controls, supernatants from psoriasis macrophages have an increased ability to stimulate and adhere to the endothelium (ICAM-1). Interestingly, regardless of the stimulus, psoriasis monocytes showed increased adhesion to both unstimulated and stimulated endothelial cells. A previous study has also found monocytes from psoriasis patients to show increased activation and integrin expression (72).

We report here that IL-36γ induced angiogenesis is dependent on VEGF-A induction. VEGF-A is perhaps the best documented inducer of angiogenesis, and its presence in psoriasis lesions is long established (73). VEGF-A and both VEGFRs are overexpressed in psoriasis lesions and serum levels of VEGF-A correlate with PASI (74–76). A genetic variant in *VEGFA* is also associated with more severe psoriasis (+405 CC) and is thought to result in increased VEGF-A production (29, 31). Interestingly, the same SNP is also associated with poor prognosis in patients with chronic heart failure (77). Importantly, it is thought that angiogenesis precedes symptomatic lesion formation; so it could be hypothesised that IL-36γ released from damaged keratinocytes would be well placed to stimulate VEGF-A synthesis and thus angiogenesis when compared with other cytokines that would have importance in a chronic lesion (78).

Angiogenesis has even been muted as a potential therapeutic target for psoriasis (20, 79, 80). Case reports have demonstrated improvements in PASI through targeting pro-angiogenic factors such as VEGF-A. Bevacizumab, a monoclonal antibody against VEGF-A used in the treatment for solid cancers, has also been shown to be effective in treating psoriasis, including one case of complete remission for a patient being treated for metastatic colon cancer (81). Case reports for tyrosine kinase inhibitors that target VEGFRs such as sunitinib and sorafenib also have produced positive results regarding psoriasis symptom reduction (82–84). Of interest, G6-31, a murine antibody against VEGF-A has demonstrated therapeutic improvement, in a mouse model of psoriasis (85). VALPHA is a fusion protein that targets both TNFα and VEGF-A and has shown to be effective in treating TPA induced psoriasis in mouse models (86).

The findings presented here also may have implications for other inflammatory diseases. Crohn's shares some immunological aspects with psoriasis, namely the IL-23/IL-17 axis activation, and in addition, a potential role for IL-36 in Crohn's is becoming apparent (6, 87). Interestingly, angiogenesis is also a feature of Crohn's (88). Similarly, IL-36 has been implicated in mouse models of respiratory infection and again linked to the IL-23/IL-17 axis, and furthermore, angiogenesis is associated with chronic lung inflammation (40, 89, 90). Whilst IL-36 is yet to be fully implicated in COPD, cigarette smoke, the causative agent of COPD, induces IL-36 from bronchial epithelial cells (91). COPD is heavily associated with Th17 cell driven inflammation (92). Psoriasis is emerging as a risk factor for COPD, and furthermore, mouse models of psoriasis show enhanced airway inflammation attributed to IL-23 signalling (93–95). For skin diseases there is emerging evidence for IL-36 to be upregulated in pathologies with neutrophil components (e.g., acne and hidradenitis suppurativa) and to some extent in all inflammatory diseases involving epidermal responses (96). Although, difficult to dissect the precise *in vivo* relevance of the IL-36-induced VEGF-A mediated angiogenesis, multiple observations point towards an important potential role. Angiogenesis does play a physiological important role in healing responses where IL-36 could have an important impact (97). In a mouse model of psoriasis, systemic anti-VEGF-A treatment has also reduced skin inflammation (85). However, VEGF-A is also induced by other skin inflammatory mediators such as TNFα, and the net effect of IL-36 remains to be shown in future *in vivo* studies.

Our data greatly support previous data suggesting a role for IL-36 in the pathology of psoriasis. IL-36 has been shown to be intimately involved in the epidermal changes characterising psoriatic lesions. This study provides further evidence of a direct relationship between the development of a Th17 psoriatic phenotype and IL-36. IL-36 acts on tissue infiltrating macrophage and actively promotes recruitment of monocytes which cumulatively amplify IL-23 expression, thus promoting polarisation of lymphocytes for increased IL-17/IL-22 expression. In addition, IL-36 directed angiogenesis is dependent on VEGF, a recognised precursor to the development of a psoriatic plaque. We therefore demonstrate a central, pivotal role of IL-36 in the development and propagation of psoriatic disease. This builds on current understanding of psoriasis pathogenesis and provides a further potential therapeutic target in managing disease. Given its potential role in establishing a psoriatic plaque this may offer an opportunity to affect the disease course through preventing a chronic disease signature being established. Thus, deciphering the exact significance of IL-36 in the psoriatic disease continuum remains an important issue for further translational and clinical studies.

ETHICS STATEMENT

This study was approved by Yorkshire and the Humber—Leeds West Research Ethics Committee with written informed consent from all subjects (PDAR study: REC 16/YH/0086).

AUTHOR CONTRIBUTIONS

CB wrote manuscript and conducted macrophage experiments. GF performed angiogenesis experiments and help write the manuscript. AB, PL, and MW delivered the clinical ethical and patient-related aspects of the projects and obtained clinical samples. PL and SP contributed to critical appraisal of results. TM generated and provided IL-36 molecules. MS, MW, and AG contributed to experimental planning, critical discussion of results obtained, as well as manuscript correction.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fimmu.2018.00200/full#supplementary-material.

FIGURE S2 | Monocytes were isolated from PBMCs using magnetic separation CD14+ beads. CD14+ purity was tested by FACs analysis with mouse anti-human CD14 FITC conjugated or mouse IgG isotype control Purity for healthy and diseased patients was >90.

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REVIEW
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The Interplay Between Keratinocytes and Immune Cells in the Pathogenesis of Psoriasis

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Psoriasis is a chronic inflammatory skin disease resulting from genetic, epigenetic, environmental, and lifestyle factors. To date, several immunopathogenic mechanisms of psoriasis have been elucidated, and, in the current model, the cross talk between autoreactive T cells and resident keratinocytes generates inflammatory and immune circuits responsible for the initiation, progression, and persistence of the disease. Several autoantigens derived from keratinocytes (i.e., LL37 cathelecidin/nucleic acid complexes, newly generated lipid antigens) have been identified, which may trigger initial activation of T cells, particularly IL-17-producing T cells, T helper (Th)1 and Th22 cells. Hence, lymphokines released in skin lesions are pivotal for keratinocyte activation and production of inflammatory molecules, which in turn lead to amplification of the local immune responses. Intrinsic genetic alterations of keratinocytes in the activation of signal transduction pathways dependent on T-cell-derived cytokines are also fundamental. The current review emphasizes the aberrant interplay of immune cells and skin-resident keratinocytes in establishing and sustaining inflammatory and immune responses in psoriasis.

Keywords: psoriasis, keratinocytes, immune cells, skin inflammation, innate immunity, adaptive immunity

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INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder involving both innate and adaptive immunity processes. It is caused by the infiltration of distinct effector leukocyte subpopulations in both the epidermis and dermis, which determines hyperproliferation of the epidermis with aberrant differentiation of keratinocytes (1, 2). As a consequence, the epidermis is thickened, with elongated rete ridges forming protrusions into dermis (2, 3). There has been a long debate on pathogenic functions of keratinocytes in psoriasis, and numerous studies have established that hyperproliferation and abnormal differentiation of keratinocytes is a secondary phenomenon induced by immune activation. This "immune" hypothesis, mainly based on dendritic cell (DC) and T cell pathogenic functions, has found confirmation in the efficacy of immune-targeting treatments (4–6). However, psoriasis cannot be considered uniquely as a T-cell-dependent disease, and it is now well known that keratinocytes have a crucial role in triggering the early pathogenic events, as well as in sustaining the chronic phase of the disease (1, 7). Early upstream events occurring in psoriasis include induction of innate immunity pathways and responses, and keratinocytes represent the "first-line responding" skin cells to psoriasis pathogenic environment (8). Upon activation by trigger factors, such as skin trauma and pathogens (i.e., streptococci) or drugs, respectively, keratinocytes become a source of innate immune mediators (**Figure 1A**). The latter include cationic antimicrobial peptides (AMP),

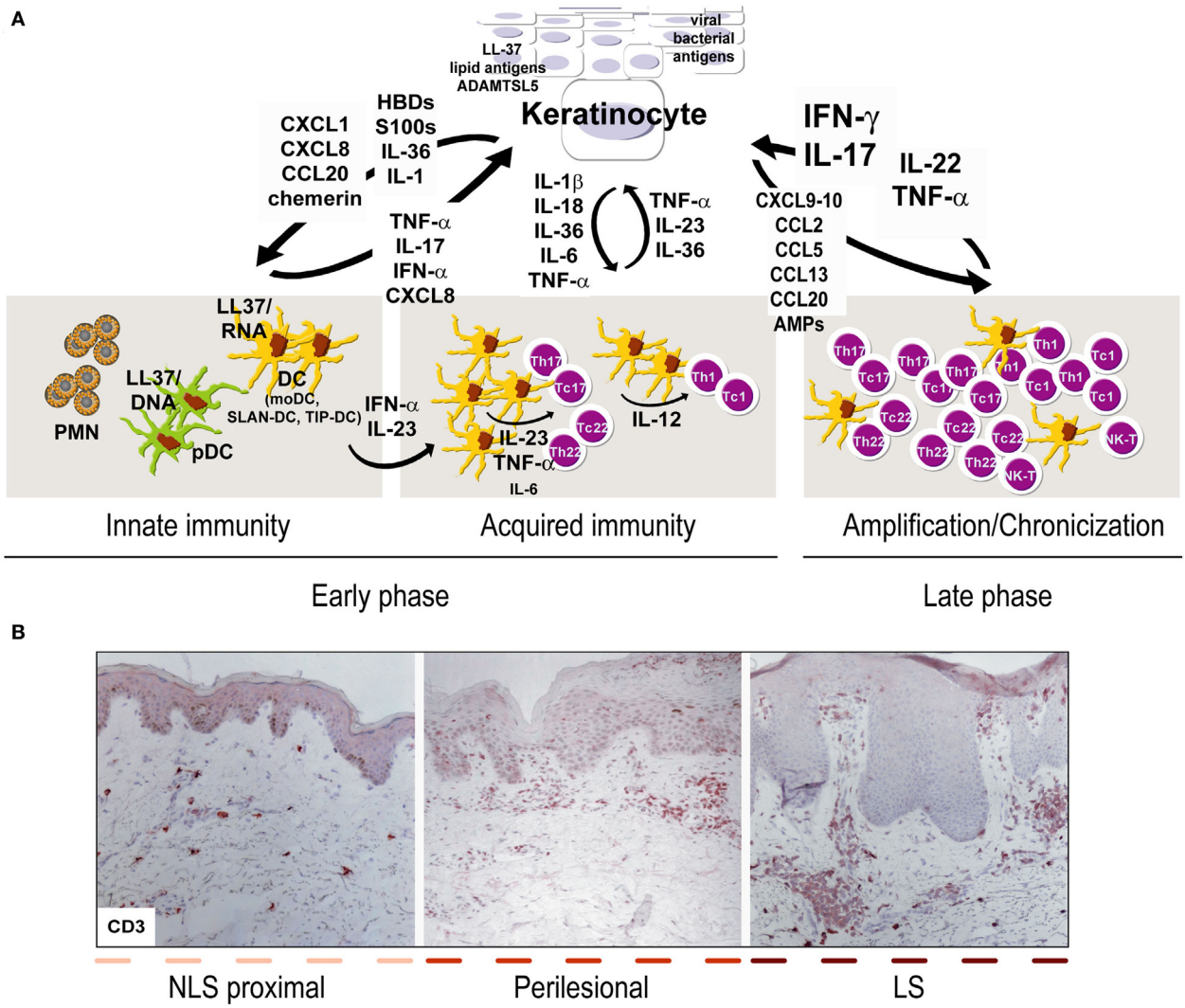

FIGURE 1 | Aberrant interplay of keratinocytes and immune cells in psoriasis. **(A)** Early upstream events in psoriasis include induction of innate immunity pathways and acquired responses, and keratinocytes represent the "key responding" skin cells by producing trigger factors, including LL37/nucleic acid complexes, lipid antigens, and ADAMTSL5, as well as pathogens of viral or bacterial origin. During this initial phase, keratinocytes also produce antimicrobial peptides (AMP), such as β-defensins (HBD) and S100 proteins, together with chemokines and cytokines of IL-1 family. Among AMP, LL37 activates plasmacytoid dendritic cells and myeloid DC (mDC), which routinely patrol uninvolved psoriatic skin, in particular, non-lesional (NLS) skin proximal to lesions, with consequent beginning of the adaptive immune phase. In early phase, SLAN-DC and TIP-DC, highly producing TNF-α, are also present. Hence, DC drive expansion of T lymphocytes, mostly Th17 and Th22 in the beginning and type-1 interferon (IFN)-γ-producing T cells, especially during the chronic phase. During acquired immunity phase, keratinocytes influence DC immune functions by producing cytokines derived from inflammasome pathway, and IL-36. T-cell infiltrate present during late/chronic phase of psoriasis establishes a cytokine milieu, mainly represented by IFN-γ, TNF-α, IL-17, IL-22, which dictates specific and pathogenic gene signatures in keratinocytes, which, thus, overexpress a number of inflammatory mediators. In parallel, under the influence of cytokines, in particular, IL-22 and IL-17, keratinocytes hyperproliferate and show altered differentiative programs. During the amplification/chronicization phase of the disease, unceasing cross-talk between keratinocytes and immune cells further amplifies inflammation and hyperplasia. **(B)** Both early and late phases of psoriasis can be found within the same psoriatic plaque, being it comprehensive of LS, perilesional and adjacent NLS areas, with markers of chronic inflammation (i.e., $CD3^+$ T accumulation in the dermis) predominantly present in LS skin [CD3 stainings have been retrieved from Ref. (48)].

cytokines of IL-1 family, and chemokines active in the recruitment of leukocyte subpopulations of innate immunity, such as plasmacytoid dendritic cells (pDC), neutrophils, mast cells, and macrophages (9, 10). Among AMP, the cathelicidin LL37 has been associated with the development of psoriasis, through its capacity to activate pDC and myeloid DC (mDC), with consequent initiation of the adaptive immune phase (10, 11). DC drive expansion of T lymphocytes, typically T helper (Th)17 and Th22 in the initial phase and interferon (IFN)-γ-producing T cells during the chronic phase of the disease (**Figure 1A**). T-cell infiltrate present in active psoriatic skin establishes a cytokine milieu that dictates specific gene signatures in keratinocytes, which, thus, overexpress several inflammatory mediators amplifying local immune reactions (12, 13). Unceasing cross-talk between keratinocytes and

adaptive immunity cells further intensifies inflammation and may be essential in disease chronicity (**Figure 1A**). In addition, intrinsic or genetic alterations of keratinocytes in the activation of key signaling pathways induced by immune cell-derived cytokines may be responsible for the typical unbalance between proliferation and differentiation processes, as well as inflammatory signatures observed in psoriatic epidermis (14, 15).

In this review, we will illustrate the multiple functions of keratinocytes during the initiation, maintenance, and amplification of the immune and inflammatory programs associated with psoriasis. A critical role of keratinocytes in triggering and sustaining the innate and adaptive immune responses will be discussed.

PSORIATIC KERATINOCYTES AS INDUCERS OF INNATE IMMUNE RESPONSES IN THE EARLY PHASE OF PSORIASIS DEVELOPMENT

Much efforts have been devoted to understanding the primary pathogenic mechanisms and the cell components responsible for the onset of the disease. Growing evidences propose a fundamental role for keratinocytes. At this initial phase, pDC and neutrophils infiltrate skin plaque lesions, and psoriatic keratinocytes are deeply involved in their recruitment and activation (16). Important studies aimed at identifying the initial trigger of psoriasis demonstrated that injured keratinocytes enable pDC-, concomitantly to mDC-driven immune responses through LL37/nucleic acid complexes, highly released in psoriatic epidermis after skin trauma (11, 17). These multimeric LL37–nucleic acid complexes induce overproduction of type I IFN by pDC as well as TNF-α and IL-6 by mDC (11, 18). A similar mechanism for pDC activation has been described for human β-defensin (HBD)2 and HBD3, other two AMP released by psoriatic keratinocytes (19). Also, macrophage populations, highly present in early lesional skin, can be activated by keratinocyte-derived LL37, which promotes their differentiation toward a proinflammatory signature by recognizing the P2×7 purinergic receptor (20). Remarkably, keratinocyte-derived LL37 also regulates the expression of cytokines of IL-1 family, including IL-36γ, by keratinocytes themselves in paracrine and autocrine loops (10). Furthermore, LL37 induces CXCL8 and CXCL1 chemokines through IL-36R signaling in psoriatic keratinocytes, which would, in turn, determine the recruitment and the burst of neutrophils in lesional skin, typical of the early phase of psoriasis (10). Importantly, LL37 induces CXCL10 and CCL20 in psoriatic keratinocytes and is most likely responsible for the first flare of the acquired immunity established in later phase of psoriasis by DC and Th17 (10).

Other chemotactic factors are expressed by keratinocytes during the induction phase of psoriasis. For instance, the chemokine chemerin, which is also abundantly released by dermal fibroblasts, is responsible for migration and accumulation of BDCA-2$^+$ pDC into pre-psoriatic skin, as well as in early psoriatic lesions. Through secretion of IFN-α, pDC activate pathogenic T-cells by inducing the maturation of mDC, and by initiating the mononuclear Th1 responses, which persist in adaptive immunity (21, 22). Chemerin expression by keratinocytes and fibroblasts is lost during later stages of plaque development, and pDC disappear in fully developed psoriasis plaques (16).

Of note, the production of IFN-α by early immigrating pDC favors acquired immune responses also by suppressing IL-1 production by keratinocytes, and, therefore, by shortening skin inflammation (23). In addition, the pathogenicity of IFN-α is also supported by the findings showing its signaling signature in keratinocytes of psoriatic plaques (24), and that psoriasis is exacerbated if patients with psoriasis are treated with IFN-α for unrelated diseases, such as viral infections or tumors, or by imiquimod (IMQ) that induces IFN-α release by pDC *via* toll-like receptor activation (25, 26).

Keratinocyte-derived IL-1α represents an additional inducer of innate immune responses in psoriasis, and it can favor neutrophil and monocyte accumulation during early psoriasis papule formation. IL-1α functions as an alarm signal and is heavily induced in necrotic psoriatic keratinocytes, especially by factors such as cytosolic DNA (27). Concomitantly to IL-1α, self-DNA induces assembling of psoriatic inflammasome containing the DNA sensor AIM2, and, thus, promotes the production and release of IL-1β and IL-18, other two pathogenic member of IL-1 family (27). Interestingly, cathelicidin LL37 can interfere with DNA-sensing inflammasomes, suggesting an antagonistic function for this peptide in the autoinflammatory pathways associated to early psoriatic processes. Critical alterations in the IL-1/IL-1 receptor system have also been found in lesional psoriatic skin (28, 29), with IL-1α and IL-1β being abundant in psoriatic lesions concomitantly to the soluble isoform of the IL-1 receptor antagonist and IL-1-RII decoy receptor. These observations suggest the impairment of negative regulatory mechanisms of IL-1 system in psoriasis. Consistently, transgenic overexpression of IL-1α and IL-1 type I receptor in the skin leads to a pathogenic phenotype in mice resembling psoriasis (30). Other than activating innate immune responses, IL-1α has found to promote keratinocyte and endothelial cell proliferation and activation (31).

KERATINOCYTE INVOLVEMENT IN ADAPTIVE IMMUNE RESPONSES IN PSORIATIC SKIN

Psoriasis lesional skin shows many inflammatory CD3$^+$ T cells, which progressively accumulate in both the upper dermis and epidermis (1, 2), thus determining the typical epidermal hyperplasia and inflammation picture (**Figure 1B**). Immunophenotyping of T cells shows that they are mostly activated memory T cells expressing cutaneous lymphocyte antigen (CLA) and belongs to distinct subsets of CD4$^+$ and CD8$^+$ lymphocytes, Th1/Tc1, Th17/Tc17, and Th22/Tc22 (2). DC activation is determinant in driving T cell responses, even if keratinocytes can trigger and condition DC responses, and, thus, influence acquired immunity in psoriatic skin.

First, keratinocytes have been described as active producers of causative antigens of psoriasis, and the position of dermal mDC at the dermal–epidermal junction, as well as in the epidermis would favor the capture by mDC of keratinocyte-derived

extracellular antigens for presentation to T cells, and intracellular antigens *via* cross-presentation. Recently, three autoantigens identified in keratinocytes were found to be involved in the pathogenesis of psoriasis. Among them, LL37 is recognized as autoantigen by circulating $CD4^+$ and $CD8^+$ T cells with a cytokine and skin-homing receptor profile typical of psoriatic skin T cells (IFN-γ^{high}, IL-17^{high}, CLA^+, $CCR6^+$, and $CCR10^+$), in up to 75% of psoriatic patients (32). Most recently, phospholipase A2 group IVD was identified in psoriatic keratinocytes as important player in the generation of psoriasis autoantigens (33). The latter include non-protein neolipids that are recognized by CD1a-restricted T cells, thereby inducing the production of IL-22 and IL-17A (34). These lipid antigens could be transferred from keratinocytes to neighboring antigen-presenting cells through released exosomes, similarly to what observed for $tryptase^+$ mast cells of psoriatic lesions (34). Finally, the disintegrin and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5), a protein modulating microfibril functions (34, 35) and identified as autoantigen presented by melanocytes in a HLA-C*06:02-restricted fashion (36), has been also recently localized in keratinocytes throughout the psoriatic epidermis (34, 37). Keratinocytes could also activate pathogenic T cells by presenting viral or bacterial products. A relevant presence of human papillomavirus-5 DNA and reactive antibodies against virus-related particles have been found in psoriasis (38). Infections by *Streptococcus* commonly associate with psoriasis, and streptococcus-derived superantigens can be presented to T lymphocytes by MHC class II-bearing keratinocytes. Psoriatic antigens are also supposed to be keratinocyte-derived molecules sharing structural homology with streptococcal proteins, which could, therefore, induce autoreactive T-cell responses against skin components (39).

Second, DC function and maturation in the psoriatic context is also depending on mediators released by keratinocytes during the innate immunity phase. Among them, IL-36 cytokines, a subgroup of IL-1 family, comprising the IL-36α, β, and γ agonists, strongly upregulated in psoriatic lesional epidermis, were found to influence DC function (40, 41). In humans, IL-36 cytokines activate mDC by increasing the proportion of cells with a strong CD83, CD86, and HLA-DR membrane expression and induce the secretion of IL-1β and IL-6, thus promoting the Th17 differentiation (42). Consistently, keratinocyte/DC cross talk mediated by IL-36 was essential in driving IMQ-induced psoriasiform dermatitis in mice. In this model, IL-36 signaling controlled the aberrant IL-23/IL-17/IL-22 axis and disease development (43). Similarly, IL-18 highly released by keratinocytes downstream to inflammasome pathway is involved in the recruitment of IL-18R-bearing DC to inflammatory sites characterized by Th1 responses, as in psoriasis. IL-18 from keratinocytes in synergy with IL-12 plays a role in the Th1 response, primarily by inducing IFN-γ in psoriatic lesions (44). In addition, recent evidence suggests that γδ T cells infiltrating can produce IL-17 *via* IL-23 in presence of IL-18 (45). Together with IL-18, mature IL-1β is also produced by keratinocytes as result of inflammasome activation, and influence DC-mediated immune responses. A number of evidence links IL-1β and Th17 pathways in psoriasis pathogenesis both in mice and in humans. Transgenic overexpression of IL-1β is responsible for massive presence of Th17 cells in the skin, as well as for the inflammatory psoriasisform phenotype of mice (46). Furthermore, the IL-23-dependent differentiation of human Th17 cells relies on the copresence of IL-1β (27).

KERATINOCYTES ARE A RESERVOIR OF INFLAMMATORY MEDIATORS, WHICH SUSTAIN PERSISTENCE OF PSORIASIS LESIONS

During the chronic phase of psoriasis, expansion and activation of T and DC subpopulations in lesional areas establishes a definite cytokine micromilieu, mostly represented by IFN-γ, IL-17, TNF-α, and IL-22. Keratinocytes bear receptors for these cytokines and potently respond by further releasing cytokines. Under the effects of these cytokines, keratinocytes also show altered proliferative and differentiation programs, as well as enhanced resistance to apoptosis (1, 47–49).

Each cytokine regulates distinct responses in keratinocytes with a certain degree of overlap in gene expression induction/inhibition. Transcriptional profiling studies conducted on lesional psoriatic skin showed that the IFN-γ-signature predominates, with the upregulated expression of approximately 400 genes dependent on signal transducer and activator of transcription 1 (STAT1), the preferential IFN-γ molecular node (50). *In vitro*, IFN-γ represents the most potent stimulus for keratinocyte inflammatory and immune activation, as it regulates the expression of approximately 1,200 genes (51). Importantly, the IFN-γ capability to induce inflammation in psoriasis was demonstrated by a study showing that IFN-γ injection in clinically uninvolved psoriatic skin determines a transcriptomic profile and inflammatory cell infiltration similar to lesional skin (51). Indeed, the same results were obtained with healthy volunteers, indicating that other cytokines specifically contribute to the psoriatic phenotype (51). IFN-γ induces in psoriatic keratinocytes key-disease mediators, such as chemokines attracting monocytes and Th1 and Th17 cells (CCL2, CCL5, CXCR3 ligands), DC (CCL13, CCL20), or $CCR10^+$ skin-homing CLA^+ T cells highly infiltrating psoriatic skin (1). HBDs and S100 proteins are also potently induced by IFN-γ in keratinocytes, alone or in synergy with TNF-α. Most of the IFN-γ-induced effects in keratinocytes are potentiated by TNF-α, which signals mainly by activating NF-κB, a transcription factor regulating gene expression frequently in collaboration with STAT1. TNF-α induces expression of ICAM-1 on keratinocytes, permitting the adhesion of circulating leukocytes. Moreover, TNF-α stimulates keratinocyte production of several chemokines active on neutrophils, T cells, and DC (CXCL1, CXCL2, CXCL8, CCL2, CCL5), as well as pro-inflammatory cytokines, such as IL-6 and IL-1, which help maintain Th17 cells (1, 2). Importantly, TNF-α induces IL-36γ in psoriasis lesions, which in turn promote expression of AMP and chemokines recruiting neutrophils and Th17 cells, as well as interfere with terminal differentiation and cornification process of psoriatic epidermis (52). The increased production of IL-36γ is also associated with the presence of Th17 lymphokines in psoriatic skin lesions, as IL-17 and IL-22 strongly induce its expression (52, 53).

Keratinocytes are also strongly influenced by IL-17 and upregulate chemokines (CXCL1, CXCL3, CXCL5, CXCL8, CCL20), AMPs (LCN2, HBD-2, S100A7), and immunomodulatory molecules, such as ICAM1, in response to this cytokine. In addition, IL-17 stimulates LL37 autoantigen production (54–56) and delay terminal differentiation of keratinocytes. IL-17 activates NF-κB, possibly through the IKBζ transcriptional regulator, as well as Act-1 intracellular pathway, which is required for IL-17 induction of keratinocyte host defense genes and inhibition of differentiative programs in keratinocytes (57). IL-17 also induces IL-19, a member of IL-20 family, which has mitogenic effects on keratinocytes themselves (58).

Th17- or Th22-derived IL-22 cytokine also acts pathogenically on psoriatic keratinocytes by promoting release of chemokines (CXCL1, CXCL2, CXCL8, CCL20), AMPs (HBD-2, HBD-3, S100 proteins), as well as inducing their proliferation and de-differentiation (59, 60). Binding of IL-22 to its receptor, the expression of which in the skin is confined to keratinocytes, mediates epidermal acanthosis through the activation of STAT3. These observations may explain the increased STAT3 expression in the epidermal compartment and the pathogenicity of STAT3 overexpression in the epidermis of transgenic mice (61). Although studies demonstrated a central role of IL-22 in psoriasis pathogenesis, this cytokine induces a limited panel of genes compared to IL-17, as detected in human lesional psoriatic skin (55), and antibodies neutralizing IL-22 failed to show a therapeutic effect in patients with psoriasis.

Collectively, IFN-γ, IL-17, TNF-α, and IL-22 can cause keratinocyte production of chemokines, cytokines, and AMPs, as well as concur to derange proliferative and differentiative programs of the epidermis. This becomes a self-amplifying loop, where these products and altered homeostasis act back on T cells, DC, and neutrophils to perpetuate the cutaneous inflammatory processes.

CONCLUSION

Currently, there are no meaningful hypotheses to explain certain typical features of psoriasis including the sharply demarcated presentation of the lesions and the more frequent localization of the lesions in certain anatomical sites (i.e., scalp, extensor body areas), although a skin/keratinocyte-specific mechanism may be involved. In contrast, the fluctuant behavior of psoriasis with phases of remission and recrudescence may be related to prevailing regulatory and effector immune mechanisms. Activated T lymphocytes are required for the development and persistence of immune responses in psoriatic skin, even though psoriasis cannot be considered uniquely as a T cell-dependent disease. In fact, the presence of type-17, type-1, and type-22 subtypes in inflamed skin could be the result of a common response to antigens, also effective in subjects not predisposed to psoriasis. Indeed, this paradox could be explained either by the presence of other not yet defined psoriasis-related cytokines in skin lesions, or by the intrinsic aberrant response of psoriatic keratinocytes to cytokines or other effector molecules. Several studies showed that genetic defects in keratinocytes are fundamental for psoriasis development. An increasing number of specific single-nucleotide polymorphisms (SNP), found in genes controlling T-cell commitment and keratinocyte inflammatory activation as well as proliferation and differentiation processes in the epidermis, has been associated with psoriasis (14). Among them, a number of SNPs were found in genes encoding molecules involved in IL-17 or TNF-α responses, even though functional studies correlating their presence to keratinocyte susceptibility to these cytokines are lacking and controversial. For instance, allelic variants were found in *NFKBIZ*, *TRAF3IP2*, and *TNFAIP3* genes (62) encoding IKBζ, Act-1 and the Act1-dependent A20 protein, respectively, all involved in IL-17 molecular signaling (57). However, no clear evidence linking these SNPs to enhanced or reduced responses of keratinocytes to IL-17 exist. In fact, Act-1 gene variants overexpressed in human keratinocytes could decrease as well as enhance Act-1-mediated IL-17 signaling, depending on the SNP type. Variants of *NFKBIZ* could also influence keratinocyte response to TNF-α since IKBζ is a transcriptional cofactor of p50 subunit of NF-κB, the main mediator of TNF-α signaling. In addition, NF-κB activity is regulated by CARD14, a protein of "signalosome" complex involved in activation of innate immunity molecules (i.e., IL-36γ, CXCL8, and CCL20), whose gene shows different allelic variants associated to psoriasis (63). Although a recent study clarified the impact of a CARD14 missense variant (Card14ΔE138), whose expression in mice determined a severe psoriatic phenotype (64), the effects of CARD14 SNPs on TNF-α- or IL-17-induced responses in keratinocytes remain to be defined.

Future studies must consider these genetic aspects, especially those concerning the relationship between genetic determinants and keratinocyte inflammatory responses to psoriasis-related cytokines. Moreover, genetic data need to be further integrated with analyses of the cytokine milieu specifically characterizing the psoriatic patient. This will permit to predict the responsiveness of psoriatic patient to a specific therapy, thus implementing a personalized medicine approach.

AUTHOR CONTRIBUTIONS

Each author has contributed in the ideation and writing of the manuscript, and each author has checked the final version of the paper.

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Long Non-Coding RNAs Play a Role in the Pathogenesis of Psoriatic Arthritis by Regulating MicroRNAs and Genes Involved in Inflammation and Metabolic Syndrome

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Psoriatic arthritis (PsA) is an inflammatory arthritis, characterized by inflammation of enthe-ses and synovium, leading to joint erosions and new bone formation. It affects 10–30% of patients with psoriasis, and has an estimated prevalence of approximately 1%. PsA is considered to be primarily an autoimmune disease, driven by autoreactive T cells directed against autoantigens present in the skin and in the joints. However, an autoinflammatory origin has recently been proposed. Long noncoding RNAs (lncRNAs) are RNAs more than 200 nucleotides in length that do not encode proteins. LncRNAs play important roles in several biological processes, including chromatin remodeling, transcription control, and post-transcriptional processing. Several studies have shown that lncRNAs are expressed in a stage-specific or lineage-specific manner in immune cells that have a role in the development, activation, and effector functions of immune cells. LncRNAs are thought to play a role in several diseases, including autoimmune disorders. Indeed, a few lncRNAs have been identified in systemic lupus erythematosus, rheumatoid arthritis, and psoriasis. Although several high-throughput studies have been performed to identify lncRNAs, their biological and pathological relevance are still unknown, and most transcriptome studies in autoimmune diseases have only assessed protein-coding transcripts. No data are currently available on lncRNAs in PsA. Therefore, by microarray analysis, we have investi-gated the expression profiles of more than 50,000 human lncRNAs in blood samples from PsA patients and healthy controls using Human Clariom D Affymetrix chips, suitable to detect rare and low-expressing transcripts otherwise unnoticed by common sequencing methodologies. Network analysis identified lncRNAs targeting highly connected genes in the PsA transcriptome. Such genes are involved in molecular pathways crucial for PsA pathogenesis, including immune response, glycolipid metabolism, bone remodeling, type 1 interferon, wingless related integration site, and tumor necrosis factor signaling. Selected lncRNAs were validated by RT-PCR in an expanded cohort of patients. Moreover, modulated genes belonging to meaningful pathways were validated by RT-PCR in PsA PBMCs and/or by ELISA in PsA sera. The findings indicate that lncRNAs are involved in PsA pathogenesis by regulating both microRNAs and genes and open new avenues for the identification of new biomarkers and therapeutical targets.

Keywords: psoriatic arthritis, gene expression, long non-coding RNAs, gene module, protein–protein interaction network

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INTRODUCTION

Psoriatic arthritis (PsA) is a chronic, immune-mediated, asymmetric inflammatory arthritis characterized by inflammation at tendon or ligament insertion sites into bone (enthesitis) and by synovitis, eventually leading to joint erosions and new bone formation (1).

Up to 30% of patients with skin psoriasis may develop PsA and its prevalence is estimated in 1% in the general population. PsA shares genetic and clinical features with other forms of seronegative spondyloarthritis (2, 3). Diagnostic criteria for PsA have not been validated, but the Classification Criteria for PsA (CASPAR criteria), published in 2006, define PsA for the purpose of enrolling patients in clinical trials and provide guidance to clinicians (4, 5). Therefore, the diagnosis of PsA is mainly performed on clinical features after the exclusion of other seronegative arthritides and no diagnostic tests are available so far.

The pathogenesis of PsA is still poorly understood and both autoinflammation and autoimmunity are believed to play a pivotal role in the disease. Synovial tissue in PsA is characterized by T-cell infiltrate, by marked angiogenesis, and by synovial hyperplasia with increased secretion of cytokines and proteases, which may amplify the local inflammatory process eventually leading to joint destruction (6). Tumor necrosis factor-alpha (TNF-α) is a very important inflammatory mediator and has been implicated in the pathogenesis of articular damage in PsA (6). TNF-α inhibitors are currently used in PsA treatment; however, a high percentage of PsA patients does not respond to TNF-α antagonists (1, 7). Therefore, other cytokines have recently become targets of biological agents, such as interleukin-12 (IL-12), interleukin-23 (IL-23), and interleukin-17 (IL-17) (1, 7). Indeed IL-17 plays a fundamental role in disease development and progression (8).

We have reported the findings of the transcriptome analysis in paired synovial tissue and peripheral blood cells of patients with PsA (9). The upregulation of Th-17 cells related genes and of type I interferon (IFN) inducible genes in PsA patients strengthened the hypothesis that PsA has a strong autoimmune origin, since the coactivity of type I IFN and IL-17 pathways is typical of autoimmunity (9). Moreover, we confirmed these findings with a miRNA microarray analysis in PBMCs of PsA patients showing that pathway enrichment analysis on gene targets of deregulated microRNAs (miRNAs) revealed signaling pathways typically implicated in PsA, such as TNF, mitogen-activated protein (MAP) kinase, and wingless related integration site (WNT) cascades (10).

By this study we wanted to provide a more in-depth knowledge on the epigenetic mechanisms that regulate the PsA pathogenesis by analyzing the expression profiles of long non-coding RNAs (lncRNAs) in the same cohort of patients that we studied in our previous work (10). lncRNAs are important molecules that regulate gene expression through multiple mechanisms and are involved in immune and inflammatory pathways (11). As far as we know, no study has yet taken into consideration lncRNAs expression profiles in PsA patients and only a few data have been reported the deregulation of some lncRNAs in psoriasis (12, 13).

Moreover, in this study we offer a sophisticated and integrated analysis of lncRNAs, miRNAs, and gene expression profiles in PsA patients that allows to identify lncRNAs that regulate transcripts effectively modulated in the disease and that are involved in pathogenetically relevant molecular pathways.

MATERIALS AND METHODS

Patients

We studied a cohort of 10 patients (6 males and 4 females, mean age: 53.5 years) affected by PsA, attending the Unit of Autoimmune Diseases, at the University Hospital of Verona, Italy. All patients fulfilled the CASPAR criteria for the diagnosis of PsA: inflammatory musculoskeletal involvement combined with at least three features: (1) evidence of current psoriasis, personal history of psoriasis, and family history of psoriasis in unaffected patients; (2) affected nails (onycholysis and pitting); (3) dactylitis; (4) negative rheumatoid factor (RF); and (5) radiographic evidence of new juxta-articular bone formation (excluding osteophytes) (4). All the patients underwent clinical examination and laboratory evaluation comprehensive of inflammatory markers, such as C-reactive protein and erythrocytes sedimentation rate; RF and anti-cyclic citrullinated peptide antibody detected by ELISA test; antinuclear antibody detected by indirect immunofluorescence on HeLa-derived HEp-2 cells; and genetic screening for the association with the allele HLA-B27. All patients underwent the following instrumental investigations: ultrasonography with Power Doppler to investigate subclinical enthesopathy and synovitis in asymptomatic patients, conventional radiography, magnetic resonance imaging, and scintigraphy. The radiological features of peripheral PsA included asymmetric distribution, participation of distal interphalangeal joints, periostitis, bone density preservation, bone ankylosis, and pencil-in-cup deformity.

The patients were affected by cutaneous or nails psoriasis and were enrolled in the study at diagnosis of peripheral PsA before starting immunosuppressive treatment.

All the participants to the study signed a written informed consent and the local Ethical Committee of the University Hospital of Verona, Verona, Italy, had approved the study protocol. All the investigations have been performed according to the principles of the Helsinki declaration.

Microarray Analysis

Blood samples were collected in BD Vacutainer K2EDTA tubes using a 21-gauge needle. PBMCs were obtained upon stratification on Lympholyte® cell separation density gradient (Cedarlane, Burlington, ON, Canada). PBMCs composition was similar between patients and controls. Total RNA extraction from PBMCs was performed with miRNeasy mini kit following manufacturer's protocol (Qiagen GmbH, Hilden, Germany). 500 ng of total RNA were used for sample preparation starting from 5 ml of blood. cRNA preparation, samples hybridization and scanning were performed following the Affymetrix (Affymetrix, Santa Clara, CA, USA) provided protocols, by Cogentech Affymetrix microarray unit (Campus IFOM IEO, Milan, Italy). All samples were hybridized on Human Clariom D (Thermo Fisher Scientific) gene chip and were analyzed using the Transcriptome Analysis Console 4.0 software (Applied Biosystem, Foster City, CA, USA by Thermo Fisher Scientific, Waltham, MA, USA).

The Human Clariom D arrays allow to interrogate more than 540,000 transcripts sourced from the largest number of public databases starting from as little as 100 pg of total RNA.

The Signal Space Transformation-Robust Multi-Array Average algorithm was applied to background-adjust, normalize, and log-transform signals intensity.

Relative gene expression levels of each transcript were validated applying a One-Way analysis of variance ($p \leq 0.01$) and multiple testing correction. Coding genes and lncRNAs that displayed an expression level at least 1.5-fold different in the test sample versus control sample ($p \leq 0.01$) were carried forward in the analysis.

The targets (including microRNAs and genes) of all the lncRNAs that satisfied the above-mentioned FC and *p*-value criteria were screened using NPInter v3.0.[1] This database allows the efficient recovery of all lncRNAs interactions experimentally validated by high-throughput experimental technologies (14, 15).

The list of gene targets of miRNAs that were targeted by significantly modulated lncRNAs was obtained using the FunRich database[2] (16).

Protein–Protein Interaction (PPI) Network Construction and Network Clustering

The Search Tool for the Retrieval of Interacting Genes (STRING version 10.5[3]) was used to obtain PPIs pairs that were validated by experimental studies (17) and to construct the PPI networks. Network topological analysis was performed using the Cytoscape software[4] (18).

High-flow areas (highly connected regions) of the network were detected using the MCODE plugin of Cytoscape, based on the thresholds of *k*-core = 3 and node score cutoff = 0.2.

Gene Functional Classification and Enrichment Analysis

Genes were functionally classified into canonical biological processes (BPs) on the basis of the gene ontology (GO) annotations.[5]

Biological processes and Pathways enrichment analysis was performed employing FunRich (hypergeometric *p*-value ≤0.05).

Real-Time PCR

lncRNAs Modulated in PsA

For each sample, 500 ng of total RNA was treated with 1 unit of DNase I Amplification Grade (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's protocol. First-strand cDNA was generated using the SuperScript IV First-Strand Synthesis System (Invitrogen; Carlsbad, CA, USA) with random hexamers, according to the manufacturer's protocol. Real-time PCR was performed in triplicate with PowerUp™ Sybr® Green reagent (Applied Biosystems; Foster City, CA, USA) in a QuantStudio 6 Flex system (Applied Biosystems; Foster City, CA, USA). Relative expression levels were calculated for each sample after normalization against the geometric mean of the housekeeping genes GAPDH and beta-actin (ACTB) expression. The ΔΔCt method was used for comparing relative fold expression differences. The data are expressed as fold changes with respect to healthy.

Genes Modulated in PsA

First-strand cDNA was generated using the SuperScript III First-Strand Synthesis System for RT-PCR Kit (Invitrogen), with random hexamers, according to the manufacturer's protocol. PCR was performed in a total volume of 25 µl containing 1× Taqman Universal PCR Master mix, no AmpErase UNG, and 2.5 µl of cDNA; pre-designed, gene-specific primers, and probe sets for each gene were obtained from Assay-on-Demande Gene Expression Products (Applied Biosystems).

Real-time PCR reactions were carried out in a two-tube system and in singleplex. The real-time amplifications included 10 min at 95°C (AmpliTaq Gold activation), followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. Thermocycling and signal detection were performed with 7500 Sequence Detector (Applied Biosystems). Signals were detected according to the manufacturer's instructions and the relative expression levels were calculated as it has been previously described (9).

Detection of Soluble Mediators in Sera of PsA Patients

Serum levels of glypican-4, IFN-γ, Wnt-2, mTOR, TNF-α, SPD-1, NFKB p65, NOTCH1, omentin, and adiponectin were detected using commercially available ELISA kits that were supplied by antibodies-online (glypican-4, Wnt-2, and mTOR), LifeSpan BioSciences (TNF-α, NFKB p65, and omentin), IBL International (IFN-γ), R&D (adiponectin), and Ray Biotech (sPD-1).

Plasmid Construction and Luciferase Reporter Assay

The plasmids to knockdown LINC00909 and LINC00657 were purchased from GenePharma (Shanghai, China).

Human HEK (human embryonic kidney) 293 T cells (1.5×10^4) grown in a 96-well plate were co-transfected with 150 ng of empty vector, 50 ng of firefly luciferase reporter comprising the lncRNAs mut vectors, (Promega, Madison, WI, USA) using Lipofectamie 2000 (Invitrogen, USA). Cells were harvested 48 h after transfection and analyzed using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol.

RESULTS

High-Throughput Long Non-Coding RNA and Gene Expression Profiling in Peripheral Blood Mononuclear Cells of PsA

In order to evaluate the potential role played by lncRNAs in PsA pathogenesis, we performed a gene array study using the Clariom D human gene chip that enabled us to analyze, at the same time, both conventional gene and lncRNA expression profiles.

[1] http://www.bioinfo.org/NPInter/ (Accessed: November 16, 2017).
[2] www.funrich.org/ (Accessed: December 15, 2017).
[3] http://string-db.org/ (Accessed: February 06, 2018).
[4] www.cytoscape.org (Accessed: February 15, 2018).
[5] www.geneontology.org (Accessed: January 19, 2018).

We compared the lncRNA expression profiles of PBMC samples obtained from 10 PsA patients with 10 PBMC samples obtained from age and sex matched healthy donors and we found that 259 lncRNAs satisfied the Bonferroni-corrected p value criterion ($p \leq 0.01$) and the fold change criterion (FC $\geq |1.5|$), displaying robust and statistically significant variation between PsA and healthy controls samples (Table S1 in Supplementary Material). The study was implemented by the analysis of conventional gene expression profiles in the same PsA samples and we found that 1,922 differently expressed genes satisfied the above-mentioned criteria. The complete list of modulated genes can be found in Table S2 in Supplementary Material. In both cases, the arrays were validated by real-time PCR. LncRNAs LUCAT1 and TRIM55-1 were validated by real-time PCR in the entire series of patients analyzed. Significantly different expression levels were found for all tested lncRNAs in PsA as compared to healthy controls (see Figure S1 in Supplementary Material).

Moreover, real-time PCR analysis for seven lncRNAs was carried out in an expanded panel of PsA patients (20 patients) and healthy controls (20 subjects). A significant modulation of all these lncRNAs was found in all tested patients thus confirming gene array results (see **Figure 1**).

To gain meaningful insights on the potential role played by modulated lncRNAs in PsA pathogenesis, the complete list of modulated lncRNA was filtered, extracting only those transcripts for which a bona fide target annotations was present in NPInter. By this method 92 lncRNAs were selected (Table S3 in Supplementary Material) and, simultaneously, the list of all gene and microRNA targets of the selected lncRNAs, experimentally validated by high-throughput technologies, was extracted from the same database.

To corroborate our results we narrowed down our analysis to modulate lncRNA that targeted genes that were significantly modulated in the array and miRNAs that we found deregulated in our previous analysis of PsA PBMCs from the same cohort of patients (10) (**Table 1**). In particular, we found that 15 of these miRNAs modulated in PsA (hsa-miR-130a-3p, hsa-miR-148a-3p, hsa-miR-151a-5p, hsa-miR-17-5p, hsa-miR-186-5p, hsa-miR-199a-3p, hsa-miR-199a-5p, hsa-miR-28-5p, hsa-miR-3135b, hsa-miR-320c, hsa-miR-320d, hsa-miR-331-3p, hsa-miR-423-5p, hsa-miR-451a, and hsa-miR-92a-3p) were targeted by selected lncRNAs. We then extracted from the FunRich database the annotated gene targets of the above-mentioned miRNAs selecting only transcripts that also resulted when modulated in the Clariom D array (Table S4 in Supplementary Material). **Table 2** recapitulates the above-selected lncRNAs and targets.

In summary, we selected only those lncRNAs, that targeted miRNAs and genes with evidence of modulation in our PsA samples to trace, with a good confidence, lncRNAs–miRNAs–genes interactions that are expected to be established in the course of PsA.

To validate our results we conducted a LINC00909siRNA and a LINC00657siRNA silencing in human 293 T cells, to explore whether this knockdown altered the expression levels of selected miRNAs targeted by LINC00909 (miR-148a-3p and mir-28-5p) and by LINC00657 (miR-130a-3p and miR-17-5p). We observed that the silencing of the two lncRNAs significantly increased the level of expression of their targeted miRNAs.

The registered percentages of increase were: 70 ± 3.8 and 78 ± 1.4% for miR-148a-3p and mir-28-5p, respectively, and 75 ± 2.3 and 80 ± 1.53% for miR-130a-3p and miR-17-5p, respectively.

PPI Network of Modulated lncRNAs in PsA

The lncRNA expression profiling of PsA PBMCs was integrated with a network analysis. We, therefore, inspected, by a bioinformatic methodology, all the functional and experimentally validated interactions among the protein products of genes targeted by lncRNAs and by the 15 above-mentioned miRNAs (230 genes) that were selected as previously described. Then, taking in account of these interactions, we constructed a PPI network which showed a good PPI enrichment p-value (0.00041). In the obtained network 229 of the above-mentioned genes (nodes), were connected by 195 pairs of interactions (edges). Since 229 out of 230 of lncRNAs and miRNAs gene targets that we used as input for the network analysis, resulted connected in the PPI network, we could observe that the selected lncRNAs may act in an integrated fashion in the disease.

We then imported in Cytoscape the PPI network, adding to its scaffold the lncRNAs-genes, miRNAs-genes, and lncRNAs–miRNAs interactions that we selected as we explained above. Thus, we obtained an implemented network that is showed in **Figure 2**. A topological analysis of this network was performed to highlight lncRNAs that were highly connected to the PPI network genes (i.e., targeting a large number of PPI-network genes) also considering all the lncRNA–gene interactions mediated by the selected miRNAs (i.e., lncRNA–miRNA–gene interactions). By this approach we could select seven lncRNAs, including EPB41L4A-AS1, LA16c-360H6.3, LINC00657, LINC00909, RP11-1100L3.8, RP11-539L10.3, and RP11-403I13.5 that displayed the highest connectivity in the network. As shown in **Figures 3A–G** (where genes of the PPI network are ordered around a circle based on their degree of connectivity, i.e., number of edges) the above-mentioned lncRNAs directly or indirectly targeted genes that, in most cases, were distributed in areas of the network characterized by a high degree of connectivity. This observation indicated that, since these lncRNAs targeted highly interconnected genes, they may have a broad effect in the disease that goes beyond the modulation of single target gene(s).

Modular Analysis of Genes and lncRNAs Modulated in PsA

In a second part of our analysis we wanted to dissect the global impact of lncRNAs on the entire PsA transcriptome (i.e., on the 1,922 modulated genes in PsA). Since the targeting of genes with more interacting partners may have a broader impact on the global transciptome than the modulation of few isolated genes, we aimed to inspect if the modulated lncRNAs may display connections with highly interacting genes in PsA. To this purpose, we built the PPI network that included the protein products of all modulated genes in PsA that showed experimentally validated interactions obtaining a wide network containing 1,637 nodes and 9,415 edges and showing a very good PPI enrichment p-value ($p < 10–16$). Then we performed a modular analysis to find areas

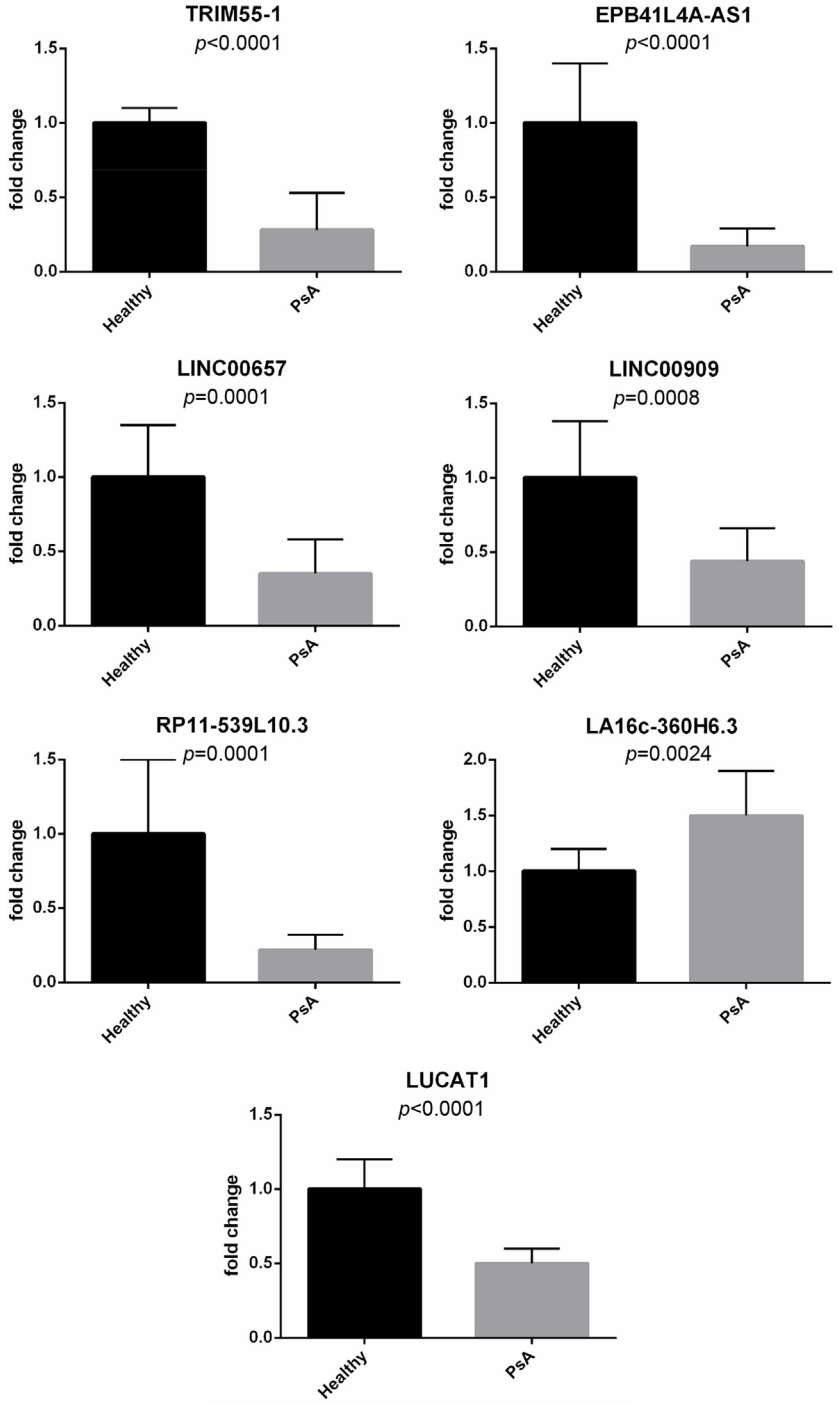

FIGURE 1 | Expression of selected long non-coding RNAs in an expanded panel of psoriatic arthritis patients (20 patients) and healthy controls (20 subjects). Bars indicate SD.

of the network that included the most highly connected genes (modules). By this analysis we could extract 16 modules (M1–M16, see Table S5 in Supplementary Material). All the 16 modules were imported in Cytoscape along with all the previously selected interactions with miRNAs and modulated lncRNAs that we also considered in the network analysis described in the previous paragraph. The graphical visualization of genes connected in modules and their interactions with lncRNAs showed that the majority of the 16 modules (10/16) were directly or indirectly targeted by modulated lncRNAs, including M1, M2, M4, M5,

TABLE 1 | Selected modulated long non-coding RNAs in psoriatic arthritis patients versus healthy controls.

ID	Fold change	*p*-value	Gene symbol	mRNA accession
TC0500008318.hg.1	−2.37	0.0026	EPB41L4A-AS1	ENST00000413221.2
TC0700007000.hg.1	−2.02	0.0015	HOTAIRM1	ENST00000616712
TC0600008510.hg.1	−2.25	0.0087	KCNQ5-IT1	ENST00000445310
TC0700013567.hg.1	2.05	0.0013	LINC00174	ENST00000416366
TC0700007277.hg.1	2.46	0.0001	LINC00265	ENST00000340510.4
TC1500007707.hg.1	−1.58	0.0089	LINC00593	ENST00000558385.1
TC2000008995.hg.1	−2.27	0.0017	LINC00657	ENST00000565493
TC1800009043.hg.1	−1.84	0.0025	LINC00909	ENST00000577806
TC0200007199.hg.1	2.27	0.0007	LINC00486	ENST00000414054
TC0100009691.hg.1	−1.97	0.0003	RP11-403I13.5	ENST00000443018.1
TC0200010127.hg.1	−3.24	<0.0001	RP11-171I2.4	ENST00000605334.1
TC0200011420.hg.1	−2.68	0.0005	AC133528.2	ENST00000433036.1
TC0400009914.hg.1	−2.37	0.0027	RP11-539L10.3	ENST00000513179.1
TC0500009465.hg.1	−1.74	0.007	RP11-779O18.3	ENST00000523005.1
TC0800007847.hg.1	−14.9	<0.0001	AC084082.3	ENST00000517961.2
TC1100011278.hg.1	1.91	0.0003	RP11-867G23.3	ENST00000501708.1
TC1200006772.hg.1	−1.86	0.0044	RP11-75L1.1	ENST00000541404.1
TC1200010732.hg.1	−3.11	0.0058	RP11-1100L3.8	ENST00000564363.1
TC1400006719.hg.1	−2.54	0.0066	RP11-468E2.5	ENST00000558478.1
TC1400009275.hg.1	−1.61	0.0027	RP11-930O11.2	ENST00000560296.1
TC1600009188.hg.1	2.21	0.0021	LA16c-360H6.3	ENST00000574245.1
TC1700007241.hg.1	2.64	0.0008	RP11-283C24.1	ENST00000578585.1
TC2100007843.hg.1	−1.78	0.0016	AF131217.1	ENST00000430247.1
TC2200008462.hg.1	−3.38	0.0058	RP3-430N8.10	ENST00000602955.1
TC1500010312.hg.1	−2.52	0.0064	RP11-815J21.2	ENST00000561409.1
TC1800007426.hg.1	−3.49	0.0032	RP11-1151B14.4	ENST00000591360.1
TC1900011833.hg.1	1.81	0.0031	CTB-25B13.12	ENST00000588225.1
TC1900007159.hg.1	−1.77	0.0084	CTB-55O6.10	ENST00000590715.1
TC1200008393.hg.1	−1.74	0.0028	RP11-981P6.1	ENST00000552778.1
TC1200008425.hg.1	−2.1	0.0024	RP11-796E2.4	ENST00000499685.2
TC1400009962.hg.1	−2.61	0.0006	RP11-471B22.2	ENST00000555853.1
TC1600006833.hg.1	2.16	0.0024	RP11-77H9.5	ENST00000564919.1
TC1400009667.hg.1	1.88	0.0092	RP4-693M11.3	ENST00000557304.1
TC1000009009.hg.1	−1.6	0.0098	RP11-498B4.5	ENST00000433600.1
TC1400010386.hg.1	1.71	0.0064	CTD-3051D23.4	ENST00000553344.2
TC1200008527.hg.1	−2.91	0.0065	RP11-256L6.3	ENST00000551849.1
TC0200007485.hg.1	1.61	0.0048	AC016722.4	ENST00000429761.1
TC1400007302.hg.1	1.95	0.0041	CTD-2002H8.2	ENST00000557322.1
TC0500008150.hg.1	−2.4	0.0066	CTD-2260A17.1	ENST00000512856.1
TC0600010636.hg.1	−1.89	0.0005	RP3-406P24.3	ENST00000415144.1

M8, M10, M11, M12, M14, and M15 (**Figure 4**). Moreover, we could observe that the vast majority of modules was targeted by the above-mentioned lncRNAs that included LINC00909 (targeting 9/10 modules) LINC00657 (targeting 8/10 modules), EPB41L4A-AS1, RP11-539L10.3 (targeting 7/10 modules), LA16c-360H6.3, RP11-403I13.5, and RP11-1100L3.8 (targeting 5/10 modules) (Table S6 in Supplementary Material).

Functional Analysis of the Targeted Modules and Their Targeting lncRNAs

To dissect the possible role played by the module-targeting-lncRNAs we performed a functional classification of genes included in the 10 targeted modules, based on the GO classification criteria. **Figure 5** recapitulates all the most relevant GO BPs in which the genes of targeted modules are classified.

M1 was the most targeted module and 35 lncRNAs showed direct or indirect connections with it (see Table S6 in Supplementary Material). The 88 genes included in this module were mostly related to translation, mRNA splicing, and protein ubiquitination GO BPs. Among genes included in protein ubiquitination, nine genes belonged to the "proteasome-mediated ubiquitin-dependent protein catabolic process" BP, including CUL1, CUL3, FBXW5, FBXW7, FBXL19, HECTD3, NEDD4L, RNF126, and UBE2A. Interestingly, it has been recently observed that ubiquitin–proteasome-mediated protein degradation plays a role in modulating the balance between bone formation and bone resorption, since it is involved in signaling pathways that are crucial for bone homeostasis like RANK/NF-κB pathway and the Wnt/β-catenin pathway (19). In addition CUL3 is also associated with the "Wnt signaling pathway" BP. 12 genes were targeted in the M1, including CUL5, TRA2B, SRRM1, ASB6, HNRNPUL1, CPSF7, HNRNPC, NUDT21, UPF1, and the above-mentioned FBXL19, NEDD4L, and FBXW7. UPF1 was the most targeted gene in M1. It is critical for the activity of Regnase-1 also called MCPIP1 (monocyte chemotactic protein-1-induced protein-1), a crucial molecule that regulates immune response avoiding over-activation of the immune system (20). Interestingly, the gene FBXL19 represents

TABLE 2 | Long non-coding RNAs (LncRNAs) and their targets modulated in psoriatic arthritis.

LncRNAs	Gene targets	miRNA targets
LINC00174	CPSF7	
LINC00265	IGF2BP2	
LINC00593	UPF1	
LINC00657	CPSF7, FXR1, HNRNPC, NUDT21 UPF1, ZC3H7B	hsa-miR-130a-3p
		hsa-miR-17-5p
		hsa-miR-186-5p
		hsa-miR-199a-3p
		hsa-miR-199a-5p
		hsa-miR-28-5p
		hsa-miR-320c
		hsa-miR-320d
		hsa-miR-331-3p
		hsa-miR-423-5p
		hsa-miR-451a
LINC00909	UPF1	hsa-miR-130a-3p
		hsa-miR-148a-3p
		hsa-miR-28-5p
		hsa-miR-320c
		hsa-miR-320d
LINC00486	FXR1, UPF1	
EPB41L4A-AS1	CPSF7, UPF1	hsa-miR-130a-3p
		hsa-miR-17-5p
HOTAIRM1	CPSF7, UPF1	
KCNQ5-IT1	CPSF7, UPF1	
RP11-403I13.5		hsa-miR-92a-3p
RP11-171I2.4	CPSF7	
AC133528.2	CPSF7	
RP11-539L10.3		hsa-miR-148a-3p
		hsa-miR-199a-3p
RP11-779O18.3	HNRNPC, UPF1, ZC3H7B	
AC084082.3	CPSF7, NUDT21, UPF1	hsa-miR-331-3p
RP11-867G23.3	UPF1, IGF2BP2	
RP11-75L1.1	UPF1	
RP11-1100L3.8	UPF1	hsa-miR-17-5p
		hsa-miR-423-5p
RP11-468E2.5	UPF1	
RP11-930O11.2	UPF1	
LA16c-360H6.3	IGF2BP2	hsa-miR-17-5p
RP11-283C24.1	UPF1	
AF131217.1	UPF1	
RP3-430N8.10		hsa-miR-331-3p
RP11-815J21.2		hsa-miR-186-5p
RP11-1151B14.4	UPF1	
CTB-25B13.12	UPF1	
CTB-55O6.10	CPSF7, UPF1	
RP11-981P6.1	CPSF7, UPF1, ZC3H7B	
RP11-796E2.4	UPF1	hsa-miR-3135b
RP11-471B22.2	CPSF7, IGF2BP2, UPF1, ZC3H7B	
RP11-77H9.5	CPSF7, HNRNPC, IGF2BP2, UPF1	
RP4-693M11.3	UPF1	
RP11-498B4.5	UPF1	
CTD-3051D23.4	IGF2BP2	
RP11-256L6.3	NUDT21, UPF1	
AC016722.4	CPSF7, UPF1, ZC3H7B	
CTD-2002H8.2	CPSF7, HNRNPC, NUDT21, UPF1, ZC3H7B	
CTD-2260A17.1		hsa-miR-151a-5p
RP3-406P24.3	CPSF7	

a susceptibility locus for PsA (21). Finally, other two genes of M1, TRIM32, and HERC5 play a role in the innate immune response and, in particular, HERC5 activates IRF3 (22).

M2 was targeted by 6 lncRNAs and included 40 genes that were mainly involved in cell division, inflammatory response (i.e., CCR7, CXCR1, CXCR2, PF4, and PPBP/CXCL7), tumor necrosis factor (TNF)-mediated signaling pathway (i.e., TNFRSF1B, TNFSF12, TNFRSF12A, and PSMA7), and Wnt signaling pathway (i.e., AXIN1, DVL3, GNB2, PPP2R5C, PSMA4, PSMB1, PSMC4, PSMD4, and RNF146) BPs. Interestingly, the expression of CXCL7 is increased in Rheumatoid arthritis (AR) synovia (23). Moreover AXIN2 another gene included in M2 is related to the intramembranous ossification BP. Eight genes were targeted in M2, including ADCY3, GNAI1, PSME3, PSMF1, RELA, TRAF3, and the above-mentioned TNFRSF1B and CCR7. In particular, the expression of CCR7 is stimulated by TNF-α and interleukin 1-beta in osteoclasts (24). Moreover, RELA is involved in peripheral regulatory T cell-induced tolerance (25) and TRAF3 is required for T cell and invariant natural killer T cell effector functions (26). In addition, TRAF3 is also involved in limiting osteoclastogenesis, indeed RANKL-induced degradation of TRAF3 enhances TNF-induced osteoclastogenesis (27).

M4 was targeted by seven lncRNAs and included 57 genes that were mainly involved in cell division, DNA repair, endocytosis, mitochondrial translation, mRNA export from nucleus, regulation of glycolytic process, and transcription. In addition, this module included two genes (i.e., MRE11A and XRCC6) associated with the BP "positive regulation of type I interferon production" and the gene POU2F2/OCT2 that increases the differentiation of antibodies secreting activated B cells (28). In this module the genes GBF1, MYBL2, RAD23A, and WASL were targeted.

M5 was targeted by the RP11-403I13.5 lncRNA and included 19 genes. Protein targeting to ER and translation were the most represented BPs in this module.

M8 was targeted by four lncRNAs (EPB41L4A-AS1, LINC00657, LINC00909, and RP11-539L10.3) and in this module seven genes were connected, including BRPF3, TAF12, TAF2, TAF7, TBPL1, TRIM24, and BPTF almost associated with the "translation" BP. Among these, BPTF is important for the homeostasis of T cells and is crucial for the maintenance and function of regulatory T (Treg) cells (29).

M10 was targeted by 8 lncRNAs and included 49 genes. These genes were associated with many BPs, including cell proliferation, signal transduction, transcription, Wnt signaling pathway (i.e., BCL9L, FZD6, LRP6, ATP6V0C, BCL9, and PYGO2), vasculogenesis (i.e., ENG and TGFBR2), bone resorption (i.e., TCIRG1), negative regulation of bone mineralization (i.e., SRGN), osteoblast differentiation (i.e., LGR4), and osteoblast development (i.e., LRP5). Several genes were associated with BPs related to glucose homeostasis like, for example, PPARGC1A (cellular glucose homeostasis), GSK3A (cellular response to insulin), IGF1R (insulin receptor signaling pathway), RAF1 (insulin secretion), and RAP1A (positive regulation of glucose import). In addition other genes were classified into BPs related to adaptive immune response like T cell differentiation (i.e., RIT1 and TCF7), and T cell cytokine production (KDELR1) and B cell homeostasis (SOS1). In M10 12 genes were targeted, including SHOC2, CCNL1, CDK13, MED16, MMS19, and the above-mentioned FZD6, PYGO2, KDELR1, PPARGC1A, RAP1A, SOS1, and TGFBR2.

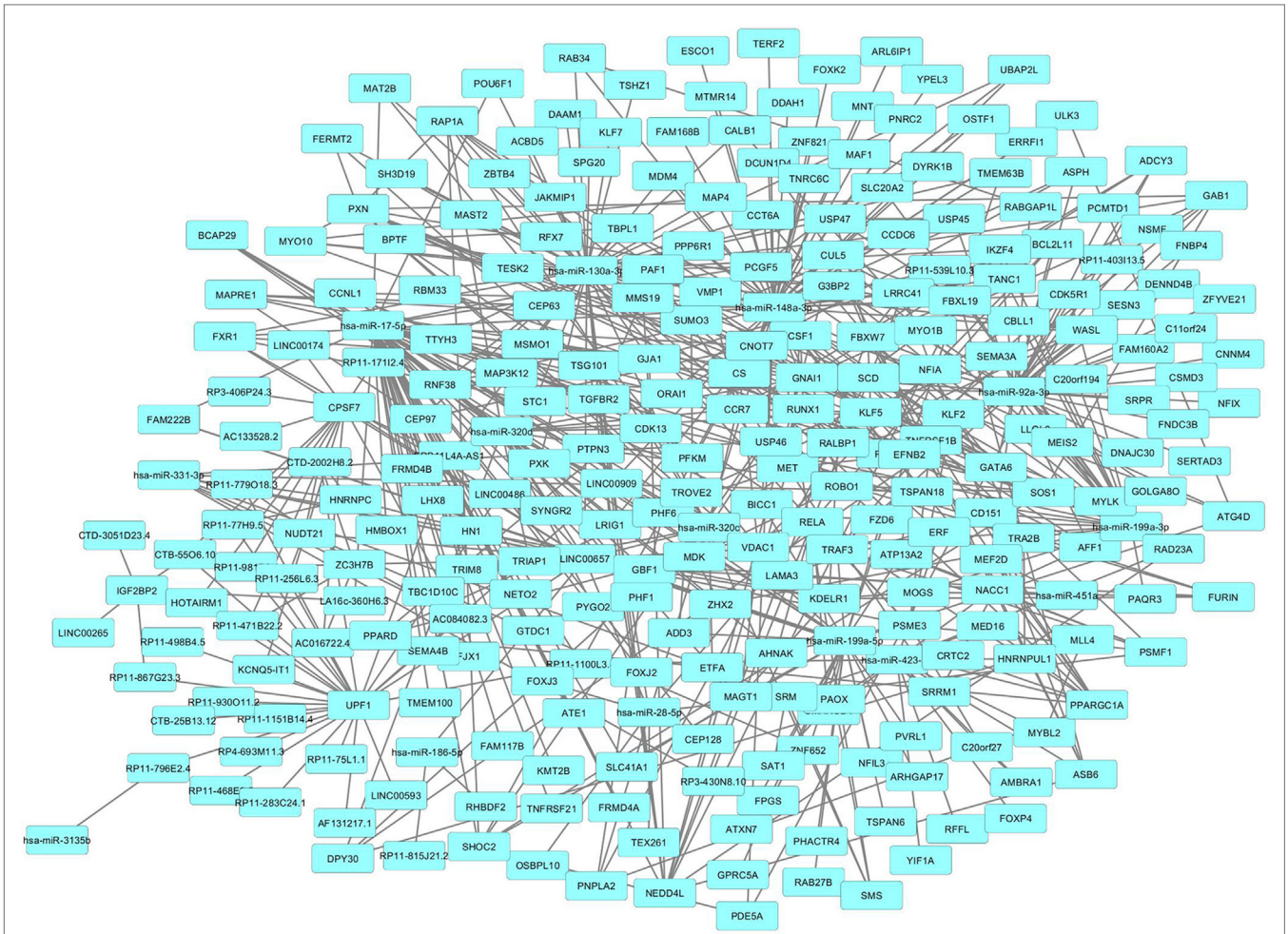

FIGURE 2 | Protein–protein interaction (PPI)-network of differently expressed genes in psoriatic arthritis (PsA) targeted by the selected long non-coding RNAs and microRNAs modulated in PsA.

M11 was targeted by 2 lncRNAs and included 28 genes. A large number of these genes were assigned to "lipid metabolic process" (i.e., PLCD3, PPAP2B, SLC27A1, and TBL1X), "phospholipid metabolic process" (i.e., AGPAT2, LPCAT3, LPCAT4, and PPAP2C), and to "phosphatidylinositol biosynthetic process" (CDIPT, MTMR14, PI4K2B, and PIK3C2B) BPs. Interestingly, two genes namely CARM1 and FASN were associated with "endochondral bone morphogenesis" and "osteoblast differentiation" BPs, respectively. In this module, TERF2, YPEL3, and the above-mentioned MTMR14 were targeted.

M12 was targeted by 6 lncRNAs and included 30 genes. We observed that several genes of this module classified in BPs related to the immune response, including SPI1/PU.1 (lymphoid progenitor cell differentiation), CD244 and KLRF1 (innate immune response), CD226 (positive regulation of natural killer cell cytokine production), CD7 (T cell activation), CD247 (T cell receptor signaling pathway), GBP1 (regulation of T cell receptor signaling pathway), CD79A (B cell activation), GBP1, GBP2, and CIITA (type I interferon signaling pathway), HCST, LILRB2, and KLRB1 (regulation of immune response), GZMA (immune response) and SPI1/PU.1 (lymphoid progenitor cell differentiation), and CIITA (positive regulation of MHC class I and of MHC class II biosynthetic process). In particular, the transcription factor SPI1/PU.1 is crucial for the development of interleukin-9-producing helper T cells (Th9) (30, 31) that have been recently involved in the PsA pathogenesis (32).

Moreover five genes, including DAG1, COL14A1, LAMA2, LAMA3, and LAMC1 were associated with the "extracellular matrix organization" BP. LAMA3 and FAM168B were targeted in this module.

M14 was targeted by three lncRNAs and included 14 genes that were mainly associated with protein targeting to mitochondrion (TOMM20 and TOMM40), protein import into mitochondrial inner membrane (TIMM22), ATP biosynthetic process (ATP5I), apoptosis (BCL2L11 and CASP4), and in Wnt signaling pathway (TLE1 and TLE3) BPs. In this module the genes BCL2L11 and VDAC1 were targeted.

Finally, M15 was targeted by 6 lncRNAs and included 44 genes. In this module, we observed the presence of genes classified in several BPS, including cell division (TUBA1C, TFDP1, CEP192,

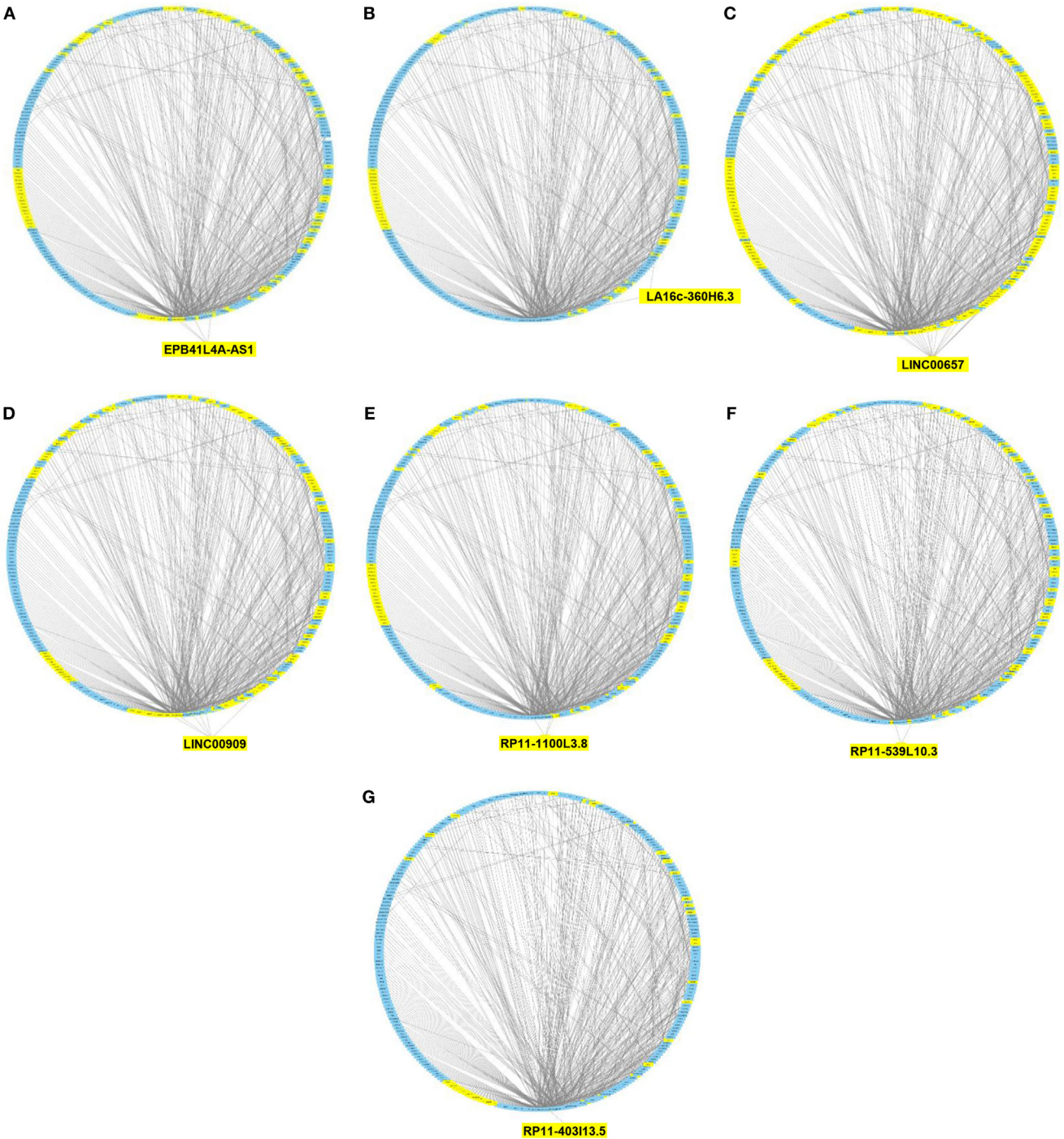

FIGURE 3 | Protein–protein interaction (PPI) network of differently expressed genes targeted by selected microRNAs and long non-coding RNAs (lncRNAs). Graphical representation of the connections among genes in the PPI network and EPB41L4A-AS1 **(A)**, LA16c-360H6.3 **(B)**, LINC00657 **(C)**, LINC00909 **(D)**, RP11-1100L3.8 **(E)**, RP11-539L10.3 **(F)**, and RP11-403I13.5 **(G)**. In the PPI network all genes are ordered around a circle based on their degree of connectivity (i.e., number of edges). Genes directly or indirectly targeted by the lncRNAs are highlighted in yellow.

CEP63, and MAPRE1) glycolytic process (PFKL, PFKM, and PKM), Wnt signaling pathway (MARK2, CTNNBIP1, EIF2C1, and TNRC6C), osteoblast differentiation (ASF1A and MAPK11), and regulation of type I interferon-mediated signaling pathway (CDC37). Genes targeted in this module were: AHNAK, CALB1, CCT6A, CEP63, CEP97, GAB1, MAPRE1, PFKM, and TNRC6C.

CALB1 plays a role in differentiation and matrix formation in osteoblasts (33), GAB1 is important for normal postnatal bone

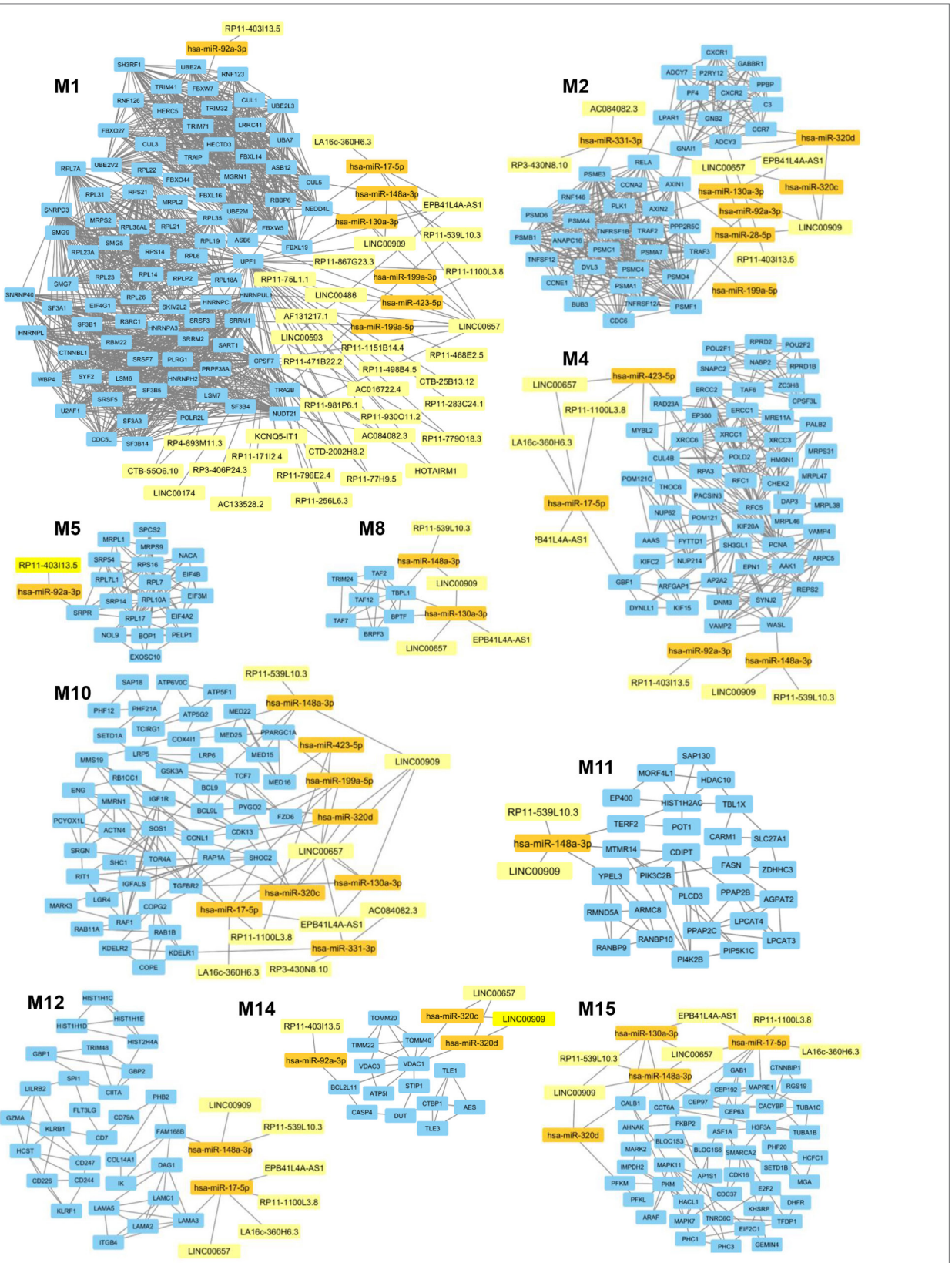

FIGURE 4 | Gene modules that are targeted by the selected long non-coding RNAs (lncRNAs) modulated in psoriatic arthritis. In each module (M) genes are represented by light blue rectangles whereas targeting microRNAs and lncRNAs are represented by orange and yellow rectangles, respectively.

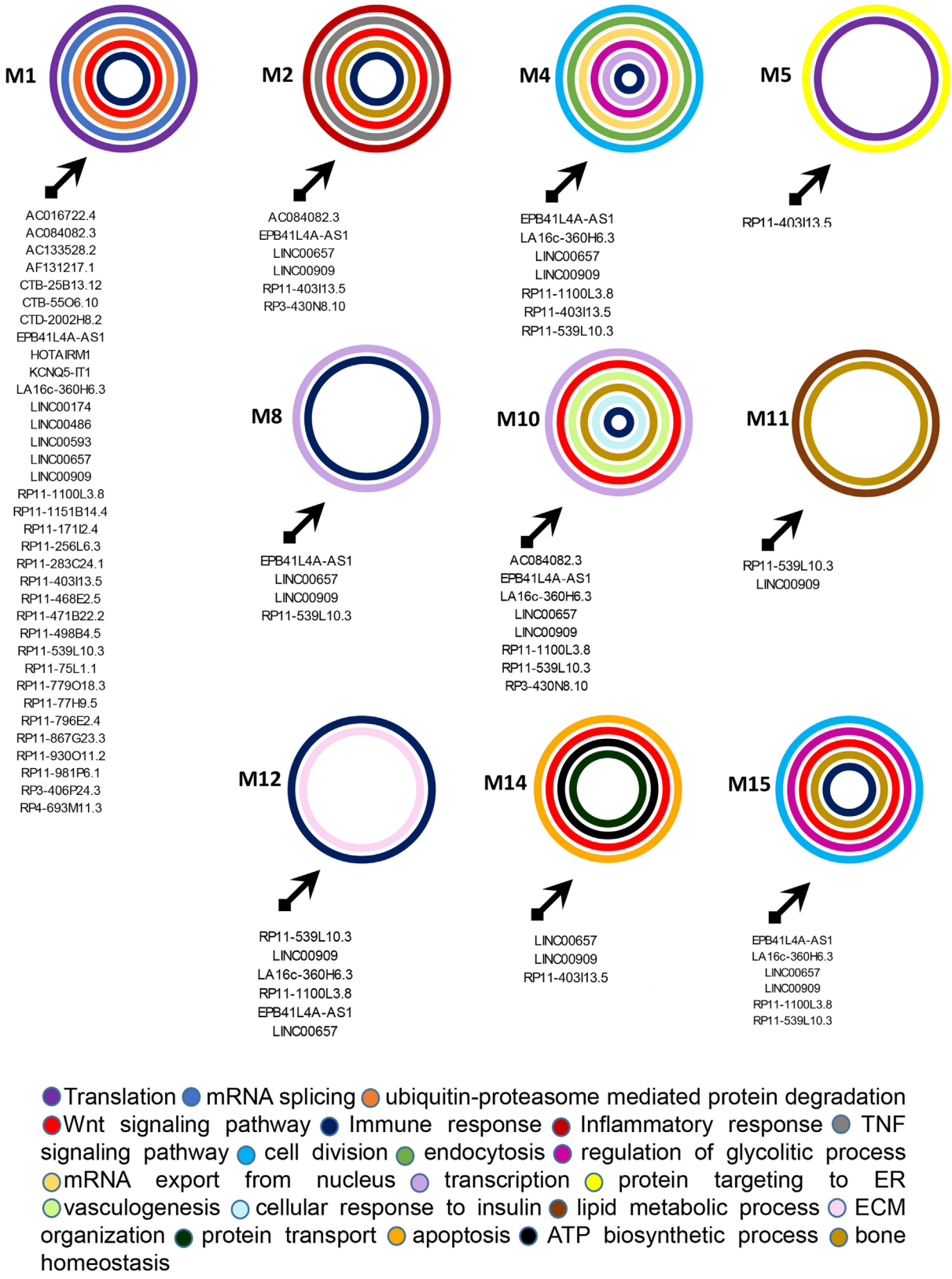

FIGURE 5 | Functional classification of genes included in the targeted modules. Graphical representation of long non-coding RNAs targeting the gene modules and of the most relevant biological processes (BPs) in which genes in each module can be classified. The BPs are represented by colored circles (each color corresponds to a different BP). The size of circles does not correspond to the number of genes that are classified in each BP.

homeostasis (34) and MAPRE1 regulates cell–cell adhesion-induced osteoblast differentiation (35).

Since disease can be viewed as the result of perturbations of complex signaling networks, a pathway enrichment analysis was performed, in each module, using FUnrich. This analysis showed that the most represented enriched pathways were related to immune response (including signaling of both adaptive and innate immune response, type I interferon and gamma interferon signaling), bone homeostasis, metabolism, and cell adhesion.

The results obtained are shown in **Figure 6**, which shows a schematic representation of the most relevant enriched signaling pathways ($p \leq 0.05$).

To provide experimental evidence that pathways identified by the throughput analysis of modulated genes and lncRNAs in PsA are active in the disease, we performed a two step validation of the presented results. Indeed, first we validated by real-time PCR the modulation of genes ascribed to several signaling pathways, including SH3GL1, EPN1, and NUP214 (mTOR signaling), TRAF2, TNFRSF1B, and RELA (TNF/NF-κB signaling), MAPK7 and MAPK11 (toll-like receptors signaling), TCF7, BCL9, and LRP6 (Wnt signaling), MED15, ENG, and ACTN4 (glypican signaling), GSK3A, IGF1R, and PARGC1A (insulin signaling), and CIITA IFI27 and MYD88 (type I interferon signaling). All the tested transcripts were overexpressed in PsA patients when compared to healthy subjects (see Figure S2 in Supplementary Material).

Second, in the sera of patients with PsA, we evaluated the presence of several molecules associated with the pathways modulated in PsA. Indeed, we decided to quantify the serum levels of glypican-4, IFN-γ, Wnt-2, mTOR, TNF-α, sPD-1, NFKB p65, NOTCH1, omentin, and adiponectin. **Figure 7** shows the concentration of these molecules in the sera of the PsA patients. The serum levels of all the tested molecules were significantly increased in PsA patients when compared to healthy donors with the exception of adiponectin and omentin that were significantly decreased in PsA sera.

DISCUSSION

Psoriatic arthritis is a chronic inflammatory arthritis that affects 10–30% of patients with skin psoriasis. IL-17, TNF-α, and type I IFNs play a fundamental role in the pathogenesis of the disease and monoclonal antibodies targeting IL-17 and TNF-α along with IL-12/IL-23 are the main biotechnological treatments that have a dramatic good response in PsA and in skin psoriasis. As we have previously reported (9), the involvement of the mentioned cytokines is typical of an autoimmune process and support the hypothesis of the autoimmune origin of the disease. In particular, IL-17 is able to synergize with TNF-α, IL-22, and other cytokines, including IL-6 and IL-8, in sustaining the inflammatory process at different sites and in favoring the development of comorbidities that are typical of psoriasis, such as PsA, metabolic syndrome, obesity, and cardiovascular disease.

Long non-coding RNAs control gene expression at multiple levels, including epigenetic regulation, chromatin remodeling, and post-transcriptional gene regulation. Accumulating evidences indicate that lncRNAs can be involved in different types of immune-mediated human diseases, including autoimmune diseases (11). The role played by lncRNAs in PSA pathogenesis has not been elucidated yet and a comprehensive analysis of lncRNA expression profiles in PsA has not been performed. We, therefore, aimed to identify lncRNA expression signatures associated with PsA, analyzing the expression of a vast array of lncRNA in PBMC cells from PsA patients by microarray analysis.

The applied criteria for the selection of modulated lncRNAs included a multiple step process that combined the lncRNAs expression analysis to the conventional gene expression and miRNAs profiling in the same cohort of patients.

By this approach we were able to select deregulated lncRNAs that were as much as possible correlated to the PsA transcriptome.

Through a complex network analysis of the whole set of molecular interactions among modulated genes and lncRNAs we further wanted to select those lncRNAs that targeted modules of most highly connected genes in the PsA interactome, since they may have a major impact on PsA gene modulation. That, for example, has led us to favor lncRNAs with high connectivity rather than poorly connected lncRNAs (even) with higher fold change. The functional analysis of the above-mentioned highly connected modules revealed that the modulated lncRNAs targeted genes involved in BPs that play a pivotal role in the PsA pathogenesis such as, for example, immune response (including B and T cell activation) inflammatory response, TNF, Wnt and type I interferon-mediated signaling, bone resorption, bone mineralization, and glyco-lipid metabolic process (see Results), thus indicating that the selected lncRNAs regulated the three major aspects of the disease, including skin involvement, osteo-articular features, and metabolic syndrome.

As it has recently acquired in molecular biology, diseases are now viewed in the context of signaling pathways perturbation. We, therefore, performed a pathway enrichment analysis of all targeted gene modules and, by this approach we reached a non-ambiguous identification of important pathways that can be modulated by the selected lncRNAs. Our analysis confirmed that these lncRNAs play a role in the regulation of signaling pathways associated with both adaptive and immune response and with autoimmune diseases (i.e., type I interferon signaling pathway). In addition, they regulate important signaling involved in bone homeostasis including glypican, syndecan, and Wnt pathways.

Moreover, these results revealed the presence in PsA of lncRNAs that modulate the signaling pathway associated with influenza, thus highlighting the possible role played by influenza viruses in triggering PsA and suggesting the possible involvement of an epigenetic control. The selected lncRNAs were involved in the modulation of lipid metabolism pathway (see Results, module M11), and it is well known that dyslipidemia is frequently associated with PsA (36) and, in particular, patients with PsA have been shown to have elevated cholesterol levels (37) and increase risk of cardiovascular disease.

Another deregulated signaling pathway is the S6K1 (S6 kinase 1) and mTOR (mammalian target of rapamycin) pathway (see Results, modules M1, M4, and M5), a nutrient sensing system that respond to nutrient overload leading to insulin resistance, obesity, and diabetes (38, 39). These observations suggest that lncRNAs may also

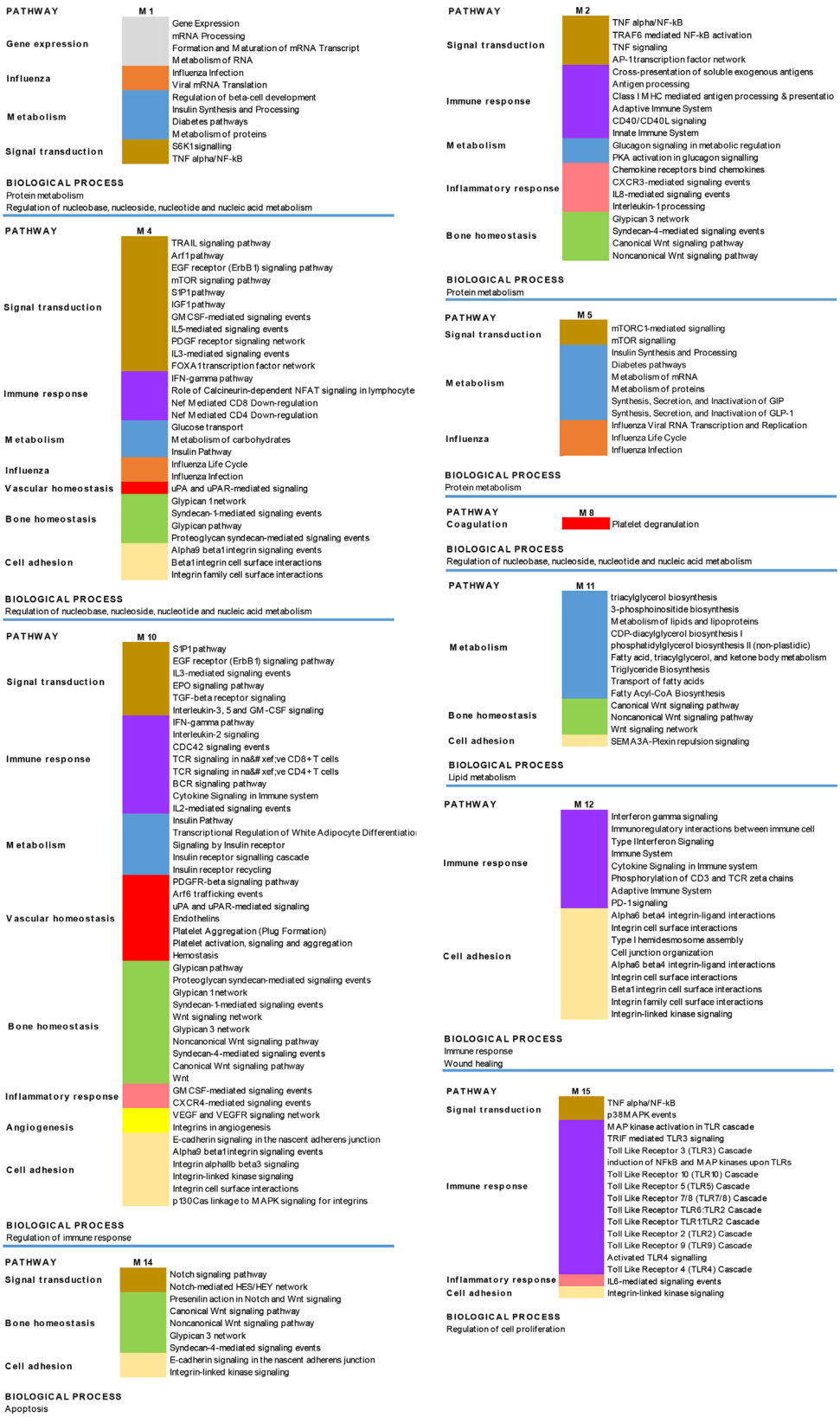

FIGURE 6 | Biological processes and pathways enrichment analysis of the targeted modules. Selection of the different enriched pathways ($p < 0.05$) in the 10 targeted modules (M). Pathways that are enriched in more than one module are labeled by the same color in the different modules.

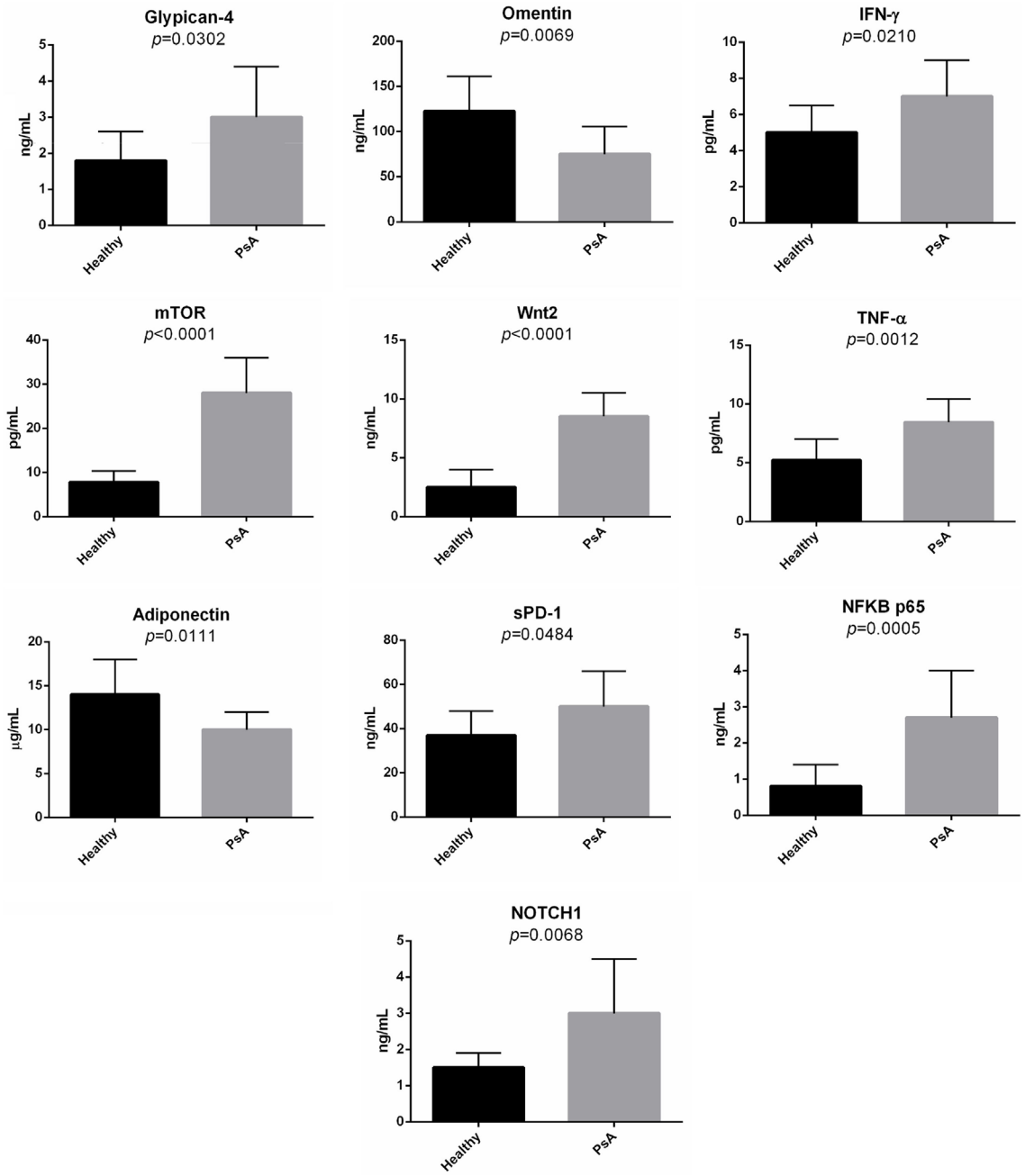

FIGURE 7 | Serum levels of selected molecules in psoriatic arthritis (PsA) patients and in normal subjects. The histograms represent the mean of the results obtained in 20 healthy donors and in 20 PsA patients.

be involved in the metabolic syndrome frequently associated with PsA. Hyperuricemia is another feature of metabolic syndrome and is also known to be more frequent in psoriatic subjects than in the normal population. In humans, purines are metabolized into uric acid, which is a strong stimulator of innate immunity. In this regard, hyperuricemia may increase uric acid crystallization in and around joints, thereby inducing PsA in psoriatic subjects, thus representing an independent risk factor for PsA. Activation of pathways modulation of genes involved in related to nucleobase, nucleoside, nucleotide, and nucleic acid metabolism (modules M1, M4, and M8) may reflect this particular aspect of PsA.

Finally, lncRNAs control several pathways involved in cell adhesion (see Results, modules M4, M10, M11, M12, M14, and M15). The modulation of these pathways may contribute to the increased mucosal permeability of the gut that has been observed in PsA, even in the absence of bowel pathology (40). Indeed, bowel disease is six times more frequent in patients with PsA. Since an altered intestinal barrier is a feature of PsA, the presence

of gastrointestinal involvement in PsA should be carefully investigated during the diagnostic work up.

The detection of significantly different serum levels in PsA patients compared to healthy subjects of several soluble mediators, ascribed to the main pathways targeted by the selected lncRNAs, strongly suggests that they modulate signaling networks that are active in the disease. In particular, the increased serum levels of glypican-4 and the decrease of adiponectin and omentin highlight the typical metabolic aspects of PsA patients.

Indeed glypican-4 has been considered a novel adipokine that boosts insulin signaling and, interestingly glypican-4 levels are significantly increased in obese patients with insulin resistance (41). Moreover, both adiponectin and omentin have been showed decreased in sera from psoriatic patients (42, 43) and decreased levels of both molecules have been associated with, and involved in, metabolic syndrome (44, 45).

In conclusion, this study is the first report on lncRNAs that may exert an epigenetic control on PsA pathogenesis. The original approach that integrates genes and lncRNAs expression profiles presented in the study, allows to identify deregulated lncRNAs that modulate crucial molecular signaling associated with the typical features of the disease. These findings further support that PsA is an autoimmune disease with systemic inflammation associated with obesity and metabolic syndrome leading to cardiovascular disease.

Finally, we may suggest that these lncRNAs may be useful to design novel therapeutic strategies in PsA.

ETHICS STATEMENT

All the participants to the study signed a written informed consent and the local Ethical Committee of the University Hospital of Verona, Verona, Italy, had approved the study protocol. All the investigations have been performed according to the principles of the Helsinki declaration.

AUTHOR CONTRIBUTIONS

APU, CL, and MD conceived and designed the experiments. MD, APE, PF, GP, and ET performed the experiments. MD and APE analyzed the data. GP and ET contributed reagents and collected the patients' samples. MD wrote the paper with input from APU and CL.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.01533/full#supplementary-material.

TABLE S1 | Long non-coding RNAs modulated in psoriatic arthritis patients versus healthy subjects.

TABLE S2 | Genes modulated in psoriatic arthritis patients versus healthy subjects.

TABLE S3 | Long non-coding RNAs modulated in psoriatic arthritis patients versus healthy subjects with available target annotations.

TABLE S4 | Target genes of the selected microRNAs that are targeted by long non-coding RNAs modulated in psoriatic arthritis.

TABLE S5 | Genes modulated in psoriatic arthritis patients versus healthy subjects and included in the modules.

TABLE S6 | Long non-coding RNAs modulated in psoriatic arthritis that target modules.

FIGURE S1 | Expression of selected long non-coding RNAs in psoriatic arthritis (PsA) patients by real-time PCR. Real-time PCR of LUCAT1 and lnc-TRIM55-1 in PsA and healthy samples included in the microarray. Bars indicate SD. $*p < 0.05$; $**p < 0.01$; Student *t*-test.

FIGURE S2 | Expression by real-time PCR of genes involved in signaling pathways that are modulated in psoriatic arthritis patients compared to healthy subjects. Bars indicate SD.

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REVIEW
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Potential Role of Cytochrome c and Tryptase in Psoriasis and Psoriatic Arthritis Pathogenesis: Focus on Resistance to Apoptosis and Oxidative Stress

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Psoriasis (PsO) is an autoimmune disease characterized by keratinocyte proliferation, chronic inflammation and mast cell activation. Up to 42% of patients with PsO may present psoriatic arthritis (PsA). PsO and PsA share common pathophysiological mechanisms: keratinocytes and fibroblast-like synoviocytes are resistant to apoptosis: this is one of the mechanism facilitating their hyperplasic growth, and at joint level, the destruction of articular cartilage, and bone erosion and/or proliferation. Several clinical studies regarding diseases characterized by impairment of cell death, either due to apoptosis or necrosis, reported cytochrome c release from the mitochondria into the extracellular space and finally into the circulation. The presence of elevated cytochrome c levels in serum has been demonstrated in diseases as inflammatory arthritis, myocardial infarction and stroke, and liver diseases. Cytochrome c is a signaling molecule essential for apoptotic cell death released from mitochondria to the cytosol allowing the interaction with protease, as the apoptosis protease activation factor, which lead to the activation of factor-1 and procaspase 9. It has been demonstrated that this efflux from the mitochondria is crucial to start the intracellular signaling responsible for apoptosis, then to the activation of the inflammatory process. Another inflammatory marker, the tryptase, a trypsin-like serine protease produced by mast cells, is released during inflammation, leading to the activation of several immune cells through proteinase-activated receptor-2. In this review, we aimed at discussing the role played by cytochrome c and tryptase in PsO and PsA pathogenesis. To this purpose, we searched pathogenetic mechanisms in PUBMED database and review on oxidative stress, cytochrome c and tryptase and their potential role during inflammation in PsO and PsA. To this regard, the cytochrome c release into the extracellular space and tryptase may have a role in skin and joint inflammation.

Keywords: psoriatic arthritis, psoriatic disease, autoimmunity, oxidative stress, apoptosis, cytochrome c and tryptase

INTRODUCTION

Psoriasis (PsO) is an inflammatory skin disease characterized by plaques of thickened and scaling skin due to keratinocyte proliferation, chronic inflammation linked to the presence of several innate and acquired immune cells, and mast cells activation (1). Psoriatic arthritis (PsA) is a chronic inflammatory arthritis that may be present in up to 42% of individuals affected by PsO (2). PsA is clinically characterized by inflammation of periarticular (e.g., enthesis) and articular structures. PsO and PsA share common pathophysiological mechanisms: the marked tortuosity of blood vessels and infiltration of plasma cell and mononuclear cells are observed in both the psoriatic plaque and PsA articular space (3). For both diseases, the pathogenesis is multi-factorial with underlying autoimmune mechanism (4). Genetic predisposition, as human leukocyte antigens (HLA)-Cw0602 and the HLAB27 allele, and an altered immune response can induce inflammation of skin and joints (5). Cytokines, as Tumor Necrosis Factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-17, and IL-18, are over-expressed in skin lesions, in peripheral blood, synovial membrane, and synovial fluid of PsA patients (6). Moreover, keratinocytes and fibroblast-like synoviocytes (FLS) exhibit similar resistance to apoptosis, one of the key mechanism that may facilitate psoriatic plaque growth and the hyperplasic progression of FLS in the synovium, destruction of articular cartilage and bone damage. PsA pathogenesis is only partially understood and the interest in the pathophysiological role of the synovium is recently growing (7). Pro-inflammatory mediators, responsible for joint inflammation, may be secreted at skin level and blocking this pathological communication may represent a new fascinating therapeutic objective. The primum movens seems to be the activation of the innate immunity. Several markers have been studied, among them, elevated S100A8 and S100A9 levels were observed in fluid samples from inflamed tissues in PsA synovium and in skin psoriasis in patients affected by both PsA and PsO (8). To this regard, inflammatory mediators, at an early stage, may predict articular involvement helping in preventing joint damage. Recently, the involvement of oxidative stress in PsO and PsA pathogenesis has been considered (9). Disruption of redox signaling, caused by oxidative stress, brings molecular damage which inevitably impacts on angiogenesis, inflammation, and function/activation of dendritic cells, lymphocytes, and keratinocytes (10). Cytochrome c, whose structure is shown in **Figure 1A**, is floating into the peripheral mitochondrial membrane and mediates the electron transfer (eT) throughout the respiratory chain (11–14). Reactive oxygen species (ROS) formation induces the release of cytochrome c into the cytosol (15, 16), where it binds to the apoptosis protease activation factor (APAf-1) forming with ATP or dATP, the apoptosome; this complex activates procaspase 9, which triggers an enzymatic cascade that brings to cell apoptosis (17–19). Several clinical studies regarding diseases characterized by cell death, either due to apoptosis or necrosis, reported cytochrome c release from the mitochondria into the extracellular space and finally into the circulation. Elevated cytochrome c levels in serum were found in chronic and acute diseases, including inflammatory arthritis, myocardial infarction and stroke, and liver diseases (20–22). Its role as an inducer of skin and joint inflammation has been proposed. In particular, a link between cytochrome c, skin inflammation, and keratinocytes proliferation was demonstrated during ROS production (23). ROS dysregulation and cytochrome c release have been associated with PsO, and were reported to be the cause of associated skin inflammation (24). During inflammation, another relevant pathway involves tryptase, a trypsin-like serine protease produced by mast cells and stored in intracellular vesicles. It is a catalytically active tetramer made of identical subunits, each having the catalytic triad residues (25) (shown in **Figures 1B,C**) and represents the major protein present in mast cells granules (26, 27). The tetrameric structure and the presence of heparin are necessary *in vivo* for the tryptase function (28). Tryptase activates, both *in vivo* and *in vitro*, a number of cells involved during innate and adaptive immune response, through proteinase-activated receptor-2 (PAR-2), thus contributing to the perpetuation of inflammation (29). The activation of PAR-2 brings about, in some circumstances, apoptosis inhibition (30). Several new interesting pathogenetic ways have been recently assumed in Psoriatic disease pathogenesis (23, 25). This review aims at discussing the role played by two new inflammatory mediators, as cytochrome c and as tryptase, in regulating extrinsic and intrinsic apoptotic pathways in PsO and PsA pathogenesis (as described in **Figures 2, 3**). The role of oxidative stress, linked to psoriatic disease pathogenesis, will be highlightened. Moreover, the review will focus on the relationship of these two molecules with oxidative stress and their potential role as inflammatory markers in PsO and PsA.

POTENTIAL ROLE OF CYTOCHROME C AND TRYPTASE IN PSORIASIS AND PSORIATIC ARTHRITIS

Cytochrome c, Apoptosis and Chronic Inflammation

Cytochrome c was found to be one of the key signaling molecules during programmed cell death-apoptosis. As explained above, its translocation into the cytoplasm leads to its interaction with APAf-1 and, in presence of ATP/dATP, to the final activation of procaspase 9. These stages are crucial for the completion of the apoptosis (16). In normal conditions, the apoptosis leads to cells death in dysfunctional cells owing to the strictly regulated pathways which are vital to maintain the homeostasis. A disequilibrium in the regulation of the apoptotic way may lead to defective immune responses and, consequently, to infections, tumor growth and autoimmune diseases. There are two intracellular pathways leading to apoptosis: the extrinsic, mediated by death-receptors, and the intrinsic or mitochondrial pathway (32). ROS are known to cause mitochondrial damage and dysfunction, which can cause the rise of cytochrome c release into the cytoplasm, as part of the intrinsic pathway, and the activation of the apoptotic pathway (33). In this context, it has been demonstrated that an increase in the release of cytochrome c from the mitochondria into the cytosol, is regulated by B-cells follicular lymphoma (Bcl)-2 family proteins (33). Therefore, the regulatory effect of the Bcl-2 family is essential for

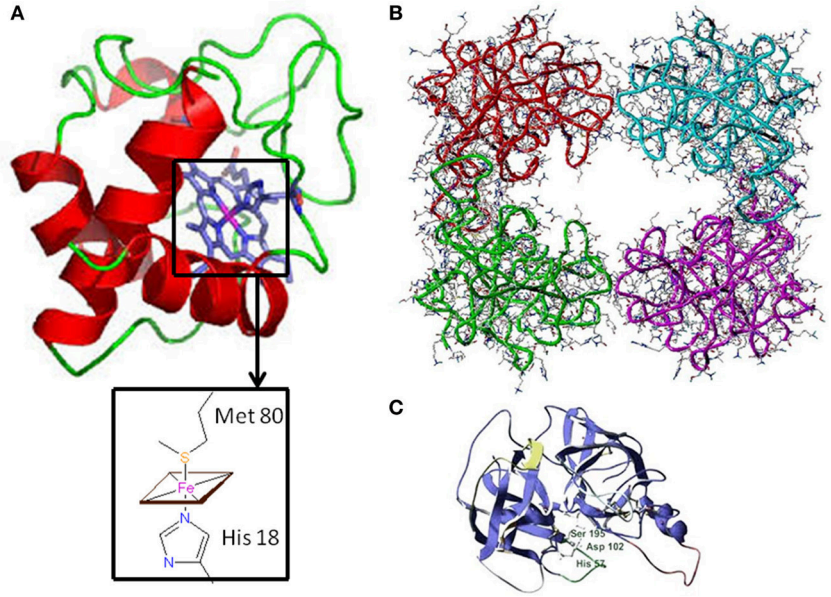

FIGURE 1 | Three-dimensional structure of Cytochrome c and Tryptase **(A)** Horse heart cytochrome c structure characterized by X-ray crystallography (6). In the inset, the fifth and sixth ligands (His 18 and Met 80, respectively) to the Fe-Heme are shown. **(B)** Human β-tryptase tetramer structure as determined by X-ray crystallography (11). **(C)** Tryptase monomer structure: the catalytic triad residues (Ser 195, His 57, and Asp 102) are highlighted.

the control of apoptosis and can determine the cell's fate (33). Schultz and colleagues described how deregulation of apoptosis, linked to the intrinsic pathway, may contribute to autoimmunity (34). They observed that mainly Fas ligand (FasL) contributes in the pathogenesis of autoimmune diseases. FasL and other (TNF) ligands bind death receptors and induce: the release of cytochrome c and the expression of both flavoprotein apoptosis-inducing factor and the second mitochondria-derived activator of caspases, known as DIABLO (inhibitor of anti-apoptotic factors and activator of procaspase) (34). The consequence is the activation of caspases with the induction of cell death through DNA and protein cleavage. Cellular damage may release potential autoantigens and may be the basis for autoimmunity and inflammation.

During autoimmune diseases, the regulation of self-reactive B cells, in order to inhibit autoantibody production, may be critical. B cells' tolerance consists in the inhibition of completely or partially autoreactive B cells by using different mechanisms. Cell death pathways are the most reliable system to eliminate autoreactive B cells and to prevent autoimmunity (35). The presence of autoreactive cells and mitochondrial markers, as cytochrome c, may be relevant also in PsO and PsA pathogenesis (22, 36). In PsO, a dysregulation of cytochrome c has been demonstrated. Indeed, a higher serum and mitochondrial levels were observed compared to healthy skin; this contributes to epidermal hyperplasia (37). Concerning PsA, the result is synovial inflammation and joint destruction (38). PsA is microscopically characterized by lining layer hyperplasia, infiltration of B and T lymphocytes, and activation of the innate immune response that lead to vascular remodeling and angiogenesis into the joint (39). The characteristic PsA synovitis has inflammatory infiltration of macrophages, lymphocytes, and plasma cells that cause FLS hyperplasia and chronically, joint and bone damage (40). A cytochrome c dysregulation may also result in an inadequate apoptosis. To this regard, both the FLS and keratinocytes display a resistance to apoptosis leading to synovial and epidermal hyperplasia which are the hallmark of synovitis and psoriatic plaques genesis (41, 42). In this context, impaired apoptosis may contribute to the perpetuation of the inflammatory process (43). For this apoptotic resistant state, Mitomycin C (MMC), which is a bacteric anti-tumor antibiotic, has been investigated as potential treatment in inflammatory arthritis. This agent has shown an inhibitory effect on fibroblast proliferation by inducing apoptosis (39). Furthermore, Yan and coll. have demonstrated that MMC can decrease cell liability and provoke apoptosis in FLS obtained from patients affected by inflammatory arthritis, as rheumatoid arthritis (RA). A possible mechanism involved in the MMC inhibitory effect on FLS may regard the intrinsic mitochondrial pathway. To this regard, MMC stimulates the production of ROS and induces the release of cytochrome c, and consequently it may activate the caspases' cascade (33).

Tryptase and Chronic Inflammation

Among cells that participate to the inflammatory status, the role of mast cells during inflammation was well demonstrated in the literature (44). Mast cells activation into joints and skin can cause the release of vasoactive mediators, such as histamine, prostanoids, and cytokines which contribute to inflammation (44, 45). Moreover, tryptase, released by mast cells, play an important role in the pathogenesis of inflammatory arthritis (46). Different pathogenetic mechanisms are linked to tryptase

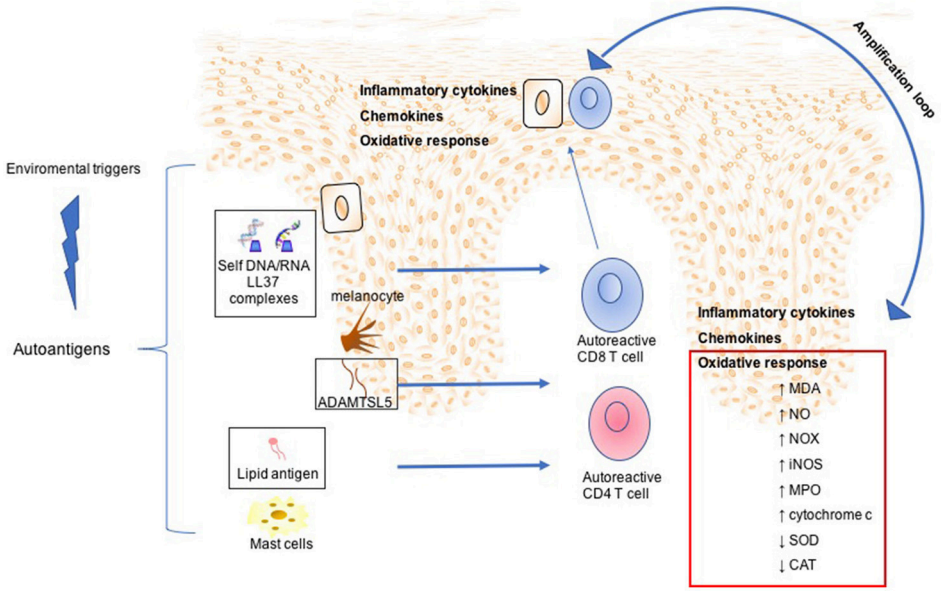

FIGURE 2 | Mechanisms involved in Psoriasis arthritis pathogenesis. In a summary view, environmental triggers induce the expression of autoantigens, such as LL37 by keratinocytes, ADAMTSL5 by melanocytes, and lipid antigen by mast cells. This leads to expansion and activation of autoreactive CD8 and CD4 T cells. Although CD4 T cells remain within the dermis producing a large plethora of inflammatory cytokines, activated CD8 T cells migrate into the epidermis. Subsequently, potentially upon recognition of autoantigens in the epidermis CD8 T cells release other inflammatory mediators, which in turn mediate skin cell homing and remain as resident memory T cells in the epidermis. All this inflammatory process is maintained and amplified by oxidative stress sustained by production of NADPH-oxidase (NOX), inducible nitric oxide synthase (iNOS) myeloperoxidase (MPO), malondialdehyde (MDA), nitric oxide (NO), and serum cytochrome c and decreased of antioxidant status such as superoxide dismutase (SOD), catalase (CAT).

release: it is expressed in high concentration in synovial fluids from patients affected by RA, PsA, and reactive arthritis (47). The link between PsA and tryptase is supported also by the presence of corticotropin releasing hormone receptor 1 in PsA synovial biopsy. This hormone and its receptor have been demonstrated in the joints of patients affected by PsA and not in the control group. This hormone can enhance the intercellular matrix degradation by inducing tryptase release by mast cells (48). Intriguingly, a mast cell subset able to produce IL-17 was detected in rheumatoid synovium (49) and in psoriasis plaques, resulting to be the prevalent IL-17 producing cells (50, 51). The relevant role of IL-17 in the pathogenesis of PsO and PsA is pointed out also by the efficacy of biological drugs as IL-17 inhibitors for both skin and joint involvement in these diseases (52–55). To note, tryptase showed to have several pathogenic roles in PsO and PsA patients: (1) it can induce the production and release of several pro-inflammatory cytokines, such as IL-1β, IL-8, TNFα, and IL-6 (56, 57); (2) it stimulates leukocytes migration into the joints by PAR-2, ICAM-1, CXCR2, and IL-8, directly causing and amplifying the inflammatory process (58–60); (3) it activates, by cleavage, proteolytic enzymes, such as prostromelysin, procollagenase, and MMP-3 which contributes to matrix degradation (61, 62). Furthermore, it has been demonstrated that mast cells proteinases, as tryptase, secreted in excess under the influence of inflammatory stimuli of specific cytokines, degrade the joint matrix *in vitro*. Tryptase damages various matrix components by the activation of matrix metalloproteinases (62). Mast cells can express several cytokines, as TNF-α and IL-1β, and various profibrotic cytokines such as TGF-α and IL-4, suggesting numerous functional roles for Mast cells during chronic inflammation (63). In particular, synovial mast cells *in vitro* release tryptase and activate latent collagenase (64), participating to the development of the typical PsA synovial hypertrophy (65).

EVIDENCE FOR AUTOIMMUNE PATHWAYS AND OXIDATIVE STRESS IN PSORIASIS

The Autoimmune Side of Psoriasis Pathogenesis

PsO is immune-mediated inflammatory cutaneous disease characterized by keratinocyte proliferation and chronic inflammation (58). Recently, evidence has supported that PsO has an autoimmune pathogenesis and three different autoantigens have been identified so far: cathelicidin LL37, a domain thrombospondin type 1 motif-like 5 (ADAMTSL5) present in metalloproteases, and lipid antigens generated by phospholipase called PLA2G4D (66, 67). LL37 is a peptide upregulated in psoriatic skin with antimicrobial properties, LL37 can bind self RNA and DNA in complexes which are able to activate plasmacytoid and myeloid dendritic cells (68). This leads to an expansion of LL37-specific T cells producing pathogenic cytokines such as INF-γ, IL17, and IL-22. LL37 presentation to CD8 and CD4 T cells is mediated by HLA-Class I, in particular (HLA)-Cw0602 and HLA-Class II molecules respectively (69). ADAMTSL5 is an autoantigen presented by (HLA)-Cw0602 in

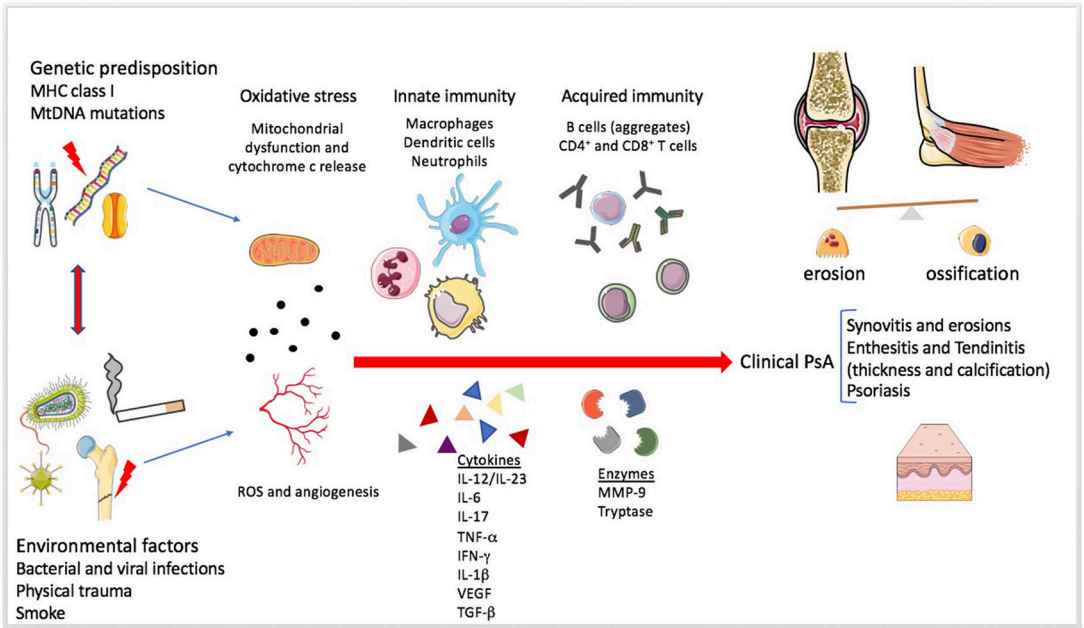

FIGURE 3 | Mechanisms involved in Psoriatic arthritis pathogenesis. The figure summarizes the pathogenesis of Psoriatic arthritis (PsA). The enthesitis seems to be the primum movens of the disease, even if the heterogeneity of systemic involvement and clinical manifestations is extremely wide (31). Genetic and environmental factors predispose a healthy individual and contribute to the development of the disease. Mitochondrial dysfunction, angiogenesis and increased production of reactive oxygen species (ROS) seem to be present since the early disease onset. The role of the innate immunity in the tolerance disruption and in the production of a pro-inflammatory milieu is very early and essential in the pathogenesis of both PsA and Psoriasis. Adaptive immunity participates later by perpetuating and further increasing the inflammation. Several soluble mediators, such as pro-inflammatory cytokines and proteases, can be found in the synovial fluid and sera of PsA patients.

PsO, the derived peptide can stimulate psoriatic T cells but not T cells from healthy individuals, resulting in IL17 production (68). Moreover, ADAMTSL5 and LL37 are increased in psoriatic lesions and are co-expressed by many immune cells, dendritic cells, neutrophils, macrophages, and T cells within skin. Both ADAMTSL5 and LL37 can be decreased by treatment with IL-17 or TNF-α inhibitors (70). Lastly, there is evidence for non-peptide autoantigen in PsO. T cells from PsA patients can recognize also lipid antigens generated in mast cells by PLA2G4D which are presented by CD1a. PLA2G4D is expressed in psoriatic skin lesions, but not in skin of healthy individuals (71). PsO pathogenesis is multifactorial, resulting from a combination of genetic, epigenetic, and environmental factors which lead to activation of an abnormal immune response. Working models for PsO suggest that several immune cells may present these antigens to autoreactive T cells with following activation and clonal expansion (60). This mechanism induces cytokines production, immune cells activation, and cell recruitment which in turn contributes to the amplification of inflammatory response and keratinocytes proliferation in PsO.

The Role of Oxidative Stress in Psoriasis Pathogenesis

In this complex pathogenesis, oxidative stress and free radical production play a role in skin inflammation (23). It was demonstrated that a reduction of antioxidant and augmented oxidant activities in psoriasis exists (72, 73). Moreover, a reciprocal amplification loop may exist between inflammation and oxidative stress in PsO. It is known that ROS production during oxidative stress activates cellular proinflammatory signaling mostly the JAK–STAT, MAPK/AP-1, and NF-κB pathways, leading to the production of cytokines, chemokines, and growth factors which are involved in the pathogenesis of psoriasis (74–77). Interestingly, activator protein-1 (AP-1) activates peroxisome proliferator-activated receptor δ (PPARδ) which is up-regulated in PsO, inducing proliferation and preventing apoptosis of keratinocytes, via the activation of heparin-binding EGF-like growth factor (HB-EGF) and activation of Protein Kinase Ba/Akt1 pathway, respectively (78, 79).

On the other hand, oxidative stress may originate by endogenous stimuli such as Th1 and Th17 cytokines, which are able to induce the production of NADPH-oxidase (NOX), inducible nitric oxide synthase (iNOS) and myeloperoxidase (MPO) and from environmental agents that may be also the cause of inflammation (80). Moreover, it has been recently demonstrated that MPO can be considered as a marker of systemic inflammation in Psoriatic disease (81).

Gabr and colleagues demonstrated a positive correlation between serum malondialdehyde, nitric oxide and serum cytochrome c levels and disease severity (measured by PASI score). On the contrary, a negative correlation with superoxide dismutase (SOD), catalase, and total antioxidant status was reported (36). Increased levels of mitochondria cytochrome c were also observed in lesional psoriatic skin compared to non-affected skin, and this increase is reversed by methotrexate

(MTX) treatment (37). Indeed, during MTX therapy, an increased cytosolic cytochrome c level and consequent cleaved caspase-9 were also observed (37). Apoptotic dysregulation has been reported in psoriatic keratinocytes, which display a resistance to apoptosis, contributing to epidermal hyperplasia (42). Accordingly, in a recent article, after UVB irradiation, psoriatic keratinocytes showed a less cytosolic cytochrome c level compared to keratinocytes from healthy skin (82). A wide type of cells, such as T cells, dendritic cells, neutrophils, keratinocytes, mast cells, NK cells and macrophages are involved in PsO pathogenesis with different roles and specific hallmarks (83). The activation of innate immune system is believed to play a key role in the initial step of plaque formation, and, in this context, mast cells contribute producing IL-22 and IL-17 (52, 84). Furthermore, a high number of mast cells are present in the affected skin as well as the associated release of tryptase and histamine (85). Tryptase positive mast cells are increased in psoriatic lesional skin compared to normal skin, and tryptase mainly is localized in the dermis, at dermis-epidermal junction and around blood vessels, as showed by Steinhoff and collaborators through immunohistochemistry experiments (45). Tryptase participates to PsO inflammation through specific cytokines and neuropeptides production. Indeed, Tryptase acts by PAR2 cleavage, which is highly expressed in mast cells and in keratinocytes of PsO skin lesions (86–88). PAR2 exerts its inflammatory effects inducing the production of proinflammatory cytokines such as TNFα, IL-1ß, IL-6, IL-8, and granulocytes macrophage-colony stimulation factor (GM-CSF) (57, 87, 89). In details, TNFα is a landmark inflammatory mediator in PsO, being one of the first target of monoclonal antibody therapy (90) and GM-CSF is a mediator for maturation of Langerhans cells and stimulates keratinocytes proliferation (91, 92). On the other hand IL-6, which is also highly expressed in PsO, is able to stimulate keratinocytes proliferation (93) and contributes to the differentiation of Th17 cells which play a key role in PsO pathogenesis (94). Interestingly, PAR2 is also able to induce skin inflammation also through neurogenic mechanism, stimulating sensory C-fibers to produce some neuropeptides such as calcitonin gene-related peptide and substance P (95).

ROLE OF AUTOIMMUNITY AND OXIDATIVE STRESS IN PSORIATIC ARTHRITIS

The Autoimmune Side of Psoriatic Arthritis Pathogenesis

The autoimmune side of PsA pathogenesis has been demonstrated by the presence of autoreactive T cells in synovium from PsA patients, which were activated by a homologous protein antigen, expressed in the synovium (4). Furthermore, the association with major histocompatibility complex class I molecules, the loci HLA-Cw6 or HLA-B27 in particular, leads to the presentation by those molecules of an autoantigen with the consequent production of antibodies against autoantigens. Additionally, the good response to immunosuppressive agents and cytokines blockers supports the involvement of autoreactive cells participating at the autoimmune process (96). Histologically, the PsA synovium presented both clonal and non-clonal T cells (97), and the link between skin and synovium T cells-clones (98) has been demonstrated. This indicates that a common antigen might drive the T cell response in both the target organs. Concerning the joint-enthesal complex, a group of cross-reactive PsA-specific antibodies, directed against peptides expressed in both the psoriatic skin and the inflamed enthesis, has been demonstrated by Dolcino and collaborators (99). Moreover, recent evidence suggests that PsA synovial biology involves several type of immune cells. The same histological heterogeneity observed in RA synovium has also been detected in psoriatic synovial tissue (100). In particular, lymphocytes infiltrates, in PsA synovium, tend to aggregate in lymphoid agglomerates, supporting the presence of an ectopic lymphoid neogenesis (LN), and autoantibody production (100). Conigliaro et al. previously reported an abnormal distribution of peripheral blood B cells in both RA and PsA patients (101). Peripheral B cells were reduced in PsA patients and their level were restored after anti-TNF treatment suggesting a role of B cells in PsA pathogenesis (101). During inflammation, several protein modifications may occur supporting the strong link between autoimmunity and inflammation. Among them, the effects of carbamylation on proteins and its effect in inflammatory arthritis has been recently investigated. Carbamylation is a post transcriptional modification on lysine, the effect on protein function and biochemical properties includes a change of the ability in polymerization, sensitivity to proteinases, and binding avidity to antibodies (102). To date, it is well known that carbamylation happens during inflammation, after the release of MPO by neutrophils, generating a pro-inflammatory loop. Carbamylated proteins are recognized by circulating antibodies, the anti-carbamylated protein (anti-CarP) antibodies that have been identified in patients with RA, also before the clinical onset of the disease (38). The presence of anti-CarP antibodies in sera from PsA patients with active disease, in the absence of rheumatoid factor and/or other known autoantibodies specificities, was demonstrated (96, 102). Interestingly, the circulating anti-CarP antibodies levels showed to be a good diagnostic test for PsA patients in comparison to healthy subject. A further support of an autoimmune origin is the discovery of a peptide antigen, called the PsA antigen, recognized by IgG derived from PsA patients' sera. This peptide shows homologies with peptides expressed both in skin and enthesis, as a further demonstration that an immunologic disequilibrium has a role in the pathogenesis of PsA (99).

The Role of Oxidative Stress in Psoriatic Arthritis Pathogenesis

The activation of innate immunity is believed to have a role in PsA pathogenesis (96). In particular, the monocyte/macrophages population has a relevant role during inflammation at the enthesal level: it participates to the tolerance disruption in PsA patients and activates the release of mediators linked to the oxidative stress (103, 104). The oxidative damage in PsA

is mainly due to a dramatic production of ROS that saturate the compensatory antioxidant enzymes and molecules, such as glutathione and SOD (105). The substantial producers of ROS are present in the mitochondrial membrane, NADPH oxidase and eT chain. The consequence of the unbalanced production of ROS is the leakage of cytochrome c from mitochondria, which activates the caspases cascade leading to apoptotic cell death (105). This reaction occurs also in cells populating inflamed joints, like synoviocytes, chondrocytes, lymphocytes and monocytes. To note, oxidative stress can lead to cyclooxygenase-2 and MMP9/13 expression and modulates apoptotic pathways and NF-κB. All of them are relevant mediators during inflammatory arthritis pathogenesis (106–109). Recent studies have highlighted the possible role of oxidative stress in PsA. Altered levels of oxidative metabolites, for example carbonyl groups, F2-isoprostanes, hydroperoxides, and sulfhydryl groups, were detected in PsA patients when compared to healthy donors (110–112). A similar study, showed a higher serum peroxide concentration and a lower antioxidant capacity in sera from patients affected by RA, PsA, and PsO in comparison with healthy individuals (113). The connection between circulating ROS and inflammation was investigated in RA, PsA, and ankylosing spondylitis patients in whom ROS in sera were higher when compared to controls (113, 114). Interestingly, after anti-TNFα treatment, a reduced value of circulating ROS was detected (114). Furthermore, the circulating peripheral blood mononuclear cells in patients with inflammatory arthritis have high levels of peroxidated lipid and altered polarization of the mitochondria membrane; these alterations correlate with patients disease activity (115). Furthermore, oxidative stress takes part also in the pathogenesis of comorbidities that may affect PsA patients. Patients with PsA have an accelerated atherosclerosis, and so a higher cardiovascular risk compared to healthy subjects (116). In this context, oxidative stress plays a relevant role in the pathogenesis of atherosclerosis: it induces the oxidative modification of LDL. The oxidized LDL takes part in many phases of atherogenesis and are strictly related to the inflammatory process (117). Moreover, oxidative modifications of LDL have been linked to the presence of TNFα and HDL may be altered during inflammation. Modified HDL lose their capacity to remove cholesterol from atherosclerotic lesions and have a reduced antioxidant activity (118). During inflammation, the release of TNFα is one of the main contributor of increased ROS production, and this is strongly related to disease activity (119). Then, the presence of ROS supports oxidative stress, further stimulating in this way cell damage and atherogenesis (113). Recent findings demonstrated that ROS were found not only into the blood stream and in circulating cells, but also into the joint. In synovial fluid of PsA patients, a higher ROS production, angiogenesis, and, DNA damage was found when compared to an osteoarthritis group (120–122); this is a further demonstration that oxidative stress has a role in the pathogenesis. Interestingly, a role of hypoxia was proven during inflammatory arthritis pathogenesis. A negative correlation between pO2 in synovial fluid of RA and PsA patients and level of macroscopic synovitis, disease activity, sublining layer thickness, and cells infiltration were demonstrated. In addition, hypoxic intra-articular environment and oxidative stress induce the production of pro-inflammatory cytokines (106). Ng and coll. confirmed this link by demonstrating a high expression of NOX in RA and PsA synovial tissue, which correlates with intra-articular pO2 and angiogenesis. A reduction of NOX expression was observed after 3 months of anti-TNFα treatment, in association with raised pO2 and a lower disease activity (123). The oxidative stress, measured as rate of mitochondrial DNA (mtDNA) mutations, is increased in RA and PsA synoviocytes; and it is correlated to high reactive ROS, reduced expression of cytochrome c oxidase, and low level of intra-articular pO2 (8). As a further evidence of the link between inflammation and oxidative stress, Harty et al demonstrated that the levels of mitochondrial DNA (mtDNA) point mutations in synovial tissue from patients with inflammatory arthritis was related to *in vivo* hypoxia and oxidative stress levels (124). The authors described a correlation between mtDNA mutation rate, the expression of TNFα and macroscopic arthroscopic signs of inflammation in RA and PsA patients. Patients were then treated with anti-TNFα, and only in anti-TNFα-responders the mtDNA mutations frequency was reduced. Moreover, the addition of recombinant TNFα in RA and PsA synoviocytes culture induces mtDNA mutations (124). These data suggest that antioxidant agents may be potentially useful as an add-on treatment to conventional therapies in the management of inflammatory arthritis and in autoimmune diseases (125, 126).

CONCLUSIONS

All studies converge to establish that in autoimmune diseases as PsO and PsA, oxidative stress, cell apoptosis and inflammation may lead to cytochrome c released from the mitochondria into the extracellular space. The future perspective given by data from the literature is that serum cytochrome c can be measured and used for diagnosing and assessing cell death during systemic diseases. The role of cytochrome c has been associated with autoimmune diseases and its release from mitochondria into the cytoplasm may be considered as a marker of inflammation and autoimmunity. Besides, several evidence suggests that mast cells activation is implicated in PsO and PsA pathogenesis. Those cells and the release of tryptase support the relevant role of innate immunity in PsO and PsA development. In this context, both cytochrome c and tryptase are detected during the inflammatory process. They may act as potential triggers in the perpetuation of the pro-inflammatory loop. In this context, mediators released from mast cells or mitochondrial activity may be used as marker of disease or as target in the treatment of autoimmune diseases. However, further studies are needed to better understand the potential contribution of cytochrome c and tryptase in autoimmune disease pathogenesis during oxidative stress and their potential

correlation with disease activity and as markers of treatment efficacy.

AUTHOR CONTRIBUTIONS

MC, FS, and LF conceived the review and wrote the introduction and the paragraph concerning psoriatic arthritis. GF, ME, AG, and RS helped in writing the part concerning cytochrome c and tryptase. EB and LB wrote the paragraph concerning psoriasis. PC, PT, FC, and LC supervised the paper. RP conceived and revised the manuscript.

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REVIEW
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Psoriasis: Classical vs. Paradoxical. The Yin-Yang of TNF and Type I Interferon

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Chronic plaque psoriasis is a common debilitating skin disease. The identification of the pathogenic role of the TNF/IL-23/T_H17 pathway has enabled the development of targeted therapies used in the clinic today. Particularly, TNF inhibitors have become a benchmark for the treatment of numerous chronic inflammatory diseases such as psoriasis. Although being highly effective in psoriasis treatment, anti-TNFs can themselves induce psoriasis-like skin lesions, a side effect called paradoxical psoriasis. In this review, we provide a comprehensive look at the different cellular and molecular players involved in classical plaque psoriasis and contrast its pathogenesis to paradoxical psoriasis, which is clinically similar but immunologically distinct. Classical psoriasis is a T-cell mediated autoimmune disease driven by TNF, characterised by T-cells memory, and a relapsing disease course. In contrast, paradoxical psoriasis is caused by the absence of TNF and represents an ongoing type-I interferon-driven innate inflammation that fails to elicit T-cell autoimmunity and lacks memory T cell-mediated relapses.

Keywords: plaque psoriasis, paradoxical psoriasis, TNF, IL-23, T_H17, type I-interferon

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INTRODUCTION

Psoriasis is a distinctly human, chronic, inflammatory skin disease, affecting 2–3% of the population worldwide, with prevalence varying considerably according to race and geographic location (1). Clinically, plaque type psoriasis, the most common form of psoriasis, is characterised by well-demarcated erythematous lesions covered with silvery-white scales. These lesions are histologically reflected by keratinocyte hyperproliferation leading to epidermal hyperplasia (acanthosis), characteristic elongation of the rete ridges (papillomatosis), thickening of the cornified layer (hyperkeratosis), and incomplete keratinocyte differentiation resulting in retention of nuclei in the stratum corneum (parakeratosis). Leukocytes, including T-cells, dendritic cells, neutrophils, and macrophages make up a considerable dermal and epidermal immune cell infiltrate. Psoriasis is caused by the interaction of predisposing genetic factors and environmental triggers leading to dysregulated innate and adaptive immune responses. Today, psoriasis is widely regarded as a T-cell-mediated autoimmune disease and skin infiltrating T lymphocytes play key effector roles by driving disease development and maintenance. Dendritic cells producing TNF and IL-23 stimulate activation of both $CD4^+$ and $CD8^+$ T-cells, which in turn migrate into the epidermis. Upon recognition of autoantigens, T-cells produce T_H17-cytokines such as IL-17A, IL-17F, and IL-22, which drive the psoriatic phenotype by inducing keratinocyte hyperproliferation. In support of this, several single nucleotide polymorphisms cluster throughout this pathway including genes in the TNF/dendritic cell activation pathway (*TNFAIP3*, *REL*, *TN1P1*, *NFKBIA*) as well as in the T-cell activation (*HLA-Cw6*, *ERAP1/ZAP70*, *ETS1*, *SOCS1*, *TNFRSF9*), and T_H17/T_C17-differentiation pathways (*IL23A*, *IL23R*) (2, 3). Consequently, antibodies targeting the pathogenic TNF/IL-23/IL-17 pathway have revolutionised psoriasis treatment over the past 15 years and are widely used in the clinic today.

In particular, TNF blockade has become the benchmark in management of numerous chronic inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, and psoriasis (4–7). As such, more than two million patients have already been treated with anti-TNFs and, with the advent of biosimilars, these figures are expected to grow further over the coming years. Yet, targeting TNF is not without consequence, as TNF is a potent pro-inflammatory cytokine known to coordinate immune responses and play an important role in limiting the spread of infectious pathogens. Thus, TNF blockade leads to an increased risk of infections and slightly increased risk for certain malignancies. However, more surprisingly, anti-TNF treatment can also induce new psoriasis-like skin lesions in about 2–5% of treated patients (8).

As anti-TNFs are amongst the most potent anti-inflammatory drugs used in the treatment of psoriasis, developing psoriasis-like skin lesions due to TNF blockade was somewhat paradoxical—hence the designation "paradoxical psoriasis."

This review aims to provide a focussed overview of the latest developments in the T-cell and cytokine networks in classical psoriasis, and contrast them to paradoxical psoriasis induced by anti-TNFs, which is clinically similar to psoriasis but immunologically distinct. Finally, these findings will be put into perspective with future avenues of research and possible clinical interventions.

CLASSICAL PSORIASIS

The Established Role of T-Helper and the Revisited Cytotoxic T-Cells

The pathogenic role for T-cells in psoriasis is well-established and stems from the following clinical observations and experimental findings: Immunosuppressive agents, such as cyclosporine, or therapies specifically targeting T-cells are efficacious in psoriasis treatment (9–12). *HLA-Cw6* represents the strongest genetic risk variant associated with psoriasis (13). Molecular analysis of psoriasis tissue showed that lesional T-cells are oligoclonal (14) and recognise epidermal autoantigens (15–18). Finally, clinically relevant xenotransplant models of psoriasis have demonstrated an essential functional role for T-cells (19–21).

T-cells migrate into inflamed skin through expression of the skin-homing Cutaneous Lymphocyte-associated Antigen (CLA) (22), LFA-1 and $\alpha_4\beta_1$ (23), and the chemokine receptors CCR8 and CCR10 (24). More specifically T_H1 cells use CXCR3 and CCR4 (25), whereas T_H17 cells use CCR4 and CCR6 (26). Among the most well-described chemokines involved in T-cell migration to the skin are CCL27 (27, 28), and CCL20 (29) produced by keratinocytes upon an inflammatory trigger. While circulating T-cells certainly play an important role in skin immunopathology, there are twice as many T-cells residing in normal healthy skin than are present in the circulation (22). Moreover, pathogenic oligoclonal T-cells remain resident in resolved psoriatic skin lesions suggesting that disease recurrence might be initiated through reactivation of skin-resident T-cells (30). Indeed, these skin-resident memory T-cells were found to be sufficient to drive psoriasis development without further recruitment of circulating cells (19, 20). Activation within the skin led to proliferation of T-cells in the dermal compartment, which preceded keratinocyte hyperproliferation. In fact, the psoriatic phenotype was only induced by migration of T-cells into the epidermis and blockade of the epidermal infiltration by T-cells prevented the development of a psoriatic lesion (20). These findings suggest that intraepidermal T-cells reflect key effector cells in psoriasis.

Traditionally, much attention has been given to differentiated $CD4^+$ T-cell subsets across chronic inflammatory diseases (31–34), including psoriasis (35). However, $CD8^+$ T-cells, which are present in healthy skin as tissue resident memory T-cells (36), have been shown to produce a similar cytokine profile (37). In psoriasis, dermal T-cell infiltrates are mostly comprised of $CD4^+$ cells, whereas the majority of T-cells in the epidermis—which represent key effector cells—are $CD8^+$ (19). Indeed, we could recently show that intraepidermal $CD8^+$ T-cells are functionally essential for psoriasis (38).

Psoriasis has been studied extensively from a genetics perspective, with HLA class I alleles known for more than 40 years to be heavily implicated (39). The *HLA-Cw6* variant is the strongest psoriasis susceptibility allele and has 10-fold higher association with early-onset severe psoriasis. As to how exactly class I HLA molecules might contribute to the pathogenesis of psoriasis is not entirely clear. But in light of the fundamental role of epidermal $CD8^+$ T-cells in psoriasis, the fact that lesional T-cells are of oligoclonal origin and $CD8^+$ T-cells recognise peptide antigens presented on MHC class I molecules suggest a role for epidermal (auto-)antigens in psoriasis. As mentioned above, epidermal $CD8^+$ T-cells in psoriasis are key effectors in psoriasis (20), and they are of oligoclonal origin (14, 30)—thus potentially recognising common antigens. Taken together with *HLA-Cw6* representing the strongest genetic risk variant associated with psoriasis, this suggests that recognition of epidermal (auto-)antigens by $CD8^+$ T-cells is pathogenic in psoriasis.

Indeed, the streptococcal M protein from *Streptococcus pyogenes* has been identified as an antigen target of primarily $CD8^+$ T-cells (40). T-cells directed against the streptococcal M-protein had the ability to react to keratin 14, which is overexpressed in psoriatic skin, due to sequence homology and antigenic similarity (molecular mimicry). Thus, the immune response to a streptococcal infection could divert T-cells toward skin antigens and cause skin pathology. Intriguingly, streptococcal throat infections are a well-known trigger factor for onset and exacerbation of psoriasis.

Other recently identified epidermal autoantigens include keratin 7 (41) and the antimicrobial peptide LL37 expressed by keratinocytes (17) as well as the melanocyte antigen ADAMTSL5 (18). Finally, CD1a-restricted lipids were also found to elicit T-cell responses in psoriatic patients (42). Interestingly, CD1a-autoreactive T-cells isolated from skin were identified as T_H22 cells producing IL-22 (43), a cytokine overexpressed in psoriasis and known to drive keratinocyte hyperproliferation.

Antigen-recognition by T-cells is thought to play a pivotal role in psoriasis, but an all-encompassing consensus on the nature of autoreactivity has yet to be reached. Despite this, all of the identified auto-antigens to date are significantly

upregulated in psoriatic skin as compared to uninvolved or healthy skin. Because the majority can be induced locally upon injury, the prevailing model postulates that skin trauma could lead to upregulation of putative auto-antigens and their recognition by tissue-resident antigen-experienced T-cells in psoriasis patients.

Cytokine Networks: The TNF/IL-23/IL-17 Axis

Nowadays, the pathogenic role of the TNF/IL-23/T_H17 axis in psoriasis is well-known and numerous biologics targeting the different cytokines of this pro-inflammatory pathway are widely used in the clinic. Yet, the arrival of TNF blockers in the early 2000s completely revolutionised the management of psoriasis and other chronic inflammatory diseases. Despite underwhelming results of anti-TNF in sepsis (44), the successful use in rheumatoid arthritis (RA) spurred trials in other chronic inflammatory diseases such as Crohn's disease, psoriasis and psoriatic arthritis (45).

TNF is known to be potently produced by immune and non-immune cells including macrophages, T-cells, dendritic cells (DC), neutrophils, and fibroblasts. One of its major roles is to mount appropriate adaptive immune responses to tumors and pathogens. This is achieved through several mechanisms. Induction of DC-maturation leads to upregulation of CD40, CD80, CD83, and CD86 thereby potentiating T-cell receptor (TCR)-mediated responses and amplifying weak antigen affinity interactions (46). It also serves to limit the immune-suppressive effects of regulatory T-cells (47) and to enhance proliferation and survival of committed effector memory T-cells. In line with these findings, TNF is critically required to mount effective $CD8^+$ T-cell responses against tumors and for the recruitment of T-cells into tumor sites (48). These pro-inflammatory effects of TNF are corroborated in psoriasis, where TNF is found to dictate the inflammatory environment in several ways (49–52). In detailed histological and molecular investigations, it was found to be mostly produced by mature conventional DCs. Blockade of TNF leads to an initial reduction of the chemokine CCL20, which preferentially recruits T_H17 cells into inflamed tissue, coinciding with loss of IL-17 and diminution of dermal and epidermal T-cells. In addition, it leads to normalisation of DC numbers and reduction of IL-23 cytokine expression, followed by normalised keratinocyte differentiation, and eventually to histological improvement and clinical response. Taken together, TNF maintains a pro-inflammatory environment that primes pathogenic T_H17 T-cells through induction of IL-23, maintaining them at the site of inflammation, and sustaining T_H17 cytokine production (53, 54).

Though TNF might contribute to increased IL-17 production by T_H17 cells (55), IL-23 directly governs T_H17 cytokine production both by critically participating in T_H17 cell polarisation as well as by stimulating production of IL-17 by differentiated T_H17 cells (56, 57). Initial supportive evidence for a functional role of IL-23 in psoriasis included the clinical efficacy of an anti-p40 monoclonal antibody (blocking both IL-12 and IL-23) in psoriasis (58) and the association of a single nucleotide polymorphism in the *IL23R* gene in psoriasis patients (55, 59). Confirmation soon followed by the successful use of IL-23-specific antibodies in clinically relevant mouse models and then in patients (60, 61). In addition, IL-4 abrogated T_H17 cell-mediated inflammation by selectively silencing IL-23 in antigen-presenting cells while sparing IL-12/T_H1 immunity (62) and resulting in therapeutic outcome. In psoriasis, IL-23 is mainly produced by activated DCs but keratinocytes and other non-immune cells probably contribute to its production. In fact, it has been shown recently that TNF-dependent epigenetic control of IL-23 expression in keratinocytes plays a role in chronic skin inflammation (63).

T_H17 cytokines, such as IL-17A, IL-17F, and IL-22, represent the key effector cytokines in psoriasis pathogenesis as they directly drive the development of a psoriatic phenotype. They induce epidermal hyperproliferation, attract neutrophils to the skin, and activate keratinocytes to produce chemokines and antimicrobial peptides, which sustain the inflammatory process (64). There are six homologous IL-17 cytokines (A through F) which are produced by either haematopoietic or non-haematopoietic cells, can signal through different combinations of receptors, and mediate distinct biological activities. The individual members are reviewed in this issue by Brembilla et al. (65). Besides the aforementioned effects in the pathogenesis of psoriasis, IL-17 can act in synergy with TNF to further potentiate expression of multiple pro-inflammatory mediators known to play a role in psoriasis, such as IL-8, beta-defensins, S100A proteins, IL-19, and CCL20 (29, 66–68). As such, concurrent inhibition of TNF and IL17 might result in more effective therapy. Though, a bi-specific dual variable domain immunoglobulin targeting both cytokines did not demonstrate increased efficacy compared to anti-TNF in RA (69), this remains to be tested in psoriasis.

While antibodies targeting IL-17 have shown great efficacy in psoriasis (53, 70), blockade of IL-22 failed to meet primary end points in clinical trials indicating distinct role for IL-17 and IL-22 in psoriasis. IL-22, which is produced by T_H17 cells (71) and exclusively by a distinct T_H22 subpopulation (72, 73), had previously been regarded as a very promising target. In psoriasis, IL-22 is found to be produced by dermal $CD4^+$ but also by epidermal $CD8^+$ T_C17 and $CD4^+$ T_H22 cells (38, 74, 75), as well as Innate Lymphoid Cells (76, 77), and mast cells (78). Interestingly, skin-resident T-cells mediating disease memory in clinically resolved psoriasis plaques were found to be epidermal T_C17 and T_H22 cells producing mainly IL-22 (75). Upon binding to its heterodimeric receptor consisting of IL-22RA1 and IL10Rβ (79), which is expressed on keratinocytes (80), IL-22 induces proliferation of keratinocytes and inhibits terminal maturation (81, 82). While transient expression of IL-22 upon skin injury promotes epidermal remodelling with re-epithelisation of skin wounds (74), its chronic expression by psoriatic T-cells drives keratinocyte hyperproliferation and epidermal hyperplasia. In line with this, in transgenic mice, IL-22 was sufficient to induce a skin phenotype that resembles psoriasis (82), and the psoriatic phenotype induced by skin injection of IL-23 was abrogated in IL-22-deficient mice (83). However, the pathogenic function of IL-22 shows redundancy with other members of the IL-20 subfamily of cytokines such as IL-19 and IL-20, potentially rendering its blockade clinically ineffective in humans.

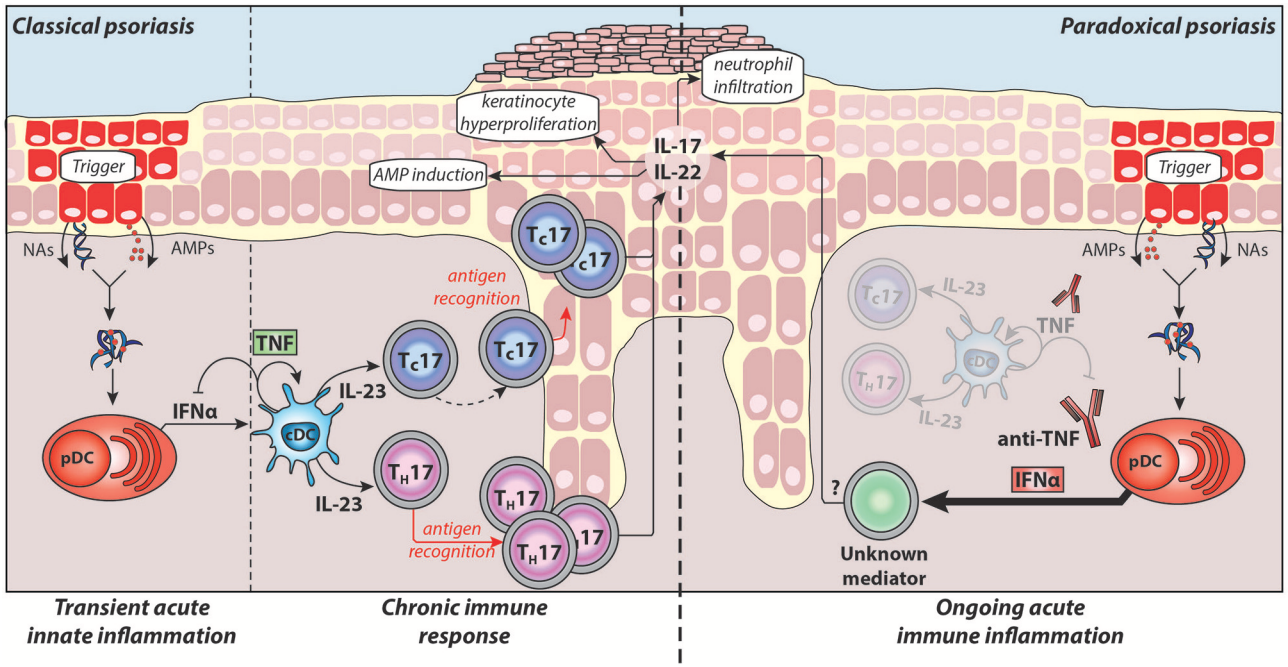

FIGURE 1 | Pathogenesis of classical plaque psoriasis and paradoxical psoriasis. Antimicrobial peptides (AMPs), which are produced by keratinocytes upon skin injury or released by neutrophils, form complexes with nucleic acids (NAs) released by dying cells. These complexes activate plasmacytoid dendritic cells (pDC) to produce large amounts IFNα during the acute/early phase of psoriasis pathogenesis. IFNα activates conventional dendritic cells (cDCs), which in turn produce TNF and IL-23. TNF induces the maturation of cDCs and pDCs, which lose their ability to produce IFNα. Thus, in classical psoriasis, early IFNα production gets relayed by TNF that controls and limits the IFNα production by pDCs via a negative feedback loop (through induction of pDC maturation). Subsequently, IL-23 and other pro-inflammatory cytokines produced by cDCs drive the activation of potentially autoreactive T-cells, which proliferate and, particularly $CD8^+$ T_C cells, migrate into the epidermis. Upon antigen recognition they produce the T_H17 cytokines IL-17 and IL-22 that induce keratinocyte hyperproliferation, attract neutrophils to the skin, and upregulate AMP production providing a positive feedback loop eventually resulting in the psoriatic phenotype (chronic/late phase). Normally, during anti-TNF therapy, the absence of TNF and consequently of downstream cytokines suppresses pathogenic T-cells thereby alleviating classical psoriasis. However, in patients developing paradoxical psoriasis, TNF blockade inhibits pDC maturation and leads to sustained IFNα production. In addition, as cDC cannot mature in absence of TNF, paradoxical psoriasis fails to elicit a T cell mediated autoimmune response. Thus, paradoxical psoriasis remains in an ongoing IFNα-driven acute immune inflammation independent of T-cells. The exact pathogenic downstream mechanism of IFNα-driven paradoxical psoriasis skin lesions remains to be fully elucidated.

Detailed knowledge about the pathogenesis of chronic plaque psoriasis and the central role for the TNF/IL-23/T_H17 pathway has led to the development of therapies targeting the pathogenic cytokines, including anti-TNFs, anti-p40 (IL-12/IL-23), anti-p19 (IL-23 specific), anti-IL-17A, and anti-IL-17 receptor antibodies. This pathway and its pathogenic mechanisms in classical plaque psoriasis are illustrated in **Figure 1**. However, less is known about instigators of psoriasis and pathogenic upstream triggers of acute cutaneous inflammation.

Type I Interferons, Setting the Tone for Autoimmunity?

Type I IFNs are key cytokines in antiviral host defence due to their ability to limit viral replication and to induce an effective antiviral immune response (84, 85). They promote maturation of myeloid DCs and priming of $CD8^+$ T-cells (86), induce T-cell proliferation (87), and sustain their survival (88). In addition, type-I IFNs stimulate differentiation of B-cells into antibody-secreting plasma cells (89). Thus, type-I IFNs are essential for the induction of an effective immune response against viruses. Although produced by all nucleated cells, they are preferentially expressed by a rare type of circulating cells called plasmacytoid dendritic cells (pDCs) (90). Upon viral recognition through endosomal Toll-like receptors (TLR) 7 and 9, pDCs produce extraordinary amounts of type-I IFN, and, therefore, have also been called professional IFN producing cells (90, 91). Under normal conditions, pDCs are not present in peripheral tissues, but they get recruited to the skin in case of infection, injury, autoimmunity, and cancer (64). Skin wounding induces rapid skin infiltration of pDCs and transient expression of type-I IFNs, which accelerate re-epithelialisation (92).

As self-nucleic acids are abundantly released into the extracellular environment during apoptotic and necrotic cell death, it is essential that pDCs avoid inappropriate activation by host-derived nucleic acid, but retain the ability to quickly respond to viral DNA and RNA. To achieve this, TLRs that sense nucleic acids are located intracellularly within endosomes, which prevents activation by extracellular self-DNA/RNA but allows immune response to viruses that actively invade the cells. Moreover, extracellular nucleases rapidly degrade nucleic acid released by dying cells without affecting DNA or RNA contained within viruses (93). Thus, under normal circumstances

TABLE 1 | Classical vs. Paradoxical psoriasis- differences, similarities, and treatment strategies.

Characteristics	Classical psoriasis	Paradoxical psoriasis
Clinico-phenotypic presentation	Well-demarcated erythematous plaques covered with silvery-white scales.	Presence of different psoriatic patterns including plaque-type, guttate, pustular forms as well as eczematiform presentation. Palmoplantar zones affected more often. Non-cicatricial alopecia regularly noted.
Histo-pathological appearance	Characteristic psoriatic histology: Epidermal hyperplasia (acanthosis), papillomatosis, hyper-/parakeratosis, dermal, and epidermal immune cell infiltrates.	Three different patterns: -classical psoriatic pattern -eczematiform pattern with spongiosis -lichenoid pattern with interface dermatitis often all these patterns are simultaneously present at variable degrees.
Recurrence	Relapsing.	Non-relapsing (upon cessation of anti-TNF).
Genetic associations	Many known (and established): *HLA-Cw6*, *IL12B*, *IL23A*, *IL23R*, and various components along type-I interferon signalling, NF-KB signalling, and other signalling pathways.	Few proposed: *IL23R* (an allele that is protective concerning classical psoriasis), and *FBXL19*, *CTLA4*, *SCL12A8*, *TAPI* which have an unclear role in paradoxical psoriasis and the outcome of the allele is undetermined.
Role of TNF	Driven by TNF.	Induced by blockade of TNF.
Role of adaptive immunity	T-cell mediated. Intraepidermal and dermal (autoimmune) T_H/T_C17-cells found throughout skin lesions.	T-cell independent. Significant reduced numbers of intraepidermal $CD8^+$ T_C-cells as compared to classical psoriasis.
Role of innate immunity	Transiently driven by pDC derived type-I IFN during the early phase of psoriasis development. Mature cDCs and neutrophils present in large numbers in skin lesions of chronic/late phase of classical psoriasis.	Driven by unabated type-I IFN produced by non-maturing pDCs. Immature dendritic cells, and neutrophils often present in lesions. Role for other cell types not known (particularly in mediating the psoriatic phenotype).
Pathogenic mechanism	Chronic (autoimmune) T_H/T_C17-mediated inflammation	Unabated, ongoing type-I IFN-driven innate inflammation, absence of T-cell autoimmunity.
Treatment avenues	-targeting TNF highly effective Various other treatment strategies validated: -targeting of IL-12/IL-23 highly effective -targeting of IL-23 highly effective -targeting IL-17A and its receptor highly effective -targeting type-I interferon is ineffective in established classical chronic plaque-type psoriasis.	-switch to different class of biologics (other than anti-TNF) often needed in severe cases of paradoxical psoriasis In the absence of detailed knowledge about the pathogenic pathways, proposition of: -use of anti-IL12/IL23 (successful in case reports) -unknown efficacy of IL-23 specific biologics -unknown efficacy of targeting IL-17A and its receptor -targeting type-I interferons and/or pDCs potentially effective

host-derived self-DNA/RNA released by apoptotic or damaged cells cannot activate TLR9 and 7. However, they can become potent triggers of pDC activation and type-I IFN production in the presence of endogenous antimicrobial peptides (AMP) such as LL37 and beta-defensins (94–96). AMPs are typically not expressed in healthy skin under steady-state conditions, but are transiently produced by keratinocytes or released by infiltrating neutrophils in response to skin wounding or infections (97–99). Their cationic and amphipathic structure allows AMPs to interact with and disrupt microbial membranes, which typically contain a high degree of negative charges (100). Besides their role as direct effector molecules against microorganisms, AMPs are also involved in the initiation of inflammation by breaking innate tolerance to otherwise inert extracellular self-DNA and self-RNA. Cationic AMPs bind to negatively charged fragments of nucleic acid to form aggregated and condensed structures that are resistant to extracellular degradation. Translocation of these complexes into endosomes and activation of TLR7 and 8 (RNA), or TLR9 (DNA) lead to sustained production of IFNα and IFNβ by pDCs (94–96).

Under physiological conditions, AMP-expression with activation of pDCs by AMP-nucleic acid complexes is transient, controlled by the damaging or infectious stimulus. By contrast, in psoriasis, the expression of AMPs is persistent and leads to sustained production of type-I IFNs by pDCs, which accumulate in the dermis of early developing psoriatic lesions (101). Subsequently, these trigger activation of myeloid DCs and autoreactive T-cells. Recent work has demonstrated that IFNα particularly drives the activation and skin infiltration of pathogenic $CD8^+$ T-cells in psoriasis (102). Moreover, IFNα conditioned DCs produce large amounts of IL-23 (103, 104) indicating an important role for type-I IFNs in

driving T_H/T_C17-mediated (auto)immunity in psoriasis. Indeed, depletion of pDCs or blocking type-I IFN signalling both inhibited psoriasis development confirming that its overexpression by pDCs reflects a critical early/acute event in the pathogenesis of psoriasis (101). This is further supported by the observations that *de novo* psoriasis or pre-existing psoriasis can be triggered and/or aggravated by IFNα therapy (105–108) and the TLR7 agonist imiquimod (109, 110). Interestingly, epidermal trauma, which induces AMP expression by keratinocytes and attracts pDCs into the skin, is also a typical trigger of psoriasis known as Koebner phenomenon.

Taken together, type-I IFNs play a critical role in the acute/early phase of psoriasis pathogenesis by (1) activating dermal myeloid DC, (2) inducing their maturation by upregulating co-stimulatory molecules and HLA molecules, and (3) participating in T_H/T_C17 polarisation of autoimmune T-cells through induction of IL-23 production by myeloid DCs (**Figure 1**).

PARADOXICAL PSORIASIS

Almost two decades of clinical experience with anti-TNFs have provided considerable advances in our understanding of the biology of TNF. More than 2 million patients have been treated with anti-TNFs so far.

Expected side effects such as increased susceptibility to infection and a slightly increased risk for malignancies have been confirmed (111–113), though the cancer risk still remains a matter of debate (114, 115). However, the observation that anti-TNFs, which are normally extremely effective in the treatment of chronic inflammatory diseases, could lead to aggravation of pre-existing autoimmune diseases and onset of new inflammatory diseases, was unexpected and a paradox. In fact, lupus-like syndrome can be observed in 0.5–1% of anti-TNF treated patients and 2–5% of patients develop psoriasis-like skin lesions, called paradoxical psoriasis (8, 116, 117). They represent important side effects in the treatment of major chronic autoimmune diseases as they potentially necessitate treatment cessation. Since the first description of paradoxical psoriasis (117, 118), numerous cases have been reported (119–121). Paradoxical psoriasis appears independently of the underlying disease or the type of anti-TNF agent used and regresses upon discontinuation of therapy, which suggests that paradoxical psoriasis does represent a side effect of TNF blockade and not *de novo* psoriasis. Though the side effect is a well-established phenomenon, its pathogenesis had remained elusive and only recently, the dysbalance of TNF and type-I IFN (yin-yang of TNF and IFNα) has been confirmed as a pathogenic mechanism underlying paradoxical psoriasis (8).

The first clues for a link between anti-TNF therapy and increased type-I IFN expression came from the observation that anti-TNF therapy induces an IFN signature in blood of juvenile arthritis patients (122). Likewise, anti-TNF treatments promote formation of anti-nuclear antibodies (123), which are associated with increased type-I IFN levels in SLE patients (124). Furthermore, anti-TNFs can induce or aggravate lupus, a well-known type-I IFN-driven autoimmune disease (125, 126). Indeed, patients with anti-TNF induced paradoxical psoriasis showed an increased IFN signature in lesional skin (127). Recently, we could confirm that TNF controls the production of type I-IFN by pDCs and that anti-TNF induces its unabated overexpression driving paradoxical psoriasis (8).

Upon activation, pDCs produce type-I IFNs first, which is relayed by their production of TNF. TNF induces maturation of pDCs, which upregulate costimulatory molecules and lose their ability to produce interferons (8, 128). Thereby, TNF limits the duration of type-I IFN production by pDCs, while conversely, TNF blockade decreases pDC maturation and extends their ability to produce type-I IFN. This supports a yin-yang model of TNF and type-I IFN. In classical plaque psoriasis, early transient overexpression of type-I IFN is replaced by a dominant TNF-driven chronic inflammation. In contrast, TNF blockade leads to an ongoing type-I IFN mediated acute inflammation in paradoxical psoriasis (**Figure 1**).

Another important distinction between the two entities is that in paradoxical psoriasis, unlike classical psoriasis, T-cells play a redundant role (8). Hence, both classical and paradoxical psoriasis are induced by pDC-derived type-I IFN. But while classical psoriasis develops into a T-cell mediated autoimmune disease, paradoxical psoriasis represents an ongoing type-I IFN-driven innate immune response that fails to elicit T cell autoimmunity. In line with this, there are no relapses of paradoxical psoriasis upon discontinuation of anti-TNF therapy, which supports lack of T-cell mediated disease memory in paradoxical psoriasis.

It remains unclear what triggers the activation of pDCs and eventually drives paradoxical psoriasis. Potentially certain environmental factors such as microbes could trigger development of paradoxical psoriasis, as a considerable number of patients have been found to have concurrent superinfections (129). Furthermore, the yin-yang of TNF and type-I IFN, the pathogenic mechanism underlying paradoxical psoriasis, is inherently true for healthy individuals as much as patients. But only 2–5% of anti-TNF treated patients develop paradoxical psoriasis indicating that there is another key determining factor such as genetic predisposition for paradoxical psoriasis. Among polymorphisms associated with psoriasis, five have been identified to also be associated to paradoxical psoriasis, and these include *IL23R*, *FBXL19*, *CTLA4*, *SLC12A8*, and *TAP1* (130) though it remains to be determined exactly how they would fit in the pathological mechanism.

Though classical and paradoxical psoriasis have similarities in their clinical presentation, many distinctions have been identified in recent years as to the pathogenic mechanism. **Table 1** summarises key similarities and differences between the two entities, and highlights potential treatment strategies.

OUTLOOK

Detailed knowledge on classical plaque psoriasis, particularly by identifying the relevant role of the TNF/IL-23/T_H17 axis in its pathogenesis, has allowed for novel, more targeted therapies.

Though newer treatments targeting IL-23 and IL-17 show better efficacy, today, anti-TNFs remain a gold-standard in psoriasis management. Yet important immunological side effects of TNF blockade, such as paradoxical psoriasis and lupus-like syndrome, may require premature discontinuation of an otherwise effective treatment option for patients. These side effects are caused by an unabated type-I IFN-driven immune response making it an intriguing target in these patients. While, anti-IFNs have not shown efficacy in chronic plaque psoriasis confirming the distinct inflammatory pathways in chronic and acute forms of classical psoriasis (131), they provided promising results in SLE. Therefore, type-I IFN blockade might be a valuable treatment option in acute forms of psoriasis such as erythrodermic or guttate psoriasis as well as in paradoxical psoriasis. However, simultaneous inhibition of the interferon pathway together with the ongoing TNF blockade might increase the infectious risk too considerably. Therefore, targeting IFN-producing pDCs (i.e., *via* anti-ILT-7, anti-BDCA2) or inhibiting TLR 7 and 9, thereby blocking pDC activation, could provide more suitable therapeutic options in patients that need continuation of their anti-TNF treatment. In this way, production of type-I IFNs by monocytes and stromal cells would remain intact and might allow sufficient immune responses toward infectious agents.

Despite considerable advances in the understanding of paradoxical psoriasis and its pathogenesis, several questions are still unanswered. Downstream mechanisms that mediate the interferon-driven psoriatic phenotype of paradoxical psoriasis remain unknown as IFNα does not directly induce keratinocyte hyperproliferation. The identification of cytokines involved and their cellular source might provide additional novel targets for therapeutic intervention. In addition, biomarkers to predict side effects such as paradoxical psoriasis and lupus-like syndrome could help optimising the management of patients with chronic inflammatory diseases.

AUTHOR CONTRIBUTIONS

AM and CC wrote and edited the manuscript and figures.

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REVIEW
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Systemic Inflammation and Cardiovascular Comorbidity in Psoriasis Patients: Causes and Consequences

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Psoriasis is a common inflammatory skin disease characterized by the appearance of red scaly plaques that can affect any part of the body. High prevalence, chronicity, disfiguration, disability, and associated comorbidity make it a challenge for clinicians of multiple specialties. Likewise, its complex pathogenesis, comprising inflammation, hyperproliferation, and angioneogenesis, intrigues numerous scientific disciplines, namely, immunology. From a clinical perspective, the severity of psoriasis is highlighted by its increased mortality, with cardiovascular diseases contributing the highest excess risk. From a scientific point of view, psoriasis has to be considered a systemic inflammatory condition, as blood biomarkers of inflammation are elevated and imaging techniques document sites of inflammation beyond the skin. While the association of psoriasis with cardiovascular diseases is now widely accepted, causes and consequences of this association are controversially discussed. This review comments on epidemiologic, genetic, and mechanistic studies that analyzed the relation between psoriasis and cardiovascular comorbidity. The hypothesis of psoriasis potentially being an independent cardiovascular risk factor, driving atherosclerosis *via* inflammation-induced endothelial dysfunction, will be discussed. Finally, consequences for the management of psoriasis with the objective to reduce the patients' excess cardiovascular risk will be pointed out.

Keywords: psoriasis, coronary heart disease, stroke, atherosclerosis, insulin resistance, endothelial dysfunction, mortality

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INTRODUCTION

Psoriasis is among the few non-communicable diseases the World Health Organization identified as a major global health problem (1). It is a common inflammatory skin disease, affecting around 2% of the population in Western countries (2). Its clinical hallmark are red scaly plaques that can affect any part of the body, but preferentially appear over elbows and knees as well as on the scalp, the umbilical, and perianal region. These lesions reflect the principal pathogenetic mechanisms underlying psoriasis, namely, inflammation, hyperproliferation, and angioneogenesis. To date, psoriasis is considered an immune-mediated disease, exhibiting an intense cross talk between components of the innate and the adaptive immune system, which then trigger the epidermal changes (3). Psoriatic inflammation goes more than skin deep, as signs of inflammation can readily be detected at sites outside the skin (4).

Comorbidity is a common feature of many immune-mediated inflammatory diseases across different medical specialties, including model diseases such as rheumatoid arthritis (5), inflammatory bowel disease (6), or multiple sclerosis (7). A wealth of data documents the association of psoriasis with numerous comorbid diseases, including cardiometabolic, gastrointestinal, and kidney diseases as well as malignancies, infections, and mood disorders (8). To this end, it is still controversial whether psoriatic arthritis should be considered an extracutaneous manifestation of psoriasis or as a separate entity, and thus comorbidity (9). Among the comorbid conditions, cardiovascular diseases are of particular importance, as they often directly impact the patients' mortality (10).

In the case of rheumatoid arthritis, currently available evidence is strong enough to allow the issue of recommendations that substantially change prevention and treatment goals for cardiovascular risk factors in these patients (11). And while the association with cardiovascular diseases also among psoriasis patients is now widely accepted, causes and consequences of this association are controversially discussed (12–14). This review comments on epidemiologic, genetic, and mechanistic studies that analyzed the relation between psoriasis and cardiovascular comorbidity. The hypothesis of psoriasis potentially being an independent cardiovascular risk factor, driving atherosclerosis *via* inflammation-induced endothelial dysfunction, will be discussed. Finally, consequences for the management of psoriasis with the objective to reduce the patients' excess cardiovascular risk will be pointed out.

EPIDEMIOLOGY

One of the first systematic analyses on disease concomitance based on more than 40,000 cases identified an association of psoriasis not only with cardiovascular disease but also with diseases that represent risk factors for atherosclerosis, such as diabetes mellitus or obesity. The authors concluded that a distinct pattern of associated diseases exists in patients with psoriasis and suggest a genetically determined selection (15). Since then, multiple epidemiologic studies have addressed the issue of cardiovascular comorbidity in psoriasis patients. And while some groups concluded that such a link does not exist (16–18), many others were able to reproduce this association recently summarized elsewhere (8, 19) (**Table 1**). Based on the currently available evidence, most experts agree that the association of psoriasis with cardiovascular comorbidity is real (20). This is also reflected by and justifies the inclusion of advice on the management of psoriasis patients with such comorbidities in guidelines and treatment recommendations (21, 22).

The fact that cardiovascular diseases are associated with psoriasis leads to the question on why that might be so. A strong argument in favor of an indirect link comes from the observation that psoriasis is associated with numerous conditions representing major cardiovascular risk factors in their own right (**Table 2**). In fact, the very first publication on psoriasis comorbidity reported an association with diabetes mellitus as early as 1897 (31). Also, the study by Henseler and Christophers cited above documents diabetes mellitus and obesity as concomitant conditions (15). Finally, Takeshita et al. recently summarized the evidence for obesity, hypertension, diabetes mellitus, dyslipidemia, and the metabolic syndrome as comorbid diseases in psoriasis (8), with the metabolic syndrome essentially representing the combined appearance of obesity, hypertension, insulin resistance, and dyslipidemia (32). These conditions are not only well-established major cardiovascular risk factors and therefore incorporated in the Framingham's Cardiovascular Disease Risk Scores used to predict cardiovascular risk in the general population (33), some of them, namely, smoking (34) and obesity (35), are also risk factors for the development of psoriasis.

TABLE 1 | Summary of studies analyzing the association between psoriasis and major adverse cardiovascular events.

Reference	Study characteristics	Key finding
Dowlatshahi et al. (16)	Population-based study (262 patients with mostly mild psoriasis, and 8,009 controls)	No increased risk for cardiovascular events
Parisi et al. (17)	Cohort study (48,000 patients and 200,000 controls)	No association of psoriasis with cardiovascular disease
Egeberg et al. (18)	Nationwide cohort study (adult population of Denmark)	Slight increase of myocardial infarction in patients with severe psoriasis
Armstrong et al. (23)	Systematic review and meta-analysis (220,000 patients and 10 mio controls)	Increased risk for myocardial infarction, stroke, and cardiovascular mortality among psoriasis patients
Gaeta et al. (24)	Meta-analysis (1.8 mio patients and 43 mio controls)	Increased risk for myocardial infarction and cardiovascular mortality among psoriasis patients
Gu et al. (25)	Meta-analysis of cohort studies (6.2 mio individuals overall)	Increased risk for myocardial infarction, stroke, and cardiovascular mortality among psoriasis patients
Horreau et al. (26)	Systematic literature review (324,000 patients and 5.3 mio controls)	Increased risk for myocardial infarction and stroke among psoriasis patients
Miller et al. (27)	Meta-analysis (500,000 patients and 29 mio controls)	Increased risk for cardiovascular disease among psoriasis patients
Pietrzak et al. (28)	Review (360,000 patients and 9.2 mio controls)	Increased risk for cardiovascular events among psoriasis patients
Samarasekera et al. (29)	Systematic review and meta-analysis (480,000 patients and 10 mio controls)	Increased risk for myocardial infarction, stroke, and cardiovascular mortality among psoriasis patients
Xu et al. (30)	Meta-analysis of cohort studies (326,000 patients and 5.2 mio controls)	Increased risk for myocardial infarction and stroke among psoriasis patients

TABLE 2 | Synopsis of arguments in favor or against the hypothesis of psoriasis representing an independent cardiovascular risk factor.

Domain	Psoriasis may be an independent cardiovascular risk factor	Psoriasis may *not* be an independent cardiovascular risk factor
Epidemiology	Dose effect: Population-based studies document a higher cardiovascular risk among patients with severe compared to those with mild psoriasis. The risk is also higher in patients with longer disease duration. In case–control studies, surrogate markers for increased cardiovascular risk are associated with psoriasis after thorough control for confounding factors.	Conventional cardiovascular risk factors such as several or even all components of the metabolic syndrome are associated with psoriasis throughout all age groups
Genetics	A comprehensive assessment of the catalog of genome-wide association studies shows that the genetic control of psoriasis is almost completely independent from both the metabolic syndrome and coronary heart disease	There may be some shared susceptibility loci between psoriasis and its comorbidities A missense mutation in the insulin-responsive peptidase links psoriasis to hypertension and diabetes mellitus
Pathophysiology	Remarkable similarities exist between the inflammatory processes in psoriatic and atherosclerotic plaques: • insulin resistance • endothelial dysfunction • T-lymphocyte driven • neutrophils involved • monocytes/macrophages involved • platelets involved	The exact role of several potentially shared components has yet to be established: • macrophages • IL-17A

Evidence in favor of psoriasis as an independent cardiovascular risk factor comes from studies showing a "dose effect" of psoriasis on the patients' cardiovascular risk (**Table 2**). A landmark study in this regard was conducted by Gelfand et al. who used the General Practice Research Database from 1987 to 2002, comprising prospective data collected from general practitioners in Great Britain. After adjusting for major cardiovascular risk factors, such as hypertension, diabetes mellitus, and hyperlipidemia, they found a slightly elevated adjusted relative risk for myocardial infarction among patients with mild psoriasis, and a substantially elevated adjusted relative risk among patients with severe psoriasis (36). Two meta-analyses also came to the conclusion that the cardiovascular risk of psoriasis patients correlates with the severity of their disease (29, 23). In line with this hypothesis, longer duration of psoriasis has also been associated with increased cardiovascular risk for the patients (37, 38). Another landmark study was performed by Ludwig et al. who quantified coronary artery calcification *via* CT scans, using the well-established Agatston score, among hospitalized psoriasis patients, thus suffering from severe psoriasis (39). Their scores were compared to controls matched for all major cardiovascular risk factors. The study showed highly significantly elevated coronary artery calcification, so that the study had to be stopped before the originally calculated number of patients had been included. This observation is even more alarming, as only patients with a negative history for current or previous heart problems were included.

The clinical relevance of psoriasis as an independent cardiovascular risk factor was quantified by Mehta et al. in a cohort study of severe psoriasis patients. There, the attributable risk of severe psoriasis on major cardiovascular events, i.e., namely, myocardial infarction and stroke, over a 10-year period was found to be around 6% (40). Others found the increased risk of such events associated with psoriasis to be comparable with that conferred by diabetes mellitus alone (41) or by rheumatoid arthritis (42). This is remarkable as the former is a well-accepted major cardiovascular risk factor, and the association of the latter with an increased cardiovascular risk already led to specific recommendations on how to address this extra risk, as pointed out above.

Taken together, despite few studies that did not show statistically significant associations between psoriasis and major cardiovascular events, a majority of studies using different methodical approaches suggests not only an association of psoriasis with cardiovascular diseases but also provides evidence for psoriasis as an independent cardiovascular risk factor (**Table 2**).

Noteworthy, chronic skin inflammation as such is not sufficient to explain the role of psoriasis as an independent cardiovascular risk factor, as a recent systematic review and meta-analysis failed to provide evidence for atopic dermatitis, another common chronic inflammatory skin disease, exhibiting comparable effects (43). A major epidemiologic study documented even the inverse phenomenon, namely, a rather significantly decreased risk for myocardial infarction, stroke, or cardiovascular death (44). Therefore, genetic and pathogenetic analyses are needed to understand this association.

GENETICS

One possibility to explain the association of psoriasis with its comorbid conditions in general and cardiovascular disease in particular is that these entities share common genetics. A genetic predisposition of psoriasis can already be postulated based on the facts that many patients have a positive family history and that the concordance rate among monozygotic twins is much higher compared to dizygotic twins (45). The first psoriasis susceptibility locus (PSORS-1) identified is located on the short arm of chromosome 6 in the region coding for major histocompatibility complex (MHC) molecules. It is still the most reproducible among all PSORS loci identified and may explain up to 50% of the heritability of psoriasis (46). Namely genome-wide association

studies (GWASs) shed more light on the genetics of psoriasis and allowed to identify at least 50 regions on the human genome which harbor at least 1 and sometimes more than 1 potential candidate gene associated with psoriasis. This database allows to group the respective genes according to pathways, with antigen presentation, interleukin-23 (IL-23) signaling, T-lymphocyte development and polarization, innate immunity, and negative regulators of immune responses as the key axes (47).

Following up on the notion that chronic skin inflammation as such does not suffice to explain increased cardiovascular risk, a GWAS showed some overlap between susceptibility loci for psoriasis and atopic dermatitis. This association was mediated by a combination of shared and opposing alleles, with the most significant effects operating in opposing directions, thus arguing in favor of distinct pathogenetic pathways of these diseases (48). This notion is supported by a much smaller study from Japan that observed only marginal associations between atopic dermatitis and susceptibility single-nucleotide polymorphisms for psoriasis (49). By contrast, rheumatoid arthritis as another chronic inflammatory disease with known cardiovascular comorbidity does exhibit a genetic architecture not too different from psoriasis: genetic variants in the MHC region account for more than 60% of the known genetic heritability of rheumatoid arthritis. Other associated genes are involved in the function of T-lymphocytes and monocytes representing the adaptive and the innate immune system, respectively. 35 rheumatoid arthritis risk loci gene products can be mapped to signaling pathways in T-lymphocytes and antigen-presenting cells (50). These include components of the tumor necrosis factor alpha (TNF-α) signaling cascade, the potential relevance of this finding will be discussed below.

Genetic variation and pathways in atherosclerosis do not show obvious overlap with the genetic signature of psoriasis or rheumatoid arthritis. Kessler et al. recently assigned 30 of 65 loci associated with coronary artery disease to 6 pathways with known pathophysiological roles in this disease, the function of the other 35 candidate genes is not sufficiently known to allow allocation to a particular pathway. These six pathways comprise inflammation, triglycerides, LDL cholesterol, blood pressure, vascular remodeling, and nitric oxide signaling (51).

Several groups have further analyzed the hypothesis of psoriasis and its comorbidities being genetically linked, following up on the observation that GWASs have shown overlap in the genetic susceptibility to different pathologies, namely, immune-mediated diseases (52). Ellinghaus et al. identified seven shared susceptibility loci for psoriasis and Crohn's disease; they went on to demonstrate a genetic overlap between five seronegative inflammatory diseases, namely psoriasis, Crohn's disease, ankylosing spondylitis, primary sclerosing cholangitis, and ulcerative colitis (53, 54). An observation pointing toward a genetic link between psoriasis and cardiovascular comorbidity was made by Cheng et al. who interpreted a missense mutation in the insulin-responsive aminopeptidase LNPEP as a potential link, namely between psoriasis, hypertension, and diabetes mellitus (55) (**Table 2**). The importance of insulin resistance in this context will be discussed in detail below. On the other hand, a comprehensive assessment of the catalog of GWASs by Gupta et al. shows that the genetic control of psoriasis is almost completely independent from both the metabolic syndrome and coronary heart disease (**Table 2**). To prove reliability of this approach, the authors were able to identified 10 common loci for the metabolic syndrome and coronary heart disease, using exactly the same data set (56).

Taken together, although some genetic overlap between psoriasis and several of its comorbid conditions exists, the observed association of psoriasis with cardiovascular comorbidity cannot satisfyingly be explained by shared genetics (**Table 2**).

PATHOGENESIS

Common Pathways of Psoriatic and Atherosclerotic Plaque Formation

To this end, epidemiological evidence in favor of psoriasis being associated with cardiovascular comorbidity and potentially functioning as an independent cardiovascular risk factor has been summarized. As genetic overlap cannot satisfyingly explain the excess cardiovascular risk of patients with severe psoriasis, mechanistic studies are needed to further clarify the link.

Often, animal models are a good starting point to unravel pathogenetic mechanisms. With regard to psoriasis, older models often highlighted the role of the adaptive immune system (57), while novel approaches such as mannan-induced psoriasis shed more light on the crucial role of the innate immune system (58). Few studies have been performed to analyze the link between psoriasis and its cardiovascular comorbidity. One such approach takes advantage of the capacity of the TLR7 agonist imiquimod to induce a psoriasis-like inflammation in murine skin. Jin et al. succeeded in inducing a state of systemic inflammation through topical application of imiquimod onto interleukin-10-deficient mice, but noticed a decrease of body weight in these mice, while psoriasis patients show a trend toward obesity (59). Shibata et al. studied imiquimod-induced psoriasiform inflammation in mice deficient of adiponectin, an anti-inflammatory adipokine, and found a more severe phenotype in these knockout mice. Furthermore, intraperitoneal injections of adiponectin had a therapeutic effect in adiponectin knockout mice (60). While this study explored an indirect link between psoriasis and cardiovascular disease *via* metabolic factors, the only direct link the author is aware of comes from a study in the type-II collagen-specific antibody-induced psoriatic arthritis model. Using this model, Sherlock et al. demonstrated that IL-23 drives inflammation in the aortic root through activation of $CD3^+CD4^-CD8^-$ T-lymphocytes (61).

Atherosclerosis as a key process in cardiovascular diseases has long been recognized as an inflammation-driven phenomenon (62, 63). This is also true for psoriasis (64, 65). The cardiologist Späh was among the first who discussed a potentially common inflammatory pathway and the idea of an integrated treatment approach (66). He stressed altered endothelial function and subsequent recruitment of leukocytes, primarily T-lymphocytes, to developing lesions as a shared early step in the process of plaque formation in atherosclerosis and psoriasis. Lymphocyte extravasation has indeed been studied in detail with the intention

to develop targeted therapies for psoriasis, but to date, none of the potential candidates was found to be sufficiently effective to validate development into a marketed drug (67, 68). Meanwhile, many more shared mechanisms of atherosclerosis and psoriasis have been studied in detail.

Elaborating on the observations described above, a role namely for T-helper-1 lymphocytes (TH1 lymphocytes) has been established in atherosclerosis as well as psoriasis (**Table 2**). Although TH1 as well as TH2 lymphocyte responses can contribute to atherosclerosis, several lines of evidence suggest a predominant role of TH1 lymphocytes. These include the TH1 phenotype and function of most T-cell clones derived from atherosclerotic plaques as well as immunohistochemical studies on such plaques (69–71). Subsequently, high circulating levels of TH17 lymphocytes and IL-17 in patients with acute coronary syndrome, a positive correlation of IL-17 with levels of high-sensitivity C-reactive protein and IL-6 (predicting an increased risk of myocardial infarction) in those patients, and the observation that IL-17 inhibition in mice significantly reduces the size of atherosclerotic plaques, were all interpreted as indicators for a role of TH17 lymphocytes in atherosclerosis (72–74). Similarly, psoriasis was initially thought to be a prototypical TH1 lymphocyte-mediated disease, with these cells activating macrophages, neutrophils and $CD8^+$ cytotoxic lymphocytes (75). Then, the role of TH17 lymphocytes was stressed in the light of clinical studies documenting the high clinical efficacy of therapies targeting the IL-17 pathway (76).

While the role of the adaptive immune system in the pathogenesis of psoriasis has been thoroughly investigated ever since the accidental observation of the therapeutic efficacy of cyclosporine A in 1979 (77), interest in the important role of the innate immune system has only recently experienced a renaissance (**Table 2**). Evidence for the contribution of neutrophils, which are predominant in pustular forms, but readily detectable also in chronic plaque type psoriasis, where they form the so-called Munro's abscesses within the epidermis, comes from *in vivo* studies as well as organotypic 3D models and has recently been reviewed elsewhere (78). Further supporting the role of neutrophils is the clinical observation by Reich et al., who reported that psoriasis treatment with the anti-IL17A antibody secukinumab resulted in the near total elimination of intraepidermal IL-17-positive neutrophils as an early therapeutic effect (79). Neutrophils are equally important in atherosclerosis, as they interact with damaged endothelium, augment leukocyte recruitment *via* secretion of chemotactic mediators, and promote the development of foam cells, a macrophage subset driving atherosclerosis (80). Neutrophil localization to developing atherosclerotic plaques has been demonstrated in mouse models (81, 82) and human atherosclerotic lesions (83); their presence in occlusive thrombi and culprit lesions of acute coronary syndrome patients suggests a role in atherosclerotic progression (84).

As for neutrophils, involvement of monocytes and macrophages has readily been demonstrated in both diseases (**Table 2**). These are regularly detectable in psoriatic lesions (85). Using a mouse model where the psoriatic phenotype is induced by topical application of the immunomodulator imiquimod, Costa et al. demonstrated that induction of the phenotype depends exclusively on hematopoietic cells. Using conditional knockout mouse strains, the active contribution of monocytes and macrophages on disease propagation and exacerbation was shown (86). With regard to atherosclerosis, Tabas and Lichtman recently reviewed how macrophages can be programmed for functions on a spectrum from inflammatory and host defense to resolution and repair in atherosclerotic plaques (87). In general, inflammatory macrophages carry out processes that promote atherosclerosis progression, including plaque necrosis and thinning of a protective fibrous cap. By contrast, resolving macrophages carry out functions that can suppress plaque progression and promote plaque regression, including clearing dead cells and secreting collagen that can form a protective scar over the lesion (88).

Besides these vigorously studied cells of the adaptive immune system, platelets seem to also actively contribute to atherosclerosis and psoriasis (**Table 2**). Platelets are widely known to have prominent functions in hemostasis and thrombosis, but their involvement in immune and inflammatory processes is now more and more recognized (89). Their potential to influence such process is not surprising, given their capacity to release a plethora of mediators and to interact with numerous cells and tissues through a variety of adhesion molecules. In psoriasis, platelet activation can be used to monitor disease activity through quantification of platelet activation markers in the patients' blood (90). Mechanistically, it is thought that activated platelets facilitate leukocyte extravasation (91). Similar effects link platelets to atherosclerosis (92, 93). The clinically relevant role of platelets in this context is underlined by the success of platelet inhibitory drugs in treating and preventing acute arterial thromboembolic events (94).

In recent years, a factor attracting particular attention in the context of psoriatic as well as atherosclerotic inflammation is IL-17A. Its role as a major driver of psoriatic inflammation is now well accepted and underlined by the numerous lines of evidence, including the development of a psoriatic phenotype in mice overexpressing IL-17A in the epidermis (95), or the high efficacy of IL-17A-blocking biologics in the treatment of psoriasis (96). By contrast, deciphering the exact role of IL-17A in atherosclerosis remains a challenging task (**Table 2**). Numerous studies documented pro-atherogenic effects. For example, in a hypercholesterolemic animal model, IL-17A inhibition reduced atheroma area and stenosis. At the molecular level, expression of the chemokine CCL5 (CC chemokine ligand 5), the cytokines IL-6 and TNF-α, several adhesion molecules including vascular cell adhesion molecule 1, and the pro-thrombotic molecule TF was reduced (74). Complementary experiments were conducted in murine models, where addition of exogenous IL-17A-stimulated pathological changes associated with increased plaque instability, while IL-17A inhibition resulted in regression of atherosclerosis (97–99). *Ex vivo* studies on human plaque fragments showed that exposure to IL-17A induced pro-inflammatory, pro-thrombotic, plaque destabilizing, and cell-attracting effects (100). On the other hand, there is also some evidence suggesting that IL-17A may have anti-atherosclerotic effects, as another mouse model characterized by increased IL-17A expression showed significantly smaller atherosclerotic lesions. In the same publication,

an association between IL-17A expression and plaque stability in human carotid artery plaques was reported (101). Moreover, a study from Simon et al. on almost 1,000 patients with acute myocardial infarction demonstrated that low serum levels of IL-17A are associated with a higher risk for major cardiovascular events (102). Overall, many experts lean toward the concept of IL-17A being primarily pro-atherogenic, although some uncertainty persists.

Psoriatic Inflammation as a Driver for Atherosclerosis

Thus, over the last decade, multiple shared pathogenetic mechanisms have been identified in psoriatic and atherosclerotic plaque formation. These similarities do not, however, explain why psoriasis might actually represent an independent cardiovascular risk factor, as suggested by the majority of epidemiologic studies. Of major importance in this regard was the notion that psoriasis cannot be regarded as isolated cutaneous inflammation, but rather represents a chronic systemic inflammatory disease. To this end, several groups have identified biomarkers of inflammation in the blood of psoriasis patients which correlate with psoriasis severity, such as C-reactive protein (103), erythrocyte sedimentation rate (104), and the platelet activation marker P-selectin (90). Documentation of vascular inflammation through (18)F-fluorodeoxyglucose positron emission tomography computed tomography (PET-CT) in psoriasis patients, pioneered by Mehta and co-workers, points into the same direction (4). More recently, the group demonstrated that psoriasis severity associates with aortic vascular inflammation detected by that method (93), suggesting that psoriatic inflammation affects blood vessels and induces inflammation in the vessel walls. Studies in mouse models confirm that chronic skin-specific inflammation can indeed induce vascular inflammation (94).

The pathogenetic link between psoriasis and cardiovascular comorbidity is likely provided through insulin resistance and endothelial dysfunction, as these are known drivers for atherosclerosis (105). Insulin resistance is typically defined as decreased sensitivity to metabolic actions of insulin that promote glucose disposal. This is not only an important feature of diabetes mellitus but also a prominent component of cardiovascular disorders, which are characterized by endothelial dysfunction (106). Conversely, endothelial dysfunction is also present in diabetes mellitus (107). In addition to its essential metabolic actions, insulin has important vascular actions that involve stimulation of the production of nitric oxide from endothelium, leading to vasodilation (**Figure 1A**). This effect has metabolic consequences, too, as increased blood flow ultimately leads to augmented glucose disposal in skeletal muscle (108). On the other hand, insulin signaling in endothelial cells regulates secretion of the vasoconstricting factor endothelin-1. Inflammation shifts this equilibrium as it induces insulin resistance *via* cytokines, which alter insulin signaling in endothelial cells, ultimately reducing the production of vasodilating nitric oxide and inducing endothelial dysfunction (109) (**Figure 1B**).

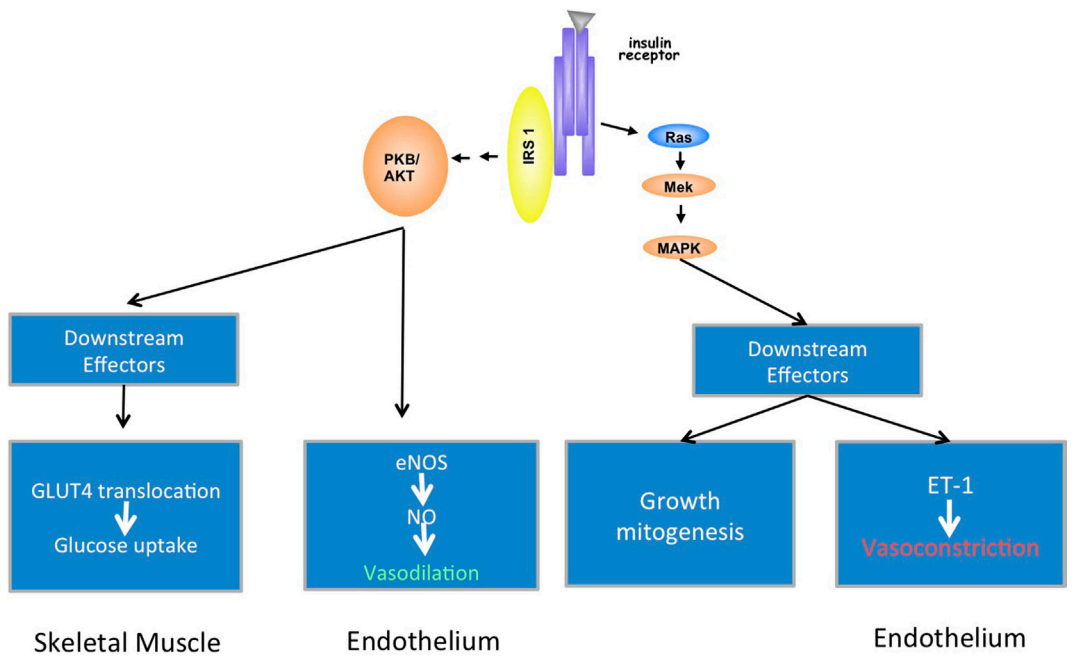

FIGURE 1 | Metabolic and vascular effects of insulin. Under physiologic conditions, the activated insulin receptor on endothelial cells leads to phosphorylation of endothelial nitric oxide synthase (eNOS) *via* activation of insulin receptor substrate (IRS) and phosphoinositide-3-kinase (a). This results in vasodilation *via* NO production. This branch also regulates GLUT4 translocation and glucose uptake in muscle cells. The mitogen-activated protein kinase (MAPK) pathway controls secretion of vasoconstrictive ET-1 in endothelial cells, and cell growth and mitogenesis in cells at large. Inflammation reduces NO production *via* blocking insulin receptor signaling at the level of the IRS-1 (b).

Noteworthy, TNF-α, a central cytokine in the pathogenesis of many chronic inflammatory diseases including psoriasis, is a major insulin antagonist (110).

The concept of the "psoriatic march" provides a framework to explain how psoriatic inflammation drives cardiovascular comorbidity *via* atherosclerosis independently from the presence of additional cardiovascular risk factors (111, 112) (**Figure 2**). According to this concept, psoriasis is a chronic systemic inflammatory disorder, as evidenced by elevated biomarkers of systemic inflammation. Noteworthy, not only classical markers for systemic inflammation have been shown to be elevated but also resistin and leptin (113, 114). These belong to a family of mediators secreted by adipocytes called adipokines. Resistin and leptin are insulin antagonizing adipokines. Collectively, the adipokine milieu in the blood of psoriasis patients is strikingly similar to that of prediabetic individuals and is a hint toward a state of insulin resistance. The diagnosis of insulin resistance is based on the so-called homeostasis model assessment of insulin resistance (HOMA-IR) index, calculated on the basis of a blood test, or an oral glucose tolerance test (115, 116). Using these approaches, two cross-sectional studies demonstrated that psoriasis patients exhibit insulin resistance at clinical levels (113, 117). Besides the insulin-antagonizing effects, numerous adipokines in general and leptin in particular may drive atherosclerosis through immunomodulation, including the upregulation of adhesion molecules on endothelial cells (118). At the level of endothelial cells, insulin resistance is thought to induce endothelial dysfunction *via* the pathway described above, resulting in vascular stiffness at the functional level. Indeed, several groups found evidence for endothelial dysfunction, using ultrasound methods. In particular, flow-mediated vascular dilation was impaired (119–122). In one of these studies, insulin resistance was assessed as well *via* the HOMA-IR and found to be significantly higher when compared to non-psoriatic controls, again stressing the link between insulin resistance and endothelial dysfunction in psoriasis (122). This cascade drives atherosclerosis, which ultimately causes cardiovascular diseases such as myocardial infarction and stroke.

Although not in the focus of this review, it is interesting to note that inflammation-induced insulin resistance may well help to explain the altered epidermal homeostasis observed in the epidermis of psoriatic plaques (123, 124), a phenomenon that might have implications beyond psoriasis (125).

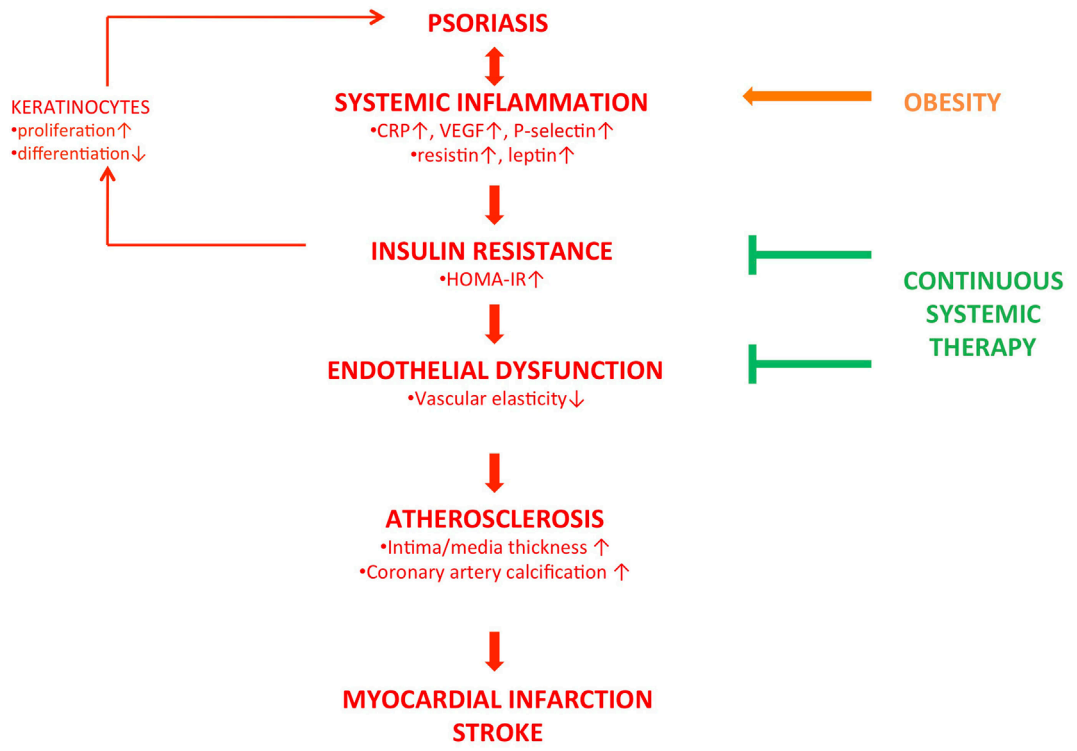

FIGURE 2 | The concept of the "psoriatic march." This hypothesis suggests that psoriasis is a systemic inflammatory condition, as numerous biomarkers of inflammation are elevated in the patients' blood compartment. Functional consequences are insulin resistance, evidenced by an increased HOMA-IR (homeostasis assessment of insulin resistance), and endothelial dysfunction, resulting in increased vascular stiffness. This provides the basis for atherosclerosis, observable through analysis of vessel wall composition *via* CTs or ultrasound. Depending on the sites of atherosclerosis, major cardiovascular events such as myocardial infarction and stroke result from this. This "backbone" (red, bold) may be developed further by adding additional "modules": insulin resistance has been shown to alter epidermal homeostasis (red, fine). Obesity, causing a state of systemic inflammation as well, is a known risk factor for psoriasis and may induce the phenotype (orange, bold). Whether systemic anti-inflammatory therapy is capable of reducing the patients' cardiovascular risk through reducing insulin resistance and endothelial dysfunction is still a matter of debate (green).

CONSEQUENCES FOR THE MANAGEMENT OF PSORIASIS

Monitoring

Most observations discussed so far support the notion that cardiovascular comorbidity is a clinically relevant problem for patients suffering from severe psoriasis. In spite of this, several studies document that these patients are not adequately monitored and treated for cardiovascular risk factors. A survey conducted between October 2010 and April 2011 among primary care physicians and cardiologists showed that most of the responding physicians (251 of 1,200 questionnaires sent out were returned) did not routinely screen psoriasis patients for cardiovascular risk factors (126). A cross-sectional study based on National Ambulatory Medical Care Survey data from 2005 to 2009 demonstrated that less than half of the psoriasis patients were screened for at least one cardiovascular risk factor (127). Finally, in a cross-sectional study of patients with hypertension in Great Britain, psoriasis patients were more likely to have uncontrolled hypertension compared with non-psoriatic individuals (128). This is in contrast to recommendations from numerous organizations and societies, who unanimously suggest such a screening (129–131). Takeshita et al. recently summarized what most experts currently agree upon, much of it being identical to what is comprised in current recommendations for the general adult population (132, 133): traditional cardiovascular risk factors should be evaluated. These include total and high-density lipoprotein cholesterol, systolic blood pressure, use of antihypertensive therapy, diabetes, and current smoking. In addition, lifestyle interventions, such as weight loss and smoking cessation, should be encouraged among patients who are obese and who are current smokers, respectively. A controlled clinical trial published a decade ago already showed the potential advantages of a more comprehensive approach to psoriasis treatment, as obese patients with moderate-to-severe psoriasis had a better response to low-dose cyclosporine A if a calorie-reduced diet was included in their treatment regimen (134).

Prevention

The state of chronic systemic inflammation in psoriasis may at least contribute to atherosclerosis *via* insulin resistance and endothelial dysfunction and therefore be partially responsible for the increased cardiovascular risk of patients with severe psoriasis. This raises the question whether continuous systemic anti-inflammatory treatments may help to reduce this excess risk. Recently, data from the CANTOS trial suggest that this is principally feasible. That trial evaluated the efficacy of the interleukin-1β-blocking antibody canakinumab in more than 10,000 patients with previous myocardial infarction and a high-sensitivity C-reactive protein level of 2 mg or more per liter on nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death. At a median follow-up of 3.7 years, the incidence rate was 4.50 events per 100 patient-years in the placebo-group, and between 3.86 and 4.11 events per 100 patient-years in the different canakinumab dosing groups. Thus, the trial documented a small, but statistically significant reduction of recurrent cardiovascular events in a high-risk population (135).

An early hint that continuous systemic anti-inflammatory treatment might reduce the cardiovascular risk of psoriasis came from a retrospective study by Prodanovich et al. who analyzed the files of more than 7,000 American veterans who had been treated over extended periods of time with methotrexate for their psoriasis. They found a significantly reduced incidence of cardiovascular diseases in those patients (136). Since then, several observational studies analyzing the effects of methotrexate or TNF-α inhibitors came to similar conclusions (137, 138), while others failed to document such protective effects (139, 140). Complementary to these studies, small controlled trials were performed, evaluating changes of biomarkers for cardiovascular risk under systemic anti-psoriatic treatment. Indeed, several groups reported amelioration of such markers under successful therapy. These include cytokines, adipokines, endothelial dysfunction, and carotid intima-media thickness (141–145).

Based on some of these observations, more ambitious projects were launched, sponsored by pharmaceutical companies. A pilot study on 30 psoriasis patients looked at vascular inflammation in the ascending aorta and carotid arteries by means of PET-CTs. In this study, decreases in vascular inflammation were observed in patients treated with adalimumab compared with placebo when data for the ascending aorta and carotid arteries were analyzed separately at 15 weeks (146). A larger study including 107 patients showed no difference over 16 weeks in the adalimumab-treated group compared to placebo and a modest increase in vascular inflammation in the carotid arteries after 52 weeks of treatment with adalimumab (147). A commentary on that publication suggested that the study might have been too small or of insufficient duration to show an effect, and that it was the carotid arteries and ascending aorta that were studied, not the coronary arteries, which might explain the negative result (20). Another study using the IL-17A-blocking antibody secukinumab was performed in a multi-center setting in Germany. A recently presented abstract on the occasion of the congress of the European Academy for Dermatology and Venereology in Geneva in 2017 documented a trend toward improvement in vascular elasticity, but no date from that trial have so far been published in a peer-reviewed manner.

Taken together, there is some evidence in favor of the idea to reduce the excess cardiovascular risk of psoriasis patients through systemic anti-inflammatory therapy. TNF-α and IL-17A are interesting targets in this regard, as the former is an important insulin antagonist, and the latter seems to exhibit primarily pro-atherogenic effects, although some anti-atherogenic effects have also been reported (148). The CANTOS study can serve as proof of concept for the principal capacity of anti-inflammatory therapy to reduce the cardiovascular risk, but it also points out that even in a high-risk population the effect size might be relatively small. This may explain why much smaller and shorter studies can easily fail to show efficacy in this regard, even more so, if relatively health patients are enrolled, as has been the case in the German trial using secukinumab. Although this approach remains intellectually appealing, it may be more effective and efficient in a

real-world scenario to address other major cardiovascular risk factors associated with psoriasis in order to reduce the patients' excess cardiovascular risk, e.g., through appropriate treatment of the metabolic syndrome or components thereof, or through lifestyle interventions (smoking!).

CONCLUSION

Psoriasis is currently regarded a chronic-recurrent, systemic inflammatory disease, driven by an intense cross talk between cells of the innate and adaptive immune system. It is associated with substantial cardiovascular comorbidity, which can only partially be explained through shared a genetic control. Evidence in favor of psoriasis as an independent cardiovascular risk factor comes from epidemiologic studies showing a "dose effect" of psoriasis on the patients' cardiovascular risk as well as from case–control studies looking at biomarkers for cardiovascular risk and specific manifestations of atherosclerosis. Inflammation-induced insulin resistance and endothelial dysfunction provide a pathogenetic link between psoriasis and atherosclerosis. Whether systemic anti-inflammatory therapy can reduce the patients' excess cardiovascular risk remains one of the hot research topics in the field. Independent of this discussion, a comprehensive approach to the management of psoriasis at least in patients with severe disease is mandatory based on current knowledge. This must include regular screening and monitoring of traditional cardiovascular risk factors as well as their guideline-oriented treatment.

AUTHOR CONTRIBUTIONS

W-HB is the sole author of this manuscript. He defined the content, performed the literature search, wrote the text, and created figures and the table.

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Potential Immunological Links Between Psoriasis and Cardiovascular Disease

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Preclinical and clinical research provide strong evidence that chronic, systemic inflammation plays a key role in development and progression of atherosclerosis. Indeed, chronic inflammatory diseases, such as psoriasis, are associated with accelerated atherosclerosis and increased risk of cardiovascular events. Contemporary research has demonstrated plausible mechanistic links between immune cell dysfunction and cardiometabolic disease in psoriasis. In this review, we describe the role of potential common immunological mechanisms underlying both psoriasis and atherogenesis. We primarily discuss innate and adaptive immune cell subsets and their contributions to psoriatic disease and cardiovascular morbidity. Emerging efforts should focus on understanding the interplay among immune cells, adipose tissue, and various biomarkers of immune dysfunction to provide direction for future targeted therapy.

Keywords: psoriasis, cardiovascular disease, inflammation, atherosclerosis, vascular inflammation, inflammatory cytokines

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INTRODUCTION

Inflammation is the hallmark of atherosclerosis (1). Preclinical and clinical research provide strong evidence that chronic inflammation is critical to the process of atherogenesis. Chronic inflammatory diseases, such as psoriasis, are associated with accelerated atherosclerosis and increased risk of cardiovascular events (2–6). Atherosclerosis is increasingly recognized as an inflammatory process, thus similarities between atherosclerosis and chronic, systemic inflammatory diseases have become an emerging focus of interest. Almost 20% patients with coronary heart disease lack conventional risk factors (7), supporting the importance of evaluating residual inflammatory risk (8). Chronic inflammatory diseases such as psoriasis have been shown to add 6% additional risk (9, 10) to the Framingham Risk Score (8, 9) highlighting the need to understand the role of immunological processes in cardiovascular disease (CVD) for better risk stratification and treatment strategies.

CHRONIC INFLAMMATION AND CARDIOVASCULAR CO-MORBIDITIES

Patients with chronic inflammatory diseases are predisposed to cardiometabolic diseases including obesity, hypertension, and dyslipidemia (11–16)—chronic inflammatory conditions common in the general population (17–19). Obesity, particularly visceral, is strongly associated with dysregulated expression of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1

beta (IL-1β), and IL-6, as well as adiponectin and leptin, contributing to metabolic derangement and insulin resistance (13, 18, 20). Atherogenic metabolic dyslipidemia is common in chronic inflammation. Abnormalities include impaired reverse cholesterol transport ability of the HDL, increased LDL particle number, and decreased LDL size (21–23). Animal and human models have demonstrated innate immunity as well as experimental *in vivo* induction of inflammation *via* bolus of an inflammatory cytokine such as TNF-α or IL-6, results in release of adipokines and generation of peripheral insulin resistance (24–27). Moreover, anti-inflammatory therapies such as aspirin, colchicine, and more recently canakinumab have been effective in CVD treatment, supporting the critical role of inflammation in CVD (28–32).

One of the most common co-morbid conditions associated with psoriasis is psoriatic arthritis (PsA). Epidemiological data indicate that almost one-third patients with psoriasis also have prevalent PsA (33). Similar to psoriasis, PsA is associated with increased prevalence of traditional cardiovascular risk factors, greater subclinical CVD assessed as vascular inflammation (VI) by 18-FDG PET/CT and ultrasound-guided carotid plaque assessment and intima-media thickness measurement, and elevated rates of major adverse cardiovascular events (MACEs) (34–38). Furthermore, like psoriasis, traditional risk factors do not fully capture the risk of CVD in PsA (39, 40).

Recently, there is growing focus on shared immunological links between atherosclerosis and several other autoimmune diseases such as systemic lupus erythematosus, inflammatory bowel disease, human immunodeficiency virus infection, rheumatoid arthritis, and psoriasis. These all carry an accelerated CVD risk, thought to be partly attributable to inflammation-driven endothelial dysfunction, lipoprotein derangement, and metabolic dysfunction stemming from chronic inflammation (41, 42). In order to speed understanding of inflammatory cardiometabolic dysfunction, psoriasis has been utilized as a human model (3) to understand the role of innate and adaptive immunity in subclinical CVD (43, 44). The clinical implications of understanding how the inflammatory processes in psoriasis contribute to cardiovascular morbidity are vast since approximately 3% of the US population has psoriasis. Furthermore, observational reports have suggested that anti-inflammatory therapies commonly used to treat psoriasis may associate with reduced cardiovascular risk (45, 46).

POTENTIAL IMMUNOLOGIC LINKS BETWEEN PSORIASIS AND CVD

Psoriasis Is Associated With Subclinical and Clinical Atherosclerosis

In the last decade, multiple studies have demonstrated an association between psoriasis and both subclinical and clinical atherosclerosis, such as VI by ^{18}F-FDG PET/CT, coronary artery calcium and non-calcified coronary plaque burden by coronary computed tomography angiography (44, 47–51). Population-based studies provide evidence of early subclinical and clinical CVD in psoriasis (2, 4, 52, 53). Research into the concept of psoriatic march (54) has led to an understanding of common cellular and molecular level links between psoriasis and atherosclerosis (55).

Common Immune Cells Between Psoriasis and Atherosclerosis

T Cells

Studies in the last two decades have established psoriasis primarily as a T-cell-mediated disorder (56–60). While initial evidence implicated a predominant role of helper T cells type 1 (Th1) through downstream activation of macrophages, neutrophils, and CD8$^+$ cytotoxic T lymphocytes (61), recent research shows the importance of the Th17 and other IL-17 producing cell types such as CD8$^+$ T cells and γδ T cells (62). Although Th1 subtype is the most studied cell-type in psoriasis, different stages of this chronic inflammatory disease employ various cells of innate and adaptive immunity (62). All the subtypes of T cells involved in pathogenesis of psoriasis are also involved in atherosclerosis (63).

Th1 Cells—Helper T Cells Type 1

Activation of the innate immune system is the key event in beginning the inflammatory cascade in psoriasis. It primarily includes differentiation of T cells into Th1 cells catalyzed by IL-12 (62). Mechanistic studies in patients with psoriasis have suggested a preference of hematopoietic progenitors toward Th1 subtype (64). Th1 cells induce psoriatic inflammation by activating neutrophils, macrophages, and CD8$^+$ cytotoxic T lymphocytes (61). Primary mediators of Th1 activity are interferon-gamma (IFN-γ), IL-2, and TNF-α, which act on keratinocytes and induce antimicrobial peptide production that subsequently continues the inflammatory cascade. Th1 cells are also critical to the process of atherosclerosis, a process thought to be primarily driven by IFN-γ, the hallmark cytokine of the Th1 response (65). In patients with unstable angina and acute coronary syndrome (ACS), Th1 cells were found to be elevated (66, 67). Furthermore, mechanistic studies have also established the role of IL-12 in the development and progression of early atherosclerotic plaques (68–70). In addition, IL-18, a Th1-promoting cytokine, has also been shown to have a role in atherosclerosis (71, 72). Finally, targeting Th1 differentiating transcription factor is shown to associate with reduced atherosclerotic plaques (73). An IL-12 stimulated activation of Th1 response with downstream release of pro-inflammatory cytokines is a common feature between psoriasis and atherosclerosis and is thought to contribute to subsequent endothelial dysfunction and T cell recruitment to the sites of atherosclerotic plaques (74). While the role of Th1 cells is profoundly studied, the function of Th2 cells remains a topic of controversy as multiple studies exist that support pro-atherosclerotic (75), atherosclerosis protective (76), and also null effect (77) of Th2 cells.

Th17 Cells—Helper T Cells Type 17

Th17 cells in psoriasis release different cytokines such as IL-17, IL-22, and TNF-α (78) and are also involved in macrophage-dependent and -independent stimulation of dendritic cells (DCs) to propagate the inflammatory response (79). They may be involved in increased production of angiogenic inflammatory mediators such as monocyte chemoattractant protein (MCP-1), nitric oxide, and vascular endothelial growth factor (80, 81). Similar to Th2 helper cells, there is conflicting data on the role of Th17 cells in atherosclerosis (82). Patients with ACS show increased Th17 cells and IL-17 compared with those with stable

angina or non-cardiac chest pain (83, 84). There is mixed evidence from mechanistic models: with some mouse models supporting the pro-atherogenic role of Th17 and IL-17 (85–87), while others have found low IL-17 mRNA in atherosclerotic plaques and overall attenuated disease development with high prevalence of Th17 cells (88–90). We later discuss the emerging role of neutrophils in the IL-17 axis, a possible mechanistic link; however, further clinical and translational research is necessary to elucidate the differential roles of Th17 and neutrophils in this pathway.

Regulatory T Cells (Treg Cells)

Regulatory T cells are a subset of T lymphocytes with a primary function to inhibit T cell activation and proliferation, through both cell-contact-dependent and cell-contact-independent anti-inflammatory cytokine (mainly TGFβ and IL-10) driven mechanisms (91). Treg inhibitory function is distinctly impaired in psoriasis (92, 93), contributing to the chronic auto- inflammation in psoriasis. ACS patients are also known to have decreased levels of circulating Treg cells with reduced efficacy and increased apoptosis susceptibility (94–97). Treg cells play an anti-inflammatory role in atherosclerosis through endothelial cell modulation, plaque stabilization by decreasing macrophages and lipid content and increasing smooth muscle cell and collagen, inhibition of pro-inflammatory cytokines, and secretion of anti-inflammatory cytokines such as TGFβ, IL-10, and IL-35 (91). Identification of common targets to reverse Treg cell dysfunction or to augment their activity in psoriasis may represent treatment mechanisms for both psoriasis and atherosclerosis simultaneously.

Finally, there are several other T cell phenotypes that have been identified in psoriasis skin lesions, such as $CD4^+$, $CD8^+$ T cells, $CD146^+$, and γδ T cells (98). However, their role in psoriasis and atherosclerosis need to be further explored. While the traditional paradigm of T cell lineages might predominate shared mechanistic links between psoriasis and atherosclerosis, there is significant heterogeneity and plasticity within the T cell subtypes. T cell predominance may change in context of subtype preponderance with the natural disease course, specifically, a switch from Th1 dominated profile in early initiation phase of psoriasis to a Th17 governed response in the chronic inflammatory phase with both involved in atherosclerosis progression (99).

Dendritic Cells

In psoriasis, DCs not only act as antigen presenters and cytokine producers but also play an important part of bridging the innate and adaptive immune systems in continuing the chronic inflammation inducing cascade (43, 79). While pDCs are important in initiation of psoriasis *via* type 1 IFN responses (62, 100), mDCs are key mediators for specific Th cell expansion *via* IL-12 and IL-23 secretion (79). While new evidence suggests a role for DCs in atherosclerotic plaque build-ups, plaque vulnerability through cholesterol metabolism and adaptive immune response modulation (101), their shared role in psoriasis and atherosclerosis needs further research.

Monocytes and Macrophages

Monocytes and macrophages are cellular hallmark of atherosclerosis (1) and are also involved in pathogenesis of psoriasis (102). While macrophages are traditionally subclassified as pro-inflammatory (M1) and anti-inflammatory (M2), they are known to be plastic and adapt to the surrounding milieu according to the underlying pathological state (103, 104). Furthermore, a preclinical *in vivo* and *in vitro* study demonstrated that chronic skin inflammation in psoriasis polarizes them toward the pro-atherosclerotic phenotype (99). These cells are involved in ACS, and their increased expression and activity is also present in vulnerable plaques (105). Novel evidence has recently suggested that a complex interplay involving neutrophil–macrophage cross-talk is crucial to the process of atherosclerosis and ACS (106–108). As these cells are involved throughout the process of atherosclerosis from plaque development to complications, such as ACS, and also play a significant role in psoriasis, further research may provide new avenues for treatment of both these conditions.

Neutrophils

Despite being the most abundant white blood cell in the circulation, neutrophils have received little attention in the pathophysiology of atherosclerosis and psoriasis. Recent mouse models and clinical trials have demonstrated the mechanistic role of neutrophils in psoriasis and atherosclerosis through the IL-17 driven keratinocyte hyper-proliferation, leading to chronic skin inflammation (109, 110). Psoriasis patients are known to have higher serum levels of IL-17 compared with healthy controls; however, the paradigm of Th17 as the predominant cellular source of IL-17 in psoriatic lesions is no longer fully valid (111). Recent studies have demonstrated that cells of the innate immune system, such as neutrophils, mast cells, γδ T cells, and innate lymphoid cells, are the main sources of IL-17 in psoriasis. Furthermore, despite controversies, IL-17 is shown to have a role in atherosclerosis in clinical and mouse model-based studies (83–85, 87).

Psoriasis increases neutrophil activation and release of neutrophil-associated proteins. Proteins associated with neutrophils such as S100A8/A9 may further provide a link between psoriasis and cardiometabolic diseases (100). S100A8/A9 (MRP8/14) is released by activated neutrophils and upregulated in psoriatic lesional skin (100, 112). We demonstrated its strong association with both skin disease severity and VI (100). Collectively, evidence suggests that neutrophils and their proteins may contribute to the early atherosclerotic milieu in psoriasis and independently predict endothelial dysfunction.

A novel subtype of neutrophils, the low-density granulocytes (LDGs), are moving to the forefront of research in psoriasis and CVD pathophysiology. LDGs are characterized by high pro-inflammatory activity, altered phagocytic function, elevated type I interferon production, and high abundance in atherosclerotic plaques and plasma of psoriasis patients (113). At the gene expression level, LDGs differ from their autologous normal-density granulocytes (NDGs) counterparts, as well as from healthy control neutrophils (114–116). LDGs also differ phenotypically from NDGs. Of these differences, the most compelling is their enhanced capacity to spontaneously form neutrophil extracellular traps (NETs). This novel defense mechanism termed NETosis goes beyond classical phagocytosis, where NETs are formed as a result of release of cytosolic granule proteins bound to nuclear material catalyzed by peptidylarginine deiminase 4 (117).

Although NETs are beneficial in antimicrobial defense, they may act as a source of autoantigens and are implicated in the development of autoimmune diseases especially psoriasis, as well as other diseases including systemic lupus erythematosus, atherosclerosis, preeclampsia, acute lung injury, deep vein thrombosis, and cancer-associated thrombosis (118–121). Cholesterol crystals are shown to trigger NETosis, further potentiating atherosclerosis by macrophage priming, Th17 activation, and immune cell recruitment in plaques (108). NETs are also shown through immunochemical stains to directly induce endothelial dysfunction and plaque rupture in human carotid plaque sections (122). NETs may be involved in the initial injury of the endothelium during atherogenesis, with recent evidence demonstrating the presence of neutrophils and NETs at sites of plaque rupture and endothelial cell erosion in human carotid plaques, features which we hypothesized would be evident in early atherosclerosis in psoriasis.

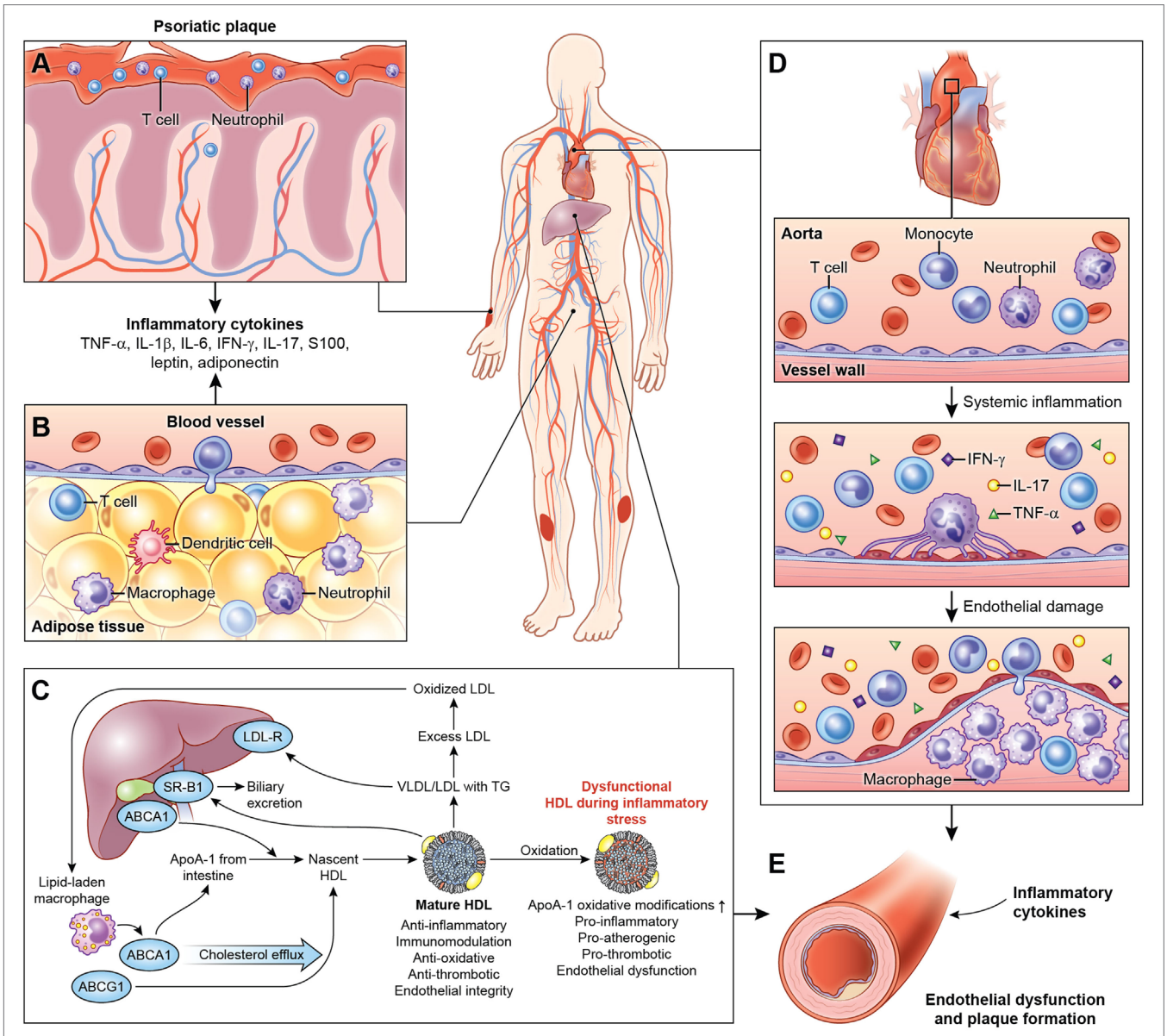

FIGURE 1 | Systemic effects of chronic low-grade inflammation in psoriasis. **(A)** Psoriasis, both cutaneous and arthritic, is a low-grade chronic, systemic inflammatory disease associated with increased circulating pro-inflammatory cytokines. **(B)** Chronic inflammation in psoriasis is associated with adipose tissue dysfunction characterized by pro-inflammatory cytokines and adipokines associated with endothelial dysfunction. **(C)** Furthermore, psoriasis exhibits a deranged lipid profile and impaired HDL function, which in combination with chronic inflammation accelerate atherosclerotic vascular disease. **(D)** The vessel wall is infiltrated through a complex interplay of pro-inflammatory cellular components, cholesterol crystals, and various lipoproteins. Over the time, with build-up of the plaque, this atherosclerotic lesion poses a significant threat to blood flow and is prone to rupture, often accelerated by inflammation leading to myocardial infarction. **(E)** Thus, psoriasis and psoriatic arthritis upregulate T-cell, neutrophil chemotaxis, and keratinocyte activation and endothelial dysfunction leading to increased atherosclerosis in blood vessels. Abbreviations: TNF-α, tumor necrosis factor-alpha; IL, interleukin; IFN-γ, interferon-gamma.

ADIPOSE DYSFUNCTION IN PSORIASIS

Systemic inflammation associated with psoriasis also contributes to inflammation of the adipose tissue (20), harboring components of the innate immune system (**Figure 1**) (63, 123). The physiological distinction between visceral and subcutaneous adiposity has been considered an important determinant in assessing CVD risk. Visceral adiposity is highly metabolically active, and its dysregulation can alter the immune cell and adipokine profile, exacerbating endothelial dysfunction. Visceral adiposity is associated with subclinical CVD measured as VI by ^{18}F-FDG PET/CT independent of cardiovascular risk factors in psoriasis (124). Furthermore, a decrease in visceral adiposity associated with an improvement of VI following 1 year of biologic anti-inflammatory therapy.

Psoriatic adipose tissue contains immune cells that influence cardiometabolic disease (20). T cells, B cells, DCs, neutrophils, mast cells, and adipose tissue macrophages (ATM) may contribute to obesity and insulin resistance, while eosinophils and Treg may protect against insulin resistance. ATM represent unique functional subset in psoriasis that are predisposed toward pro-inflammatory cytokine expression and adipose dysfunction, extending beyond the M1/M2 macrophage paradigm (20, 125, 126).

While visceral abdominal adiposity is being increasingly studied, there is emerging research that a local type of visceral adipose tissue, known as perivascular adipose tissue (PVAT), which surrounds most blood vessels (coronary arteries, the aorta, and microcirculation of the mesentery), may contribute to cardiometabolic disease (127, 128). Its anatomic proximity to the vasculature has led to research investigating the mechanisms of dysfunctional PVAT driven immune-mediated cross-talk in endothelial and vascular function under inflammatory conditions (127, 128). Mechanistic studies have demonstrated significant adipokine and chemokine (MCP-1, IL-8) production by PVAT and its ability to stimulate chemotaxis, contributing to progression of atherosclerosis (129, 130). Multiple pathways have been identified through which adipokines are implicated in CVD development—from direct vascular effects on endothelial function and smooth muscle migration to immune cell migration into the vascular wall through a potential "outside-in" inflammatory cascade (127). Recent efforts have led to a novel approach to image the PVAT and showed that it is associated with coronary inflammation in a dynamic fashion (131), with potential for prospective risk stratification.

Leptin is shown to be elevated in patients with psoriasis, to correlate with psoriasis disease severity and with indices of subclinical atherosclerosis (132, 133). We have previously exhibited an association between enhanced leptin and resistin activity with attenuated adiponectin activity in innate immune activation (24). Increased leptin and resistin promote expression of pro-inflammatory cytokines including TNF-α, IL-2, IL-6, and MCP-1, all of which are prothrombotic and drive VI through monocyte migration and macrophage activation (134). Finally, adipokines may contribute to the effect of insulin on the vasculature by contributing to changes in capillary recruitment (127).

Peri- and epicardial fat tissue are additional sources of visceral fat deposition, and a rich source of inflammatory cytokines that are associated with both subclinical and clinical coronary heart disease (128). Epicardial fat tissue has been reported to be significantly increased in psoriasis patients and may represent an independent risk factor for atherosclerosis (135).

BIOLOGIC THERAPIES

The current generation of biologic agents target cytokines critical to the pathogenesis of psoriasis, including the three known major drivers: TNF-α, IL-23, and IL-17. The majority of most effective psoriasis treatments target the IL-23/Th17 pathway. These medications include the anti-IL-17 and anti-IL23p19 agents (**Table 1**). However, as novel therapies emerge, even today, anti-TNF agents remain the standard of care in general clinical practice (43, 136). While observational data in large payer-based or veterans association-based cohorts suggest a reduced risk for MACEs primarily with anti-TNF agents, no trials assessing direct cardiovascular effects of these medications in psoriasis patients exist to date (137–140). Although effective in treating psoriasis, interestingly, these therapies have been proven of no use in rheumatoid arthritis, another chronic inflammatory disease where the IL-23/Th17 axis plays an important role. The rationale behind these contradictory findings in two major inflammatory diseases currently remains unclear (141, 142).

FUTURE DIRECTIONS

Over the last decade, remarkable progress has been made for the treatment of moderate-to-severe psoriasis, especially with the advent of biologic therapies, which target specific cytokines, immune cells, and pathways. Moreover, the recent success of CANTOS (32) has demonstrated that inflammation reduction through direct IL-1β inhibition using a monoclonal antibody, canakinumab, in the absence of lipid lowering, can reduce CV event rates. As such, the emerging field of biologic treatments

TABLE 1 | Biologic treatment options to treat psoriasis.

Biologic drug	Target cytokine	Cardiovascular effects
Etanercept Infliximab Adalimumab	Tumor necrosis factor-α	Observational data indicating better CV outcomes. RCT for subclinical cardiovascular disease (CVD) demonstrating promising results. RCT dedicated for CV events not available (139, 140, 143)
Secukinumab Ixekizumab Bimekizumab Brodalumab[a]	Interleukin-17A and interleukin-17A receptor for brodalumab	Dedicated RCT for CV events unavailable
Ustekinumab Briakinumab[a]	Interleukin-12/23p40	RCT for subclinical CVD demonstrating favorable results. Dedicated RCT unavailable (144)
Guselkumab Tildrakizumab Risankizumab	Interleukin-23p19	No data available yet for CV effects
Fezakinumab	Interleukin-22	Drug still in early development phase

[a]*Discontinued medications from the market.*

is exciting as it may provide therapeutic utility in psoriasis with added benefits of modulating CVD risk. Furthermore, completed and ongoing trials assessing the subclinical CVD in psoriasis have demonstrated promising findings (143, 144).

Finally, future research should focus on examination of complex inter-relationships between various conventional and non-conventional, inflammatory and non-inflammatory pathways to understand the heightened risk of CVD in disease conditions with underlying chronic inflammation.

CONCLUSION

Increasing evidence demonstrates an important role of immune dysfunction linking psoriasis to cardiometabolic diseases including atherosclerosis. Future efforts in patients with chronic inflammatory disease like psoriasis should focus on elucidating the complex interplay among immune cells, adipose tissue, and various biomarkers of immune dysfunction. The shared mechanistic links between psoriasis and atherosclerosis provide promising avenues in targeted treatment for both diseases, especially in light of the recent trial CANTOS (32), which demonstrated reduced incidence of recurrent cardiovascular events after treating residual inflammation in patients with known coronary artery disease.

AUTHOR CONTRIBUTIONS

AS and NM conceived and designed research. AS, AJ, HT, AD, and NM contributed to both manuscript writing and critical review.

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REVIEW
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Psoriasis, Cardiovascular Events, and Biologics: Lights and Shadows

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Nowadays, it is well established a link between psoriasis and cardiovascular (CV) diseases. A series of different overlapping mechanisms including inflammation, homeostasis dysregulation, and genetic susceptibility are thought to underlie this association. Advances in understanding the molecular patterns involved in the complex scenario of psoriasis have highlighted a tight correlation with atherosclerosis. Indeed, common profiles are shared in term of inflammatory cytokines and cell types. In the last decade, the management of psoriasis patients has been revolutionized with the introduction of biological therapies, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-12/23, and IL-17 inhibitors. In clinical setting, the effectiveness of these therapies as well as the incidence of CV events is related to the type of biologics. In particular, anti-TNF-α agents seem to reduce these events in psoriasis patients whereas anti-IL-12/23 agents related CV events reduction still remain to clarify. It has to be taken into account that IL-12/23 inhibitors have a shorter post-marketing surveillance period. An even more restricted observational time is available for anti-IL-17 agents. IL-17 is associated with psoriasis, vascular disease, and inflammation. However, IL-17 role in atherosclerosis is still debated, exerting both pro-atherogenic and anti-atherogenic effects depending on the specific context. In this review, we will discuss the differences between the onset of CV events in psoriasis patients, referred to specific biological therapy and the underlying immunological mechanism. Given the development of new therapeutic strategies, the investigation of these inhibitors impact on heart failure outcome is extremely important.

Keywords: anti-IL12/23, anti-IL17, anti-tumor necrosis factor-alpha, atherosclerosis, cardiovascular risk, psoriasis

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PSORIASIS AND CARDIOVASCULAR (CV) EVENTS

The relationship between psoriasis (Pso) and an increased incidence of major adverse cardiovascular events (MACEs) has been observed for decades, since McDonald and Calabresi first demonstrated that the risk associated with arterial and vascular diseases was 2.2 times higher in more of 300 hospitalized patients with Pso compared with controls with other dermatological conditions (1). Since

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CT, computed tomography; CV, cardiovascular; CVD, cardiovascular disease; FDG, ^{18}F-fluoro-deoxyglucose; GPRD, General Practice Research Database; HDL, high-density lipoprotein; IFN, interferon; IL, interleukin; IMT, intima-media thickness; IRs, incidence rates; LDL, low-density lipoprotein; MACEs, major adverse cardiovascular events; MCP, monocyte chemoattractant protein; MI, myocardial infarction; MTX, methotrexate; OR, odds ratio; PASI, psoriasis area severity index; PET, positron emission tomography; RBP4, retinol-binding protein 4; RCTs, random control trials; Th, T helper; TNF, tumor necrosis factor; TNFi, TNF-α inhibitors; V-CAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

then, several studies have confirmed these findings, convincingly proving that patients with Pso have an effective higher risk of developing severe CV events, such as myocardial infarction (MI) and stroke (2). In 2006, using the General Practice Research Database (GPRD) source, Gelfand et al. suggested that Pso is an independent risk factor for acute MI and cardiovascular disease (CVD), particularly in young patients, and that this risk is most significant in patients with severe disease (3). In 2007, Ludwig and colleagues also identified Pso as a possible independent risk factor for CVD development founding a significantly increased prevalence and severity of the CVD indicator coronary artery calcification factor in these patients (4). In addition, increases in the prevalence of other independent "traditional" risk factors for CVD, including smoking, excess alcohol intake, as well as obesity, hypertension, dyslipidemia, and insulin resistance (the common underlying factors of metabolic syndrome), have been also reported in psoriatic patients (5–7). However, despite the evidences, some studies failed to find a significant independent association between Pso and CVD (8, 9). In 2015, using the same population-based GPRD, Parisi et al. conducted a series of multivariable analyses on patients with incident Pso concluding that neither Pso nor severe Pso are associated with a risk of MACE even after adjustment for traditional CVD risk factors (10). To date, the debate is whether or not this link represents a causal relationship or is a predisposition due to the underlying risk factors exhibited by patients with severe Pso (9, 10). The main hypothesis is that chronic inflammation which occurs in Pso is more than skin deep and results in a "psoriatic march" driving systemic mechanisms that are shared with other chronic inflammatory diseases, including atherosclerosis (**Figure 1**) (11–14). This concept was introduced for the first time in 2011 by Boehncke and colleagues to describe how systemic psoriatic inflammation may lead to insulin resistance as well as endothelial cell dysfunction, causing atherosclerosis, the major pathological change preceding MI and stroke development (15). Indeed, psoriatic patients with altered glucose metabolism and insulin resistance showed an increased arterial stiffness compared with healthy subjects, with a positive correlation between arterial stiffness and Pso disease duration (16, 17). Understanding why Pso may be a risk factor for atherosclerosis requires a basic understanding of their shared pathogenic features. In 2012, Flammer and Ruschitzka proposed the theory of "two plaques for one syndrome" since molecular mechanisms as well as pro-inflammatory cytokine profile of psoriatic lesions are remarkably similar to that of atherosclerosis ones, with a comparable inflammatory infiltrate of T cells, macrophages, and monocytes (18, 19). In addition, both diseases display a common pattern of T-cell activation, with T helper (Th)1 and Th17 cytokine upregulation, as well as increased local and systemic expression of adhesion molecules and endothelins (19). Thus, there are displacements of inflammatory cells between lesional psoriatic skin, peripheral circulation, and atheromatous plaques of coronary vasculature caused by the releasing cytokines derived from the skin and inflammatory mediators derived from Pso lesions into the circulation, together with an upregulation of cell adhesion molecules (20). Moreover, Pso-associated pro-inflammatory cytokines, such as interferon (IFN)-γ, tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-17, have been found increased in atherosclerotic plaques and sera of patients with unstable CVD (21, 22). Similarly, increased expression of well known CV biomarkers, including monocyte chemoattractant protein (MCP)-1 and macrophage-derived chemokines, have been measured in the lesional skin and serum of psoriatic patients suggesting shared inflammatory pathways linking Pso and CVD (23). Recently, Kolliker Frers et al. have demonstrated that pro-atherogenic inflammation marker C-reactive protein (CRP) and soluble intercellular adhesion molecule-1 levels are increased also in psoriatic patients with no CV history or traditional CV risk factors, compared with healthy subjects, as well as in patients with recent-onset PsA, even in the absence of CV risks. These data reinforce the concept that that the degree of atherosclerosis tendency might be related to the amount of the inflammatory psoriatic burden and highlight the importance of primary prevention in Pso also in those psoriatic patients with no history of CV events (24).

THERAPEUTIC IMPLICATIONS

The growing body of evidences pointing to increases in MI and CV events in psoriatic patients has raised the question whether treating cutaneous disease might also prevent heart attacks and decrease CVD risk development. Moreover, the postulated hypothesis that inflammatory cascade activated in Pso contributes to the atherosclerotic process has laid the groundwork for supposing that the anti-inflammatory Pso therapy could theoretically improve also atherosclerosis and reduce the risk of MACE (25). Prodanovich et al. first reported that treatment with methotrexate (MTX), especially when used at low doses and in combination with folic acid supplementation, was able to reduce the rate of vascular disease in patients with Pso or rheumatoid arthritis (RA) (26). A subsequent meta-analysis of 10 studies confirmed these data concluding that the use of MTX resulted in 21% lower risk of CVD and 18% lower risk of MI (27). Moreover, data from a large health-care system in USA proved that decreased MI risk in psoriatic patients treated with MTX was greater than those receiving topical therapy (28). In 2013, using a population-based Danish cohort, an observational study found that the risk of MACE development decreased in psoriatic patients in treatment with MTX compared with other non-biological agents, including oral retinoids, cyclosporine, and phototherapy (29). Indeed, treatment with cyclosporine, leading to impaired renal function and hypertension, may negatively affect the MACE profile in these patients by increasing the risk of hypertension and dyslipidemia (30). For that reason, in psoriatic patients, cyclosporine should only be used for a limited period and should be substituted with another systemic agent once the skin condition is improved (20). Similarly, acitretin, the most commonly agent used to treat generalized pustular Pso, it has been associated with an increased risk of hypertension, hyperlipidemia, and CVD even if several investigations have shown that retinoids also improve and ameliorate the formation of atherosclerotic plaques (31, 32). Moreover, Boehncke et al. found that also treatment with fumaric acid esters resulted in an improvement of CV risk biomarkers in patients with moderate-to-severe plaque-type Pso. In particular, the authors

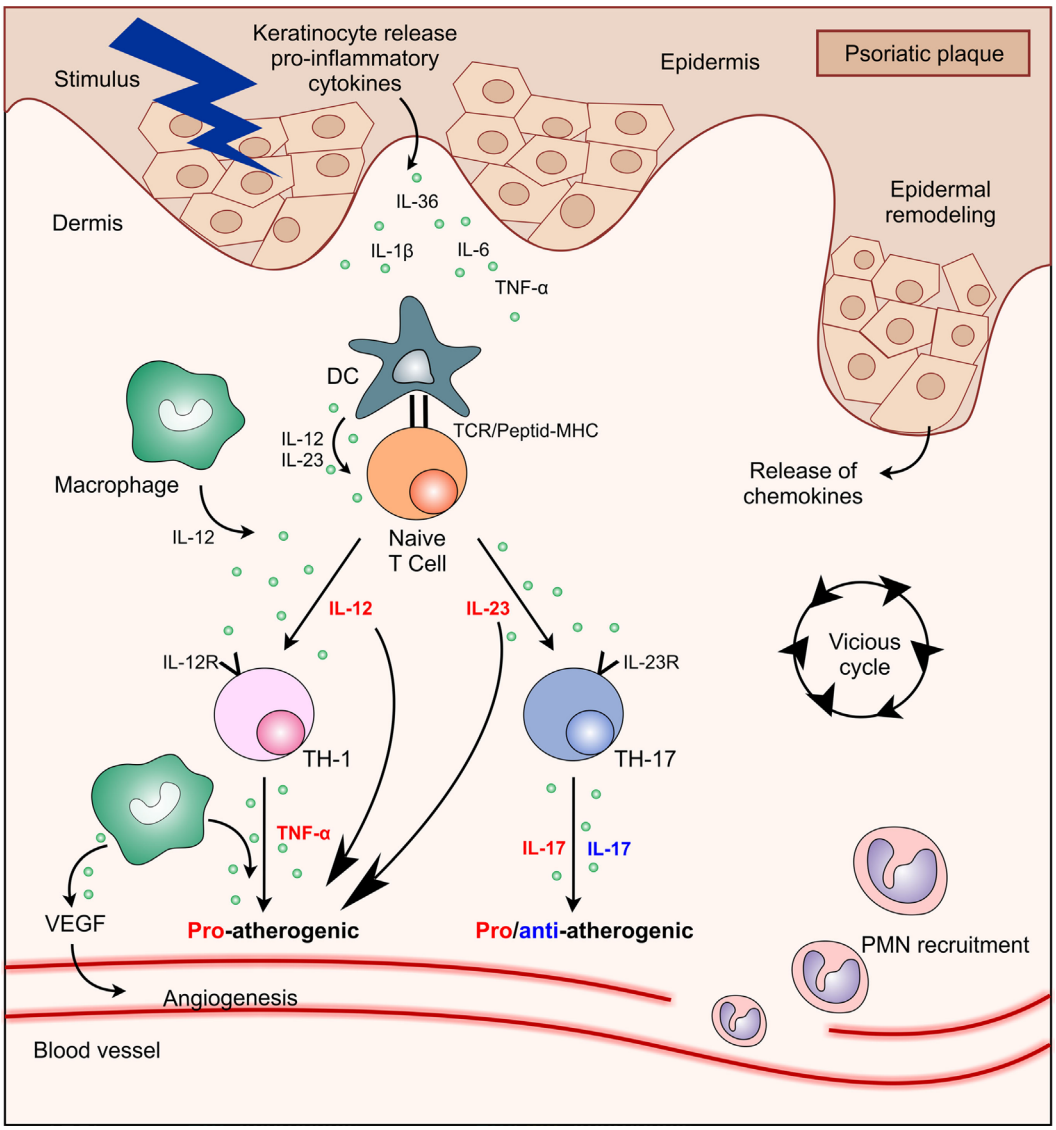

FIGURE 1 | Interactions between main cell types and cytokines present in psoriasis plaque showing their functional significance in atherosclerosis process. Abbreviations: DC, dendritic cell; IL, interleukin; MHC, major histocompatibility complex; PMN, polymorphonuclear; TCR, T cell receptor; Th, T helper; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

demonstrated that a continuous systemic therapy with fumaric acid esters, behind reducing Pso severity, was able to improve the endothelial vasodilator function, reduce serum levels of CRP, and increase the potentially cardioprotective adiponectin (33). However, it has been with the introduction of biological therapies that Pso treatment expectations and long-term control have greatly improved and the idea that these therapies, more than the systemic ones, might reduce the risk of CVD reinforced (34). To date, although several biological therapies have been approved and licensed for the treatment of Pso, their CV safety profile is not yet well established (35). Currently, it is unclear whether any of these therapies, which include TNF-α inhibitors (TNFi) (infliximab, etanercept, and adalimumab); inhibitors of the p40 subunit common to IL-12 and IL-23 (ustekinumab and briakinumab); IL-17A inhibitors (secukinumab and ixekizumab), and its receptor antagonist (brodalumab) could alter the risk of CVD development (35). Thus, the aim of this review has been to evaluate whether possible associations exist between currently licensed biological therapies and risk of MACEs in adult patients with Pso.

PSORIASIS AS IMMUNE-MEDIATED DISEASE

Psoriasis is an immune-mediated inflammatory skin disorder that affects 2.5% of the population worldwide. While the exact etiology of psoriasis is unknown, genetic and environmental

factors are important in disease development (36, 37). Moreover, the immune system plays a crucial role in the overall disease pathogenesis, with various innate and adaptive immune cells and pro-inflammatory mediators involved at different stages of the disease (38). T cells are known to be the main actors in the pathogenesis, in particular Th1 and Th17 lymphocytes contribute through the inflammatory cytokines release that promote further recruitment of immune cells, keratinocyte proliferation, and inflammation (39). More specifically, CD4+ and CD8+ T cells with an IL-17-secretory phenotype are important contributors owing to their production of the pro-inflammatory cytokines IL-17, IL-22, and TNFα (40). For over 30 years, Pso was thought to involve a Th1 response, driven by the cytokines IFN-γ and IL-12; however, the discovery of the Th17 cell population has revolutionized the complex scenario of Pso. Indeed, the IL-23/Th17 cell signaling axis effects on keratinocytes and infiltrating immune cells in the skin has shaped the current disease model of Pso. Therefore, psoriatic disease is understood as a patterned response to chronic activation of the IL-23/Th17 axis (36). Recently, it has been identified T-regulatory cells expressing IL-17A in which, Foxp3 expression is progressively lost, whereas RORγ expression is increased (39, 40). This process is upregulated by IL-23 and concurring to the chronic inflammation seen in Pso. In particular, IL-23 regulates the maintenance of Th17 cells, whereas IL-17 and TNF mediate effector functions of innate (TNF) and adaptive (TNF, IL-17) immune cells.

EFFECTS OF BIOLOGICAL THERAPY ON CV RISK IN PSORIATIC PATIENTS

Anti-TNF-α Agents

Pso is considered a T cell-mediated immune disease with a mixed Th1/Th17 cytokines environment. The interplay of these cytokines has a central role in the disease process by causing the spread of local inflammation at systemic levels. Specifically, elevated levels of TNF-α and soluble TNF receptors have been found in lesional psoriatic skin and in the serum of patients with severe Pso, which similarly reflect those detected in congestive heart failure (CHF) patients (41–43). TNF-α activation system can lead to different outcomes: (i) the development of atherosclerosis, (ii) the deterioration of cardiac functions, and (iii) the vascular smooth muscle cell remodeling (44–46). Since 2004, US Food and Drug Administration (FDA) has approved TNFi, such as etanercept, infliximab, and adalimumab for Pso treatment. Most recently, five TNFi biosimilars have been approved by FDA for Pso: infliximab-dyyb, infliximab-abda, etanercept-szzs, adalimumab-atto, and adalimumab-adbm (47, 48). The effectiveness of these biologics in the management of psoriatic patients has been highlighted since the earliest literature (49–54). Anti-TNF-α agents can reduce the CRP, the vascular endothelial growth factor (VEGF), and the chemotactic factors (e.g., VCAM-1, E-selectin, IL-8, and MCP-1) (55, 56) as well as the Th17 cell count in the peripheral blood of psoriatic patients (49, 57). In fact, TNFi stop CD4+ T cells differentiation into Th1, Th17, and Th22 cells and the consequent release of IL-17A, IL-17F, and IL-22 (47, 58). Although the evidence indicate that TNFi are effective in reducing the systemic inflammation, it is still debated whether anti-TNF-α agents can decrease CV risk in these patients or they only represent a random association. It has been reported in literature that psoriatic patients who received TNFi showed an improvement of psoriasis area severity index (PASI) score characterized by a reduction of CV risk biomarkers (e.g., CRP, VEGF, and resistin serum levels) after 24 weeks of therapy (59). Additional studies have highlighted that the vascular function was somehow restored after 10 weeks of etanercept treatment, leading to a significant reduction in CRP levels and improvements of insulin sensitivity (60, 61). The same authors also proved that TNFi statistically led to a significant reduction ($P = 0.0001$) of retinol-binding protein 4 (RBP4) circulating levels in psoriatic patients (62). RBP4 is highly linked to subclinical atherosclerosis, due to its positive correlation to carotid intima-media thickness (IMT) indicator (61, 62). Recently, it has been assumed that psoriatic patients treated with TNFi showed IMT indicator decrease associated with appropriate therapeutic responses (63). Another experimental study has reported the aortic inflammation reduction by ^{18}F-fluoro-deoxyglucose (FDG)-positron emission tomography/computed tomography (CT) in 30 psoriatic patients who received adalimumab (64). Similarly, the TNFi effects on the development of atherosclerosis has been studied in 58 psoriatic patients during a 13-month period by CT imaging (65). In addition, the patients who did not received TNFi showed a significant progression of the coronary calcification, while the TNFi-treated group did not. Another research based on echocardiographic data confirmed the improvement of the myocardial function in 18 psoriatic subjects treated with TNFi and IL12/23 inhibitors (66). Likewise, right ventricular systolic function was improved in a 30-month study of 44 psoriatic patients treated with TNFi (67). Therefore, anti-TNF-α agents might be identified as CV protective factors together with statin drug, female gender, and age (68). However, no therapeutic effect of TNFi has been demonstrated in CHF patients classified as grade III–IV by the New York Heart Association. Initially, etanercept seemed to have beneficial effects in heart failure patients (69). Subsequently, a large study consisting of 1,500 patients with symptomatic heart failure has shown no relation to mortality or hospitalizations after treatment with etanercept (70). Other clinical trials have reported a higher percentage of mortality in CHF patients who received infliximab at 10 mg/kg (higher dose than that used in Pso and RA) than the controls (71, 72). These preliminary discovers have provided the evidence for the contraindication against the use of TNFi in CHF patients. Nowadays, anti-TNF-α drugs are not contraindicated in patients with heart diseases other than CHF (34). Random control trials (RCTs) including severe psoriatic patients who received at least one of TNFi, resulted in a number of MACEs (35, 73–76). Overall, the pooled analysis confirmed that no statistically significant difference exists between patients treated with biologics or placebo (35) (**Table 1**). Considering TNFi separately, there was also no statistically significant difference in patients receiving etanercept, infliximab, or adalimumab (35). In addition, the incidence rates (IRs) of MACEs were reduced by the use of anti-TNF-α agents respect to conventional therapies (28, 29, 77, 78). The use of TNFi for patients with Pso or psoriatic arthritis was associated with a 55% reduction in the incidence of MI compared

TABLE 1 | Main randomized controlled trials and meta-analysis studies on the rates of major adverse cardiovascular events (MACEs) in psoriatic patients treated with biological agents.

Biological agents	Reference	No. of patients or trials	MACE risk (IR or OR)	Follow-up period
TNFi	Rungapiromnan et al. (35)	18 RCTs comparing TNFi (4 adalimumab, 9 etanercept, 5 infliximab) vs placebo	OR, 0.67 (95% CI, 0.10–4.63, $P = 0.69$)	8–50 weeks
Ustekinumab (anti-IL12/23p40)	Reich et al. (82, 83)	1582 ustekinumab vs 732 placebo-treated patients	IR, 0.3%; (95% CI, 0.1–0.70) vs 0.0% (95% CI, 0.0–0.5%)	20 weeks
	Tzellos et al. (84)	9 RCTs (ustekinumab vs placebo)	OR, 3.96 (95% CI, 0.51–30.41; $P = 0.19$)	30 weeks
	Rungapiromnan et al. (35)	7 RCTs (ustekinumab vs placebo)	OR, 4.48 (95% CI, 0.24–84.77; $P = 0.32$)	30 weeks
Briakinumab (anti-IL12/23p40)	Langley et al. (85)	1258 briakinumab vs 624 placebo-treated patients	IR, 1.33% (95% CI, 0.43–3.10) vs 0.60% (95% CI, 0.35–0.94)	12 weeks
	Tzellos et al. (86)	9 RCTs (briakinumab vs placebo)	OR, 4.47 (95% CI, 0.69–28.89; $P = 0.12$)	30 weeks
Ustekinumab and Briakinumab	Tzellos et al. (86)	9 RCTs (ustekinumab and briakinumab vs placebo)	OR, 4.23, (95% CI, 1.07–16.75; $P = 0.04$)	30 weeks
Secukinumab (anti-IL17)	van de Kerkhof et al. (87) Egeberg et al. (88)	10 phase II/III clinical trials 196 patients	IR, 0.35% (95% CI, 0.10–0.90) IR, 3.1% (95% CI, 1.1–8.1)	52 weeks 104 weeks
Ixekizumab (anti-IL-17)	Strober et al. (89)	7 RCTs (UNCOVER-1, -2, and -3 plus an additional 4 phase I–III studies)	38 [0.6][a]	60 weeks
Brodalumab (anti-IL17R)	Papp et al. (90)	1 phase III clinical trial (AMAGINE-1)	5 (1.0)[b]	52 weeks

IR, incidence rate; OR, odds ratio; CI, confidence interval; RCTs, randomized controlled trials.

[a]*MACE occurring in ≥1 patient, n [IR].*

[b]*n (exposure-adjusted event rate per 100 patient-years).*

with those patients who were only treated with topical therapy (28). Similarly, psoriatic patients treated with TNFi displayed a significantly lower risk of MI when compared with MTX (79). Furthermore, treatment with TNFi is associated with an 11% reduced CV risk every 6 months of additional treatment (79). On the other hand, some authors suggested that these therapies do not seem to reduce the risk of MI, because they increase patients' weight and cholesterol levels after 3 or 6 months of therapy (66, 79, 80). These results could be related to a subsequently biological effect of TNFi due to neutralization of TNF-α cachetic properties (81). On the contrary, stable lipids levels were found in psoriatic patients treated with adalimumab, etanercept, or infliximab for 13 months (65). Psoriatic patients randomized to adalimumab or non-systemic treatment (topical therapies or phototherapy) showed no changes in total cholesterol, high-density lipoprotein, low-density lipoprotein, or triglycerides after 15 weeks of treatment (64). Despite some scientific reports overshadows TNFi role on CV risk, current studies support TNFi benefic effects on cardio metabolic parameters. To conclude, although literature remains heterogeneous, in part due to methodological differences, anti-TNF-α agents exert a protective effect on CV risk.

Anti-IL-12/23 Agents

The basis for developing anti-IL-12/23 biological drugs was encouraged by evidences that mice deficient in the subunit p40, shared by both IL-12 and IL-23, are resistant to experimentally induced autoimmune conditions, such as psoriasis (91, 92). Levels of IL-12/23p40 mRNA are more elevated in psoriatic than in healthy skin (93, 94). Cytokines induced by IL-12 (such as IFN-γ) and by IL-23 (such as IL-17A, IL-17F, and IL-22) are increased in psoriatic plaques (94). Moreover, in the last two decades, these inflammatory mediators have also been shown to contribute in CVD development. Indeed, it has been shown that serum levels of IL-12 and IL-23 are augmented in patients with CVD (95, 96). IL-12 and IL-23 are presented in atherosclerotic plaque and thereby affecting the pro-inflammatory status in these patients (96–98). Taken together, these evidences suggest that targeting IL-12/23 could represent a valid therapeutic option for psoriatic disease, with benefits on cutaneous involvement and on CV comorbidities. The efficacy of anti-IL-12/23p40 biological drugs (ustekinumab and briakinumab) for psoriasis treatment (**Table 2**) has been evaluated in two phase III RCTs and in four phase III RCTs, respectively (82, 85, 99–103). The hypothesis that these agents could possibly increase CV risk while improving cutaneous disease is unexpected. Nevertheless, safety concerns have been raised regarding the possibility of an increased risk of MACEs with the utilize of anti-IL-12/23 biological agents. We will discuss on lights and shadows of this hot question. In 2011, Reich et al. (83) evaluated this point due to the fact that in the ustekinumab clinical studies target population reported a higher rate of MACEs than placebo one. In the 1,582 ustekinumab-treated patients enrolled in the phase II and III placebo-controlled psoriasis studies, five MACE events occurred [0.3%; 95% confidence interval (CI), 0.1–0.7%] respect to no events in 732 placebo-treated patients (0.0%; 95% CI, 0.0–0.5%) (83). In particular, an important numerical imbalance in MACE rate was observed in the phase II trial with a 4:1 randomization ratio (risk difference 1.2%; 95% CI, 3.9–3.7%). However, the two phase III trials did not replicate a same high risk difference (0.2%; 95% CI, 1.2–1.2 and 0.1%; 95% CI, 0.7–0.7% for PHOENIX 1 and 2, respectively) (83) (**Table 1**). During the 12/20-week follow-up periods, MACE events did not show tendency to cluster. Indeed, the five MACEs occurred at weeks 2, 6, 10, 14, and 17 (83). The standardized IR

TABLE 2 | FDA approval biological drugs for psoriasis.

Biological drug	Biological structure	Mechanism of action	FDA approval for psoriasis (year)
Etanercept	Soluble TNFR2 coupled to Fc portion of IgG1	Anti-TNF-α	2004
Infliximab	Human/mouse chimeric IgG1 mAb	Anti-TNF-α	2006
Adalimumab	Human IgG1 mAb	Anti-TNF-α	2008
Ustekinumab	Human IgG1 mAb	Anti-p40 IL-12/23	2009
Secukinumab	Human IgG1κ mAb	Anti-IL17A	2015
Adalimumab-atto (biosimilar)	Human IgG1 mAb	Anti-TNF-α	2016
Etanercept-szzs (biosimilar)	Soluble TNFR2 coupled to Fc portion of IgG1	Anti-TNF-α	2016
Infliximab-dyyb (biosimilar)	Human/mouse chimeric IgG1 mAb	Anti-TNF-α	2016
Ixekizumab	Humanized IgG4 mAb	Anti-IL17A	2016
Adalimumab-adbm (biosimilar)	Human IgG1 mAb	Anti-TNF-α	2017
Infliximab-abda (biosimilar)	Human/mouse chimeric IgG1 mAb	Anti-TNF-α	2017
Brodalumab	Human IgG2 mAb	Anti-IL17RA	2017
Guselkumab	Human IgG1λ mAb	Anti-p19 IL-23	2017

IgG, immunoglobulin G; IL, interleukin; mAbs, monoclonal antibodies; IL-17RA, interleukin receptor A; p40, subunit 40; p19, subunit 19; TNFR2, tumor necrosis factor receptor 2; TNF-α, tumor necrosis factor; FDA, Food and Drug Administration.

in these studies ranged between 0.34 and 0.52, a rate lower than that estimated for the general population in the USA or in psoriatic patients (83). Moreover, all patients who experienced MACEs had at least three established CV risk factors. Longer term analyses demonstrated that rates of CV events remained low with up to 3 years. However, the absence of a control group precludes definitive assessment of the effect of ustekinumab on MACE risk (83). Thus, data from short-term, controlled trials give only partial information regarding the possible impact of ustekinumab on CV risk: the results suggest neither harmful nor beneficial effects. However, a smaller risk increase cannot be totally ruled out. Concerning another anti-IL-12/23p40 monoclonal antibody, briakinumab, the question is even more burning. Results from one of the four clinical trials have reported more MACE events in briakinumab-treated patients compared with placebo ones over a 12-week period (101). In details, 18 MACEs had been recorded with 4 CV deaths compared with no events in placebo group. The frequency of MACE was spread in an apparently uniform manner over time. The standardized IRs were 1.33/100 patient-years (95% CI, 0.43–3.10) respect to 0.60 (95% CI, 0.35–0.94) in the placebo-controlled phase (**Table 1**). In patients with two or more CV risk factors, the IR was more elevated (2.15 events/100 patient-years). For all these reasons, the study protocol underwent an amendment (85). Interestingly, no MACEs were reported during other two published clinical trials (briakinumab vs placebo or etanercept) as well as in another trial in which briakinumab was compared with MTX with a 52-week follow-up (82, 102, 103). However, the application for authorization to market briakinumab has been withdrawn.[1] Overall, definitive conclusions did not could be performed. For this reason, two industry-independent meta-analyses of nine randomized, double-blind, placebo-controlled, monotherapy trials have been conducted to deeper examine the possible association of MACEs with anti-IL-12/23 agents (84, 104) (**Table 1**). The meta-analysis by Ryan et al. found no increased risk of MACEs in treated patients compared with placebo ones, using the Mantel–Haenszel fixed-statistical effects model (104). On the other hand, Tzellos et al. analysis showed that the odds ratio (OR) for briakinumab- and ustekinumab-treated patients was not statistically significant (OR 4.47, 95% CI, 0.69–28.89, $P = 0.12$; OR 3.96, 95% CI, 0.51–30.41, $P = 0.19$, respectively). When combined, the OR for MACEs between patients treated with biological agents and those receiving placebo was found to be statistically significant (OR = 4.23, 95% CI, 1.07–16.75, $P = 0.04$) (84). In this case, the statistical effects model used was the Peto One-Step OR method. The divergence in these results has been attributable to the use of two different methods to estimate the risk. The Mantel–Haenszel fixed-statistical model using zero-cell corrections provides estimates for all studies, including zero event ones. This make it less suitable for meta-analysis of rare events (105–108) resulting in low statistical power. MACE event rates were estimated to be 0.28, 0.35, and 0.31% for ustekinumab, briakinumab, and both, respectively (108). It has been established that in case of an event IR of 1% or less, the best statistical approach is Peto method (105). However, the Peto OR method excluding trials with zero events from the analysis can lead to an overestimation of true relative risk. Moreover, neither the analysis by Tzellos et al. nor that by Ryan et al. have been adjusted for dropouts; this likely resulted in an overestimation of true risk (106). A large 5-year post-marketing study on the use of ustekinumab for psoriasis did not support the hypothesis of an augmented risk of MACEs (109). Moreover, an imbalance in MACE event rate has not been reported in other therapeutic indications of ustekinumab, such as psoriatic arthritis and inflammatory bowel disease (110–112). In addition, to date, the FDA has not communicated any changes regarding the prescribing for ustekinumab related to CV risk. More recently, a new meta-analysis has been performed by Rungapiromnan et al. (35) to evaluate the impact of biological therapies on risk of MACEs (**Table 1**). In this meta-analysis, they have selected RCTs in which patients received only licensed dose regimens of biological therapies. For this reason, briakinumab has been excluded from the analysis. Peto ORs with 95% CIs has been applied for statistical analysis. Regarding ustekinumab, no statistically significant difference in risk of MACEs has been reported respect to placebo (OR 4.48, 95% CI, 0.24–84.77, $P = 0.32$); similarly, comparing ustekinumab 45 mg with 90 mg the OR was not statistically significant (OR 1.00, 95% CI, 0.06–16.03, $P = 1.00$) (35). The most important limitation is that these findings were mainly based on small sample sizes and short-term follow-up (ranging from 12 to

[1] Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2011/01/WC500100769.pdf (Accessed: Febraury 8, 2018).

30 weeks). This is an important aspect to considering since it has been demonstrated that during the initial stage of therapy with ustekinumab inflammatory pro-atherogenic mediators such as IL-12/23 p40 temporarily increased and then dramatically decreased at week 32 (113, 114). Thus, other post-marketing studies and novel larger randomized controlled trials will be needed to continue the surveillance to assess the potential association connecting the use of anti-IL-12/23p40 biological drugs and increased CV risk.

Anti-IL17 Agents

In the past decade, the research has been focused on Th17 cells, which during differentiation secrete IL-17. IL-17 cytokine family consists of six members (IL-17A, B, C, D, E, and F). IL-17A (referred to here as IL-17) is among them, the major isoform (115). IL-17 is produced by Th17 cells and also by other cell subtypes, including γδ T, natural killer, etc. (116). However, interest in the Th17 population in relationship to Pso increased when numbers of circulating Th17 cells and IL-17 expression levels were found upregulated in the Pso lesional skin compared with non-lesional skin (49, 117). Recently, several anti-IL-17 agents have been developed. These primarily include anti-IL-17A monoclonal antibodies, secukinumab and ixekizumab, and brodalumab, an anti-IL-17 receptor monoclonal antibody. These biological agents that target the IL-17 signaling pathway have currently been evaluated and approved for the treatment of moderate-to-severe plaque Pso. The results reported by studies on efficacy and safety of IL-17 inhibitors are very promising. They showed the superiority of these new biological agents respect to both placebo and other biologics, such as ustekinumab and etanercept. For the first time, these trials include as end point the percentage of patient that achieving 90 or 100% of clinical improvement (PASI-90 or PASI-100, respectively) (47). In addition, although IL-17 antagonists had a higher rate of any adverse events than placebo, there was no significant difference in severe adverse events. This suggests that IL-17 antagonists are well tolerated (118). Even if the effects of IL-17 therapy on CVD in psoriatic patients is yet to be fully elucidated. Indeed, the interest on MACE is a crucial and much debated point. Likewise, Th17 pathway has a crucial role in CVD (22). Indeed, IL-17 is also involved in angiogenesis process, and on the synthesis of MMPs and CRP. Thus, in theory, Th17 cells could also play a crucial role in atherosclerotic and in CVD. Reports in atherosclerosis showed a conflicting result on IL-17 and Th17 cells in disease onset and plaque stability. IL-17 may exhibit both pro- and anti-atherogenic effects, depending on the inflammatory context (115). IL-17 pro-atherogenic effects may result from the induction of pro-inflammatory cytokines or chemokines (IL-6, GM-CSF, CCL2, and CXCL1) by endothelial cells or macrophages. The IL-17 atheroprotective effects may be dued by IFN-γ decreased and to its inhibitory effect on the expression of vascular cell adhesion molecule, this molecule is important to mediating the accumulation of monocytes and T cells within the lesions. Indeed, it was showed that IL-17, IL-21, and IL-23 were found in atherosclerotic plaques and associated with increased inflammation and plaque vulnerability (119). By contrast, Taleb et al. have reported a role for IL-17 in promoting plaque stability. Indeed, IL-17 expression in human carotid lesions was related to a fibrous phenotype with a lower macrophage and higher smooth muscle cell content (120). Despite some data suggesting increased levels of Th17 cells and IL-17 in patients with acute coronary syndromes (121, 122), the bulk of evidence indicate that circulating IL-17 levels are similar in patients with or without coronary artery disease (123). Indeed, some authors have reported that the patients with IL-17 low levels were more susceptible to the risk of death and recurrent MI (124). On the other hand, several experimental evidences and biomarker studies, which indicate a link between IL17 and instability in atherosclerotic plaques, supporting the hypothesis that in part explain the high risk of MI in Pso patients (22). Indeed, Pso patients have significantly elevated IL-17 serum levels (49) and they have a high risk to developing CV comorbidities. The conception that Th17 cytokines may provide a link between Pso and CVD comes from literature data that reporting a pathogenic role of IL-17 in Pso and examining the Th17 axis contribution into atherosclerosis (125).

As regarding secukinumab, data reported that there is a favorable safety profile in patients with moderate-to-severe plaque Pso over a total follow-up period of 52 weeks in a pooled safety analysis of 10 studies. However, at baseline, more subjects in the secukinumab groups had CV risk than comparator groups. Even if overall exposure-adjusted IRs of adjudicated MACE in secukinumab-treated subjects was comparable to etanercept-treated subjects (87). During the first 12 weeks of secukinumab studies, MACEs were reported in three patients receiving secukinumab 300 mg (0.26%), in no patients receiving etanercept, and in one patient treated with placebo. Over 52 weeks, exposure-adjusted IRs of MACEs were comparable in patients receiving secukinumab 300 mg (0.42/100 subject-years), secukinumab 150 mg (0.35/100 subject-years), and etanercept (0.34/100 subject-years), despite the presence of higher baseline CV risk factors in the two secukinumab groups (**Table 1**). All patients with a MACE had CVD risks, such as hypertension, dyslipidemia, etc. (87). In summary, from this comprehensive analysis of pooled safety data from 10 studies reported that the incidences of MACE in secukinumab-treated patients was low. A very recent real-life observational study of 195 patients (**Table 1**) treated with secukinumab for up to 2 years reported that 2% of patients suffered a CV event yielding a conspicuously elevated IR compared with findings from the secukinumab phase III clinical trial program, although the absolute numbers were very low ($n = 4$) (88).

The other antibody that inhibits IL-17 is ixekizumab; recently, safety data from a 12-week induction period, a 12- to 60-week maintenance period, and from all ixekizumab-treated patients were presented. The analysis of these data essentially reported similar rates of MACE between ixekizumab and etanercept and low rates of MACE with continued exposure for ixekizumab until week 60 (**Table 1**) (89). In particular, during the induction period, the between-group rates of adjudicated MACE were similar. The MACE individual components did not change substantially with longer exposure to ixekizumab. 7 of 4,035 ixekizumab-treated patients experienced vascular death. At baseline, patients subsequently developing MACE had a

higher prevalence of risk factors for acute atherothrombotic events than patients without MACE. In conclusion, ixekizumab use was not associated with an increased risk of MACE. The safety profile reported in this analysis study is consistent with previous reports for ixekizumab (76, 126).

As regarding Brodalumab that selectively targets human IL-17RA and antagonizes the IL-17 pathway, it was considered safety from analysis of a prospective study, indeed incidences of serious adverse events in the induction phase were low among groups. There were no reports of MACEs during the induction phase, and only five reports (exposure-adjusted event rate of 1.0/100 patient-years) through week 52 (**Table 1**) (90). Conversely, in another phase III study, brodalumab treatment was compared with ustekinumab treatment in psoriasis, and the adverse effects were more recurrent in the brodalumab group than in the ustekinumab one. Indeed, in the AMAGINE-2 study, one death by stroke happened during the induction phase, and five deaths occurred through week 52; whereas in the AMAGINE-3 study, two deaths occurred: one from cardiac arrest and one from accidental death (127). However, the sizes of study populations, which were adequate for efficacy and common adverse event assessments, may have been inadequate to assess rare adverse events, which would require longer follow-up of large numbers of patients to better understand the safety profile of brodalumab.

Taken together, results from short-term safety and efficacy trials exploring anti-IL-17 therapy in psoriatic patients suggest no increased CVD risk compared with placebo or other classes of biologics used in Pso. Indeed, we may infer that IL-17 antagonists could possibly play an outstanding role in reducing CV morbidity. Even if there are several limitations in these clinical trials, e.g., patients highly selected, short duration of treatment with placebo or TNFi in relative few number of patients compared with the number of patients receiving IL-17 inhibitors. The advent of IL-17 biologics has improved significantly Pso disease (**Table 2**), but time will tell whether this IL-17 inhibition will also improve CVD outcomes in Pso.

CONCLUSION

Since systemic inflammation is currently considered the main cause of CVD risk in Pso, it has been thought that biological therapies might be helpful in treating not only cutaneous manifestations but have also positive or negative CV effects depending on the specific cytokine target and molecular mode of action. However, despite the recognized efficacy of biological agents in psoriasis treatment, little is known about their impact on risk of MACE in these patients. Data from meta-analyses have suggested that biological therapies including TNFi, anti-IL-12/23p40, and IL-17A agents did not have a significant impact on the risk of MACE in psoriatic patients over the short term. In particular, the use of TNFi is currently associated with a minor CVD risk in these patients, whereas the role of anti-IL-12/23p40 agents remains conflicting. Nevertheless ustekinumab, the only IL-12/23p40 inhibitor currently approved for Pso, results neutral on CV parameters. Regarding newer IL-17 inhibitors, early data have suggested no increased CVD risk respect to placebo or other classes of biologics used in psoriasis. However, because of follow-up short duration, many of these results should be interpreted with caution. Moreover, more studies, involving recruitment of a larger numbers of patients as well as a longer duration of treatment exposure, are needed to better evaluate the impact of biological therapies on the risk of MACEs in patients with Pso and to develop effective strategies for CVD prevention in these patients.

AUTHOR CONTRIBUTIONS

AB designed the manuscript structure, reviewed the scientific literature, and supervised the final version of the manuscript. GC designed the review structure and contributed to the writing of the manuscript. ES contributed to the writing of the manuscript and created graphical illustration. RC and AR reviewed the scientific literature and contributed to the writing of the manuscript. GF and NB reviewed the scientific literature and approved the final version of the manuscript.

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Transcription Factor Retinoid-Related Orphan Receptor γt: A Promising Target for the Treatment of Psoriasis

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Psoriasis, which is a common chronic inflammatory skin disease, endangers human health and brings about a major economic burden worldwide. To date, treatments for psoriasis remain unsatisfied because of their clinical limitations and various side effects. Thus, developing a safer and more effective therapy for psoriasis is compelling. Previous studies have explicitly shown that psoriasis is an autoimmune disease that is predominantly mediated by T helper 17 (Th17) cells, which express high levels of interleukin-17 (IL-17) in response to interleukin-23 (IL-23). The discovery of the IL-23–Th17–IL-17 axis in the development of psoriasis has led to the paradigm shift of understanding pathogenesis of psoriasis. Although anti-IL-17 antibodies show marked clinical efficacy in treating psoriasis, compared with antibodies targeting IL-17A or IL-17R alone, targeting Th17 cells themselves may have a maximal benefit by affecting multiple proinflammatory cytokines, including IL-17A, IL-17F, IL-22, and granulocyte-macrophage colony-stimulating factor, which likely act synergistically to drive skin inflammation in psoriasis. In this review, we mainly focus on the critical role of Th17 cells in the pathogenesis of psoriasis. Especially, we explore the small molecules that target retinoid-related orphan receptor γt (RORγt), a vital transcription factor for Th17 cells. Given that RORγt is the lineage-defining transcription factor for Th17 cell differentiation, targeting RORγt *via* small molecular inverse agonists may be a promising strategy for the treatment of Th17-mediated psoriasis.

Keywords: autoimmune disorder, psoriasis, T helper 17 cells, retinoid-related orphan receptor γt nuclear receptor, retinoid-related orphan receptor γt inverse agonist

INTRODUCTION

Psoriasis is an autoimmune disease with chronic skin inflammation (1), affecting over 125 million people worldwide (up to 2–4% of the world's population) (2). It is predominantly a skin disease, which can manifest itself as various phenotypes, including plaque-type psoriasis or psoriasis vulgaris, guttate psoriasis, pustular psoriasis such as palmoplantar pustulosis, and erythrodermic psoriasis.

Psoriasis vulgaris, a most common type of psoriasis, is characterized by well-defined areas of erythematous and plaques with overlying silvery scale. The main histopathological changes of psoriasis vulgaris include abnormal cell proliferation, parakeratosis, hyperkeratosis, angiogenesis, and inflammatory cell infiltration (1, 3).

Increasing evidence has shown that comorbid cardiovascular diseases are the leading causes of death among patients with psoriasis (4). In addition, a high prevalence of metabolic syndrome, psychosocial distress or psychiatric disorders, chronic kidney disease, and gastrointestinal disease has been demonstrated in individuals with psoriasis (5, 6). The global financial burden associated with the care of psoriatic patients is substantial and significant (7–10). It was reported that the annual costs for treating psoriasis in USA amounted to approximately $112 billion in 2013 (11). As for individuals, patients with psoriasis would incur a lifetime medical expense for relief of physical symptoms and emotional health (12).

THERAPEUTIC CHALLENGES FOR PSORIASIS

Based on the immunological characteristics of psoriasis, researchers have developed topical treatments, including corticosteroids, vitamin D3 analogs and Victoria A acid, and systemic therapies, including methotrexate and cyclosporine, for psoriasis. In clinic, patients with mild-to-moderate plaque psoriasis are usually treated topically with corticosteroids and vitamin D3 analogs, whereas those with moderate-to-severe psoriasis are systemically treated with methotrexate and cyclosporine (13, 14). However, these treatments exhibit low efficacies, poor tolerability, and various adverse reactions (15) (**Table 1**).

Although the introduction of biological treatments, including tumor necrosis factor (TNF)-α antagonists (Efalizumab), anti-TNF antibody (Adalimumab) (16), IL-12/interleukin-23 (IL-23) antagonists (Ustekinumab) (17), and interleukin-17 (IL-17) antagonists (Secukinumab, Ixekizumab, and Brodalumab) (18, 19), has revolutionized the short-term treatment of moderate-to-severe plaque psoriasis, the long-term use of biological therapies may cause loss of efficacy as well as severe adverse reactions, such as infection, cancer, and hepatic dysfunction (20, 21) (**Table 2**). These clinical side effects of existing treatments strongly suggest that it is still urgent to discover safer and more effective therapeutic drugs for psoriasis.

PATHOGENESIS OF AUTOIMMUNE PSORIASIS

To develop a better, safer, and more effective therapy for psoriasis, it is imperative to understand psoriatic pathogenesis. Previous studies have indicated that psoriasis is a skin disease mainly mediated by dendritic cells and T cells although macrophages, neutrophilic granulocytes, keratinocytes, vascular endothelial cells, and the cutaneous nervous system are involved in its pathogenesis (22, 23).

Epidermis-produced antimicrobial peptide LL-37 (cathelicidin), which acts as a dendritic cell activator, is upregulated in the initial phase of psoriasis (24). LL-37 stimulates dermal plasmacytoid dendritic cells to produce interferon-γ (IFN-γ), which in turn activates myeloid dendritic cells (mDCs) to secrete IL-12 and IL-23. IL-12 promotes the differentiation of Th1 cells, whereas IL-23 enhances T helper 17 (Th17) cell development. Th1 cells secrete more IFN-γ and TNF-α to further stimulate mDCs. In addition, Th17 cells secrete IL-17 to stimulate keratinocytes to over-proliferate, causing psoriasis-like lesions (25). Furthermore, the lesion cells secrete a series of chemokines, attracting more immune cells to inflamed tissue, while the damaged cells are digested by macrophages and produce LL-37, forming a positive feedback path that accelerates the development of psoriasis.

TABLE 1 | Traditional treatment for psoriasis.

Traditional treatments	Molecular mechanisms	Adverse reactions
Corticosteroids	Vascular permeability ↓ Skin edema ↓ Neutrophil infiltration ↓ Cell proliferation ↓	Skin atrophy, hair thinning, hypopigmentation, allergic contact dermatitis
Vitamin D3 analogs	Immune modulation Keratinocyte maturation ↓	Hypercalcemia, urinary calcium concentrations increased, tissue calcification
Victoria A acid	The activity of Th1 and Th17 cells ↓ Keratinocyte differentiation	External medicine: itching and burning sensation and erythema, friction at the erythema Oral administration: dry and exfoliated skin, diffuse baldness, denaturation, and skin adhesion
Methotrexate	Inhibition of the enzyme 5-aminoimidazole-4-carboxamide ribonucleotide transformylase Adenosine ↓ Tumor necrosis factor (TNF) and two nuclear factor-κB subunits ↓	Bone marrow toxicity, cirrhosis, nausea, and macrocytic anemia
Cyclosporine	T cell activity ↓	Nephrotoxicity, numerous drug–drug interactions; hypertension, hyperkalemia, increased risk of lymphoma, and squamous cell carcinoma with long-term use
Fumarates	TNF, IL-12, and interleukin-23 production ↓	Gastrointestinal disturbances, flushing, eosinophilia, and proteinuria

TABLE 2 | Biologic therapies for psoriasis.

Biologic therapies	Molecular targets	Adverse reactions
Efalizumab	Tumor necrosis factor (TNF) receptor fusion protein antagonist	Infections, certain malignancies, particularly cutaneous squamous cell carcinoma
Adalimumab	Anti-TNF human monoclonal antibody	Infections and certain malignancies, particularly cutaneous squamous cell carcinoma Serious adverse reactions: active tuberculosis, myocardial infarction, optic neuritis, pancytopenia, lymphoma, etc.
Ustekinumab	Anti-IL-12 and anti-interleukin-23 human monoclonal antibody	Nasopharyngitis, upper respiratory tract infection, headache, diarrhea, muscle pain, dizziness, etc.
Secukinumab	Anti-IL17A human monoclonal antibody	The development of *Candida* infections Special adverse reaction: neutropenia
Ixekizumab	Anti-IL-17A human monoclonal antibody	The development of *Candida* infections
Brodalumab	Anti-IL-17A receptor human monoclonal antibody	The development of *Candida* infections suicidal ideation

Recently, LL-37 has been proved to be a T-cell-reactive autoantigen in psoriasis. LL37-specific $CD4^+$ T cells can produce Th17-related cytokines (26). In summary, these results indicate that psoriasis is an autoimmune disease mediated by dendritic cells and T-cells (**Figure 1**).

THE MAIN ROLE OF PATHOGENIC Th17 CELLS IN PSORIASIS

T helper 17 cells are a distinct subset of T helper cells that mainly produce IL-17A, IL-17F, and IL-22. Mounting evidence shows that there are two subsets of Th17 lineages. A non-pathogenic subset of Th17 cells induced by TGF-β1 and IL-6 has an important role in host defense against specific pathogens by producing IL-17 and IL-10 (27). The production of IL-10 by non-pathogenic Th17 cells restrains Th17 cell-mediated pathology so that they are incapable of promoting autoimmune inflammation. On the other hand, differentiation of highly pathogenic Th17 cells from naïve T cells occurs in the presence of IL-23, IL-6, and TGF-β1 (28, 29). More precisely, exposure to IL-23 diminishes the anti-inflammatory cytokine IL-10 in developing Th17 cells (27). In addition, IL-23 stabilizes and reinforces Th17 phenotypes by increasing expression of IL-23 receptor (30, 31) and endowing Th17 cells with pathogenic effector functions (32–34). These pathogenic Th17 cells contribute to various autoimmune diseases (35, 36).

Psoriasis is primarily characterized as a Th1-driven disease because the levels of Th1 cytokines, such as IFN-γ, TNF-α, and interleukin (IL)-12, are markedly elevated in psoriatic lesions, while there is no such an increase in expression of Th2 cytokines (IL-4, IL-5, and IL-13) (37–39). With the characterization of a distinct subset of Th17 cells, the research field of psoriasis has experienced a major paradigm shift.

Indeed, previous results have confirmed that pathogenic Th17 cells play a central role in the development of psoriasis(40, 41). Pathological or immunohistochemical studies on psoriasis have shown that skin lesions are mainly infiltrated by Th17 cells. In addition, IL-23, which is produced by activated mDCs, drives naïve T cells to develop into pathogenic Th17 cells (42). IL-17, which is predominantly produced by pathogenic Th17 (43), is significantly elevated in patients with psoriasis compared with healthy subjects. Upregulated IL-17 has potent ability to recruit neutrophils (44, 45), to activate T cells, to stimulate fibroblasts (46), and to promote development of multiple lineages of macrophages (47, 48). Moreover, pathogenic Th17-secreted IL-17 induces proliferation of keratinocytes and secretion of antimicrobial peptides, cytokines, and chemokines, which in turn recruit more immune cells to inflamed tissue. This positive feedback loop between Th17 cells and keratinocytes has been proved to contribute to the chronic inflammatory phase of psoriasis (43, 49, 50). Other proinflammatory factors released by pathogenic Th17 cells, such as IL-22, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF), stimulate keratinocytes to release chemokines, further sustaining the inflammatory cycle to promote the development of psoriasis (51, 52).

RETINOID-RELATED ORPHAN RECEPTOR γt (RORγt): A LINEAGE-DEFINING TRANSCRIPTION FACTOR FOR Th17 CELLS

The differentiation of Th17 cells, similar to that of Th1 and Th2 subsets (53, 54), relies on the action of a lineage-specific transcription factor, identified as the orphan nuclear receptor RORγt (55). RORγt, encoded by RORC2, is an isoform of RORγ that belongs to the NR1F subfamily of orphan receptors, including RORα and RORβ. Previous studies have indicated that RORγt is both necessary and sufficient for Th17 cell differentiation in mouse and human $CD4^+$ T cells. Ivanov et al. reported that T cells lacking RORγt ($Rorc^{-/-}$) failed to differentiate into Th17 cells even under Th17-polarizing culture conditions, while over-expression of Rorc in naïve $CD4^+$ T cells was sufficient to accelerate the expression of Th17-related cytokines and chemokines, including IL-17A, IL-17F, IL-22, IL-26, CCR6, and CCL20. Moreover, mice lacking RORγt were much less susceptible to experimental autoimmune encephalomyelitis (EAE), and $CD4^+$ splenocytes from those mice could not induce the disease (55). A similarly crucial role for RORγt in human Th17 cells was also demonstrated (56). IL-6 and IL-23 signals strongly phosphorylated and dimerized signal transducer and activator of transcription 3 (STAT3), resulting in enhanced expression and nuclear translocation of RORγt,

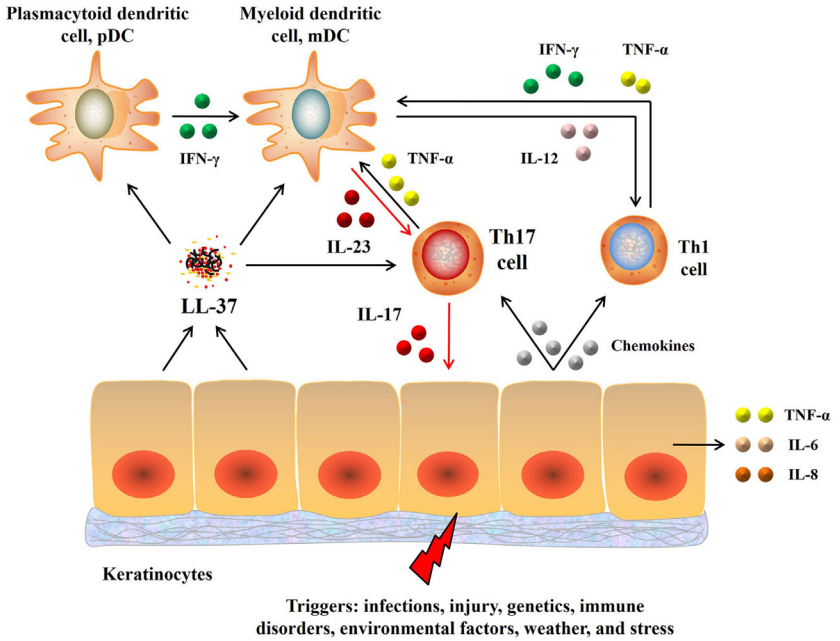

FIGURE 1 | Pathogenesis of psoriasis. Upon activation, keratinocytes secrete LL-37 that in turn activates dendritic cells, which then produce IL-23 and IL-12. IL-23 induces differentiation of naive T cells into Th17 cells that then overproduce IL-17 and IL-22. IL-17 activates keratinocytes, promotes epidermal hyperplasia and recruits proinflammatory cells, resulting in a positive proinflammatory feedback that accelerates the development of psoriasis. Moreover, IL-12 produced by dendritic cells also promotes the differentiation of Th1 cells that in turn produce Th1 cytokines, including IFN-γ. Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN-γ, interferon-γ; Th17, T helper 17; IL-23, interleukin-23; IL-17, interleukin-17.

which then promoted Th17 responses by activating Th17 gene promoters, including Il17a, Il17f, Il22, Il26, Il23r, Csf-2, Ccr6, and Ccl20. In addition, IL-23 signaling-induced transcription factor Blimp-1 enhanced pathogenic Th17 function by co-localizes RORγt and STAT-3 at *Il17a, Il23r,* and *Csf-2* enhancer sites (34, 57, 58) (**Figure 2**). Interestingly, neither IL-23 nor IL-6 alone was sufficient to effectively generate Th17 cells (59). Nevertheless, either IL-23 or IL-6 induced IL-17 production by naïve precursors in the presence of IL-1β rather than TGF-β. T-bet + RORγt + Th17 cells were generated without TGF-β and were pathogenic in an EAE animal model, indicating an alternative pathway for Th17 differentiation (59).

Taken together, previous studies have confirmed an essential role of RORγt in the differentiation of pathogenic Th17 cells. Given that pathogenic Th17 cells play such a pivotal role in the pathogenesis of psoriasis, targeting Th17 cells, especially *via* blocking RORγt, may be a good option for treating psoriasis. In addition, RORγt might be a uniquely tractable drug target by virtue of being a nuclear receptor. Therefore, RORγt can be an attractive pharmacologic target for the treatment of Th17-mediated autoimmune diseases, including psoriasis.

SMALL MOLECULES TARGETING RORγt

Retinoid-related orphan receptor γt contains identical DNA-binding domain and ligand-binding domain (LBD). Like other nuclear receptors, the binding of ligands to the region LBD causes a conformational change, which results in recruiting transcriptional co-activators as well as activating transcriptional activity.

Since RORγt receptor was initially identified as an orphan receptor, its endogenous ligands attracted more attention at first. Previous studies have revealed that several oxysterols are endogenous modulators of RORγt activity with high-affinity. For example, 7-oxygenated sterols function as high-affinity ligands for RORγt *via* directly binding their LBDs, modulating co-activator binding, and suppressing the transcriptional activity of the receptors (60). In addition, 24S-hydroxycholestrol acts as an inverse agonist that suppresses the transcriptional activity of RORγt (61).

To develop potent synthetic RORγt ligands that selectively suppress pathogenic effector functions of Th17 cells, researchers have used many strategies to screen for potentially regulatory drug candidates, as described below.

Digoxin, the cardiotonic glycoside extracted mainly from *Digitalis lanata*, has been identified as a specific inhibitor of RORγ transcriptional activity without affecting other nuclear hormone receptors, including human androgen receptor (AR) and liver X receptor α (62). It specifically inhibits IL-17 production by Th17 cells. Moreover, it is effective in attenuating EAE in mice and decreasing the disease severity in a rat model of arthritis (62–64). However, it is toxic for human cells at high doses and may cause some adverse reactions, including arrhythmia, nausea, vomiting, blurred vision, diarrhea, depression, and even lethargy. Further studies have shown that derivatives of digoxin, such as Dig(dhd) 20,22-dihydrodigoxin-21,23-diol, and Dig(sal) digoxin-21-salicylidene, specifically inhibit the differentiation of Th17 cells in

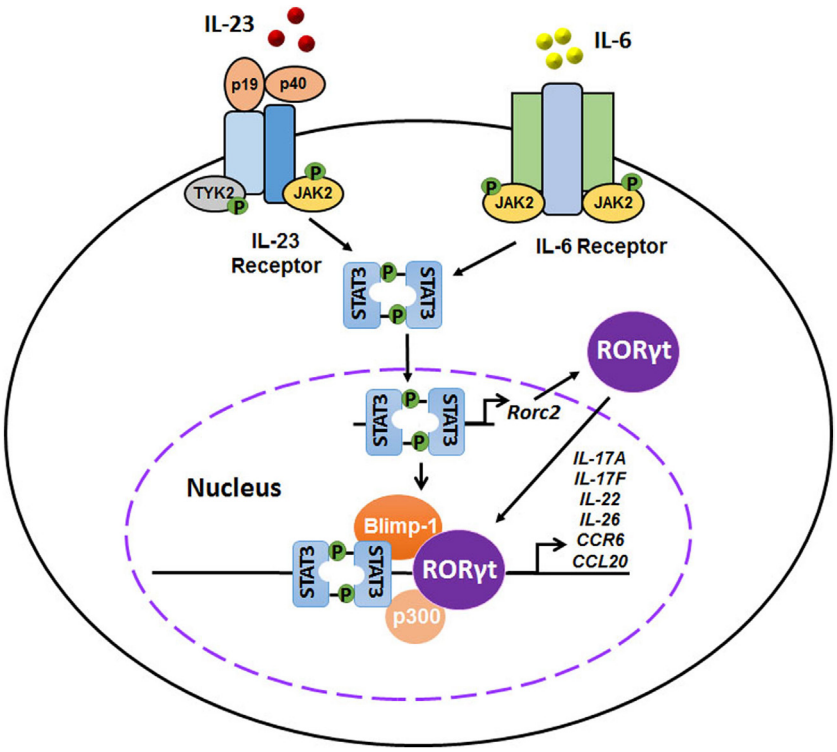

FIGURE 2 | Interplays of interleukin-23 (IL-23), IL-6, signal transducer and activator of transcription 3 (STAT3), and retinoid-related orphan receptor γt (RORγt) in the differentiation of pathogenic T helper 17 (Th17) cells. IL-23 and IL-6 signals activate the JAK–STAT signaling pathway, inducing a strong phosphorylation and dimerization of STAT3. STAT3 homodimers induce the expression and nuclear translocation of RORγt, which in turn promotes Th17 responses by activating Th17 gene promoters, including *Il17a*, *Il17f*, *Il22*, *Il26*, *Il23r*, *Csf-2*, *Ccr6*, and *Ccl20*. In addition, IL-23 signaling-induced transcription factor Blimp-1 enhances pathogenic Th17 function by co-localizes RORγt and STAT-3 at *Il17a*, *Il23r*, and *Csf-2* enhancer sites.

human CD4$^+$ T cells without significant toxicity (62), indicating that nontoxic derivatives of digoxin may be utilized as chemical templates for the development of RORγt negative regulators.

SR1001, a derivative of liver X receptor agonist, is capable of suppressing the transcriptional activity of RORα and RORγ (65). It is a high-affinity synthetic ligand that can bind the LBD of RORα and RORγ, resulting in inhibition of murine Th17 cell differentiation and IL-17 expression by inducing conformational changes that in turn suppress the receptors' transcriptional activity. Thence, SR1001 might be an attractive lead compound for drug development to treat Th17-mediated autoimmune diseases, such as psoriasis as well as RORα- and RORγ-mediated metabolic diseases (66, 67).

SR2211, a derivative of SR1001, only binds the LBD of RORγ and inhibits the transcriptional activity of RORγ without affecting RORα function (68). In addition, SR2211 suppresses the intracellular expression of IL-17 and has potential utility for the treatment of inflammatory diseases, such as experimental arthritis (69, 70). SR2211 has been shown to diminish genome-wide AR binding, H3K27ac abundance and expression of the AR target gene networks, and it could serve as a potential drug for the treatment of castration-resistant prostate cancer (71).

Ursolic acid (UA), a small molecule present in medicinal herbs such as *Prunella vulgaris* L., effectively inhibits the function of RORγt, resulting in greatly reduced IL-17 expression in both developing and differentiated Th17 cells (72, 73). However, UA also has other cellular targets, including the liver kinase B1–AMP-activated protein kinase (74), the NFE2-related factor 2 (75), nuclear factor-κB (76), and STAT3 pathway (77, 78), suggesting that it is not RORγt-specific *in vivo*.

TMP920, which can displace RORγt from its target loci, suppresses Th17 cell differentiation and Th17 signature gene expression (79). Based on TMP920, additional inverse agonists are developed, including TMP778, which exhibits an increase in potency and specificity. It predominantly affects RORγt transcription without removing DNA binding (79). Interestingly, the diastereomer of TMP778 or TMP776 displays no inverse agonist activity against RORγt. In experiments *in vivo*, TMP778 suppresses imiquimod-induced cutaneous inflammation and attenuates EAE. Furthermore, TMP778 also reduces expression of Th17-signature genes in cells isolated from the blood and skin of psoriatic patients (80).

Other RORγt inverse agonists have also been discovered. Using a scaffold hybridization strategy, a series of carbazole carboxamides are found to be potent RORγt inverse agonists (81). In addition, MG 2778, a cyclopenta[a]phenanthrene derivative, is identified as a lead compound for developing synthetic steroidal inverse agonists of RORγt (82). Furthermore, TAK-828F, a potent and selective RORγt inverse agonist, strongly inhibits Tc17 and Th17 cell differentiation from naive T cells and memory CD4$^+$

T cells without affecting Th1 cell differentiation (83). In another study, Barbay et al. have identified 6-substituted quinolines as modulators of RORγt using a RORγt-driven cell-based reporter assay. They have further elucidated the interaction between 6-substituted quinolones and RORγt in an X-ray crystal structure (84). Moreover, A213, a potent and selective antagonist of RORγt, is found to inhibit Th17 cell differentiation *in vitro*. It also attenuates psoriatic skin lesion in two different mouse models by suppressing IL-17 production (85).

Taken together, previous studies have implicated a potential therapeutic application of RORγt antagonist for the treatment of Th17-mediated diseases, including psoriasis. Especially, targeting RORγt for the treatment of cutaneous inflammatory disorders may afford additional therapeutic benefits over existing modalities, in which only one Th17 cytokine such as IL-17A is targeted. However, the small molecules targeting RORγt could generate unwanted or unexpected results given that they may exert off-target effects *in vivo*. Those molecules must undergo rigorous clinical trials prior to a clinical application to carefully evaluate their potential side effects. In addition, other types of immune cells, including type 3 innate lymphoid cells, $CD8^+$ IL-17-producing (Tc17) cells, γδT, and even Treg cells, may also express RORγt. Target RORγt could affect these cells as well. Thus, strategies targeting RORγt in Th17 cells are preferred so that we can attenuate Th17-mediated inflammation while limiting potential side effects.

SUMMARY AND OUTLOOK

Since there are many limitations of traditional and biological treatments for psoriasis, it is important to develop more effective and safer therapies of psoriasis. The finding of RORγt/Th17/IL-17 signaling pathway has provided further insights into the pathogenesis of psoriasis. Compared with antibodies targeting IL-17A or IL-17R alone, targeting Th17 cells themselves might benefit psoriatic patients to a greatest extent by impacting multiple proinflammatory cytokines (IL-17A, IL-17F, IL-22, and GM-CSF) that are likely to act synergistically to drive psoriatic inflammation. Hence, targeting RORγt *via* small molecule inverse agonists is a promising strategy for treating psoriasis *via* suppressing Th17 cell differentiation. Furthermore, small molecules disrupting RORγt are also expected to be safer than global immunosuppressive agents, such as cyclosporine. However, there are several challenges that need to be overcome. Researchers should generate safer and more potent compounds. Moreover, rigorous clinical studies are needed to assess their actual clinical efficacy and side effects since they could generate off-target effects. In conclusion, given the importance of Th17 cells and their proinflammatory cytokines in the pathogenesis of psoriasis, targeting RORγt seems to be a promising approach to treating psoriasis effectively and perhaps safely.

ETHICS STATEMENT

The epidemiological data were cited without any commercial or financial uses.

AUTHOR CONTRIBUTIONS

LT and XY wrote the manuscript; YL and HX searched the literature; ZD and GZ edited the paper.

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PSORI-CM02 Formula Increases CD4+ Foxp3+ Regulatory T Cell Frequency and Ameliorates Imiquimod-Induced Psoriasis in Mice

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Psoriasis is an autoimmune and inflammatory disease, which is estimated to affect 2–3% of the population in the world. PSORI-CM02 is an empirical formula of Chinese medicine optimized from Yin Xie Ling, which is widely used to treat psoriasis in China for decades. However, its antipsoriatic mechanisms are still not well understood. Here, we explored the therapeutic effects of PSORI-CM02 on psoriasis and its mechanisms of action in imiquimod-induced psoriasis-like mouse models and human HaCaT cells. In experiments *in vitro*, PSORI-CM02 significantly inhibited HaCaT cell proliferation in dose-dependent and time-dependent manners. Furthermore, it hindered the progression of HaCaT cell cycle and arrested HaCaT cells at G1 phase. On the other hand, our *in vivo* studies demonstrated that PSORI-CM02 dramatically reduced psoriasis area and severity index scores and lesion temperature in imiquimod-induced psoriatic mice. The antioxidative activities of glutathione, catalase, and superoxide dismutase were increased while oxidative activity of malonaldehyde was markedly decreased after treatments with PSORI-CM02. PSORI-CM02 also suppressed the mRNA expression of proinflammatory cytokines, including TNF-α, IL-6, and IL-17, and lowered their protein levels in the serum as well. In addition, PSORI-CM02 could reduce the expression of IKKα and NF-κB in psoriatic skin tissue. It also upregulated the proportion of CD4+ Foxp3+ regulatory T cells (Tregs) in both lymph nodes and spleens and promoted CD4+ CD25+ Treg proliferation *in vitro*. Taken together, our research demonstrated that PSORI-CM02 inhibited HaCaT cell proliferation by arresting them at G1 phase and alleviated systemic inflammation and psoriasis in mice *via* altering the oxidative/anti-oxidative status, tipping the balance between Th17 responsiveness and CD4+ Foxp3+ Treg generation, and suppressing the expression of proinflammatory cytokines as well as NF-κB signaling.

Keywords: psoriasis, inflammation, immunoregulation, regulatory T cell, PSORI-CM02

Abbreviations: CAT, catalase; DXM, dexamethasone acetate; GSH, glutathione; IMQ, imiquimod; MDA, malonaldehyde; PASI, psoriasis area and severity index; SOD, superoxide dismutase; Treg, regulatory T cell.

INTRODUCTION

Psoriasis is an autoimmune and inflammatory dermatologic disease, which affects about 2–3% of population in the world. Psoriatic pathology mainly includes thickening of epidermis, hyperproliferation of keratinocytes and parakeratosis in the epidermis with massive neutrophil infiltration in the dermis (1). Currently, the mechanisms underlying psoriasis have not been fully explored although it has been established that many factors, including genetic factors, environmental factors, immunological mechanisms, new blood vessel formation, lipid metabolism disorders, and unhealthy mentality, bear significant impacts on the occurrence of psoriasis (2). Nowadays, accumulating evidence suggests that patients with moderate or severe psoriasis may increase the risk of other diseases, including obesity, cancer, diabetes mellitus and the metabolic syndrome (3). Meanwhile, current treatments for psoriasis, such as topical therapies, phototherapies and conventional systemic therapies, are not completely satisfied by sufferers principally because of side effects and economic burden (4, 5).

During recent decades, Chinese herbal medicine, one of the traditional Chinese medicine that serves as a complementary and alternative medicine, has been used as popular strategies for treating psoriatic patients and aroused a remarkable and growing interest (6). Our previous researches have shown that Chinese herbal medicine can provide an effective therapy for psoriasis (7–10). PSORI-CM02, which was optimized based on Chinese herbal formula Yin-Xie Ling discovered by well-known professor Guo-Wei Xuan, is a novel formula of Chinese medicine that has been used to effectively treat psoriasis during recent years. PSORI-CM02 consists of five Chinese herbs (**Table 1**), including *Rhizoma curcumae*, *Radix paeoniae rubra*, *Sarcandra glabra*, *Rhizoma smilacis glabrae*, and *Fructus mume*. Currently, PSORI-CM02 formula is further undergoing a randomized, double-blinded and placebo-controlled clinical trial for treating stable psoriasis vulgaris with a syndrome pattern of blood stasis in our hospital.

The objective of our current work was to reveal the antiproliferative properties of PSORI-CM02 in human HaCaT cells *in vitro* and the therapeutic effects of PSORI-CM02 on imiquimod-induced murine psoriasis as well as its mechanisms of action. We found that PSORI-CM02 suppressed HaCaT cell proliferation by hindering their cell cycle progression at G1 phase, inhibited the expression of proinflammatory cytokines and NF-κB signaling, upregulated CD4+ Foxp3+ regulatory T cells (Tregs) *in vivo* and promoted their *in vitro* expansion as well while reducing IL-17 production and ameliorating murine psoriasis.

TABLE 1 | Constituents of PSORI-CM02.

Linnean classification	Botanical origin	Ratio
Curcumae Rhizoma	*Curcuma kwangsuensis* S.G. Lee et C.F. Liang	2
Radix Paeoniae Rubra	*Paeonia lactiflora* Pall.	3
Rhizoma Smilacis Glabrae	*Smilax glabra* Roxb.	5
Mume Fructus	*Prunus mume.* (Sieb.)Sieb.etZucc.	2
Sarcand Raeherba	*Sarcandra glabra* (Thunb.)Nakai	5

MATERIALS AND METHODS

Animals

BALB/c mice (male, weighing 20 ± 2 g) were purchased from the Center of Laboratory Animals of Southern Medical University (Guangzhou, China). Mice were housed in a standard housing room with controlled temperature (22 ± 2°C), relative humidity (45–55%), artificial light (12 h light/dark cycle), and provided free access to food and water under a specific pathogen-free environment. The animal protocols were approved by the Animal Experimental Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine.

Chemicals

Minimal essential medium (MEM), fetal bovine serum (FBS), and antibiotics (penicillin–streptomycin) were purchased from Gibco (Carlsbad, CA, USA). Dexamethasone acetate (DXM) was obtained from Shanghai Xinyi Pharmaceutical Factory (Shanghai, China). Imiquimod cream was obtained from Sichuan Mingxin Pharmaceutical Co., Ltd. (Sichuan, China). Eighteen chemical standards, including citric acid, gallic acid, 5-hydroxymethylfurfural, protocatechuic acid, Quercitrin, were obtained from Shanghai Aladdin Biological Technology Co., Ltd. (Shanghai, China) or Sigma-Aldrich (St. Louis, MO, USA).

Preparation of PSORI-CM02

Five Chinese herbal components (**Table 1**) contained in PSORI-CM02 formula were purchased from Guangdong Kangmei Pharmaceutical Company Ltd. (Guangdong, China). These herbs were extracted using distilled water and the extract was concentrated and stored for the future study.

Ultrahigh-Performance Liquid Chromatography (UHPLC) Analysis

Different batches of PSORI-CM02 formula were monitored for quality control purposes by UHPLC method. Briefly, PSORI-CM02 and 18 standards (**Table 2**) were dissolved with methanol–0.1%

TABLE 2 | Eighteen chemical constituents identified in PSORI-CM02.

Peak number	Formula	Identification
1	$C_6H_8O_7$	Citric acid
2	$C_7H_6O_5$	Gallic acid
3	$C_6H_6O_3$	5-Hydroxymethylfurfural
4	$C_7H_6O_4$	Protocatechuic acid
5	$C_{16}H_{18}O_9$	Neochlorogenic acid
6	$C_{23}H_{28}O_{12}$	Oxypaeoniflorin
7	$C_{20}H_{27}NO_{11}$	Amygdalin
8	$C_{16}H_{18}O_9$	Chlorogenic acid
9	$C_9H_8O_3$	p-Coumaric acid
10	$C_{16}H_{16}O_8$	5-O-caffeoylshikimic acid
11	$C_{11}H_{10}O_5$	Isofraxidin
12	$C_{21}H_{22}O_{11}$	Neoastilbin
13	$C_{21}H_{22}O_{11}$	Astilbin
14	$C_{21}H_{22}O_{11}$	Neoisoastilbin
15	$C_{21}H_{22}O_{11}$	Isoastilbin
16	$C_{21}H_{22}O_{10}$	Engeletin
17	$C_{18}H_{16}O_8$	Rosmarinic acid
18	$C_{21}H_{20}O_{11}$	Quercitrin

formic acid. Chromatographic separation was carried out with an Accela™ UHPLC system, which was comprised of a UHPLC pump and a PDA detector with a scanning from 200 to 400 nm and recorded at 214 nm. The HPLC conditions were set as following: Column: Kintex® C18, 150 mm × 2.1 mm, 2.6 μm particle size (Phenomenax, USA); Mobile phase components: A was water with 0.1% formic acid and B was methanol; Flow rate: 250 μL/min; injection volume: 10 μL; gradient: 0–45 min, linear gradient of 10–35% A, 45–50 min, 35–46% A, 50–60 min, 46–85% A.

Cell Culture

HaCaT cell line was purchased from American Type Culture Collection (Manassas, VA, USA). HaCaT cells were cultured in MEM (Gibico, USA) medium with 10% FBS (Gibico, USA) and 100 mg/mL and 100 IU/mL penicillin/streptomycin in a humidified atmosphere (5% CO_2, 37°C).

HaCaT Cell Proliferation Assays *In Vitro*

In vitro HaCaT cell proliferation was measured using MTT assays. Briefly, HaCaT cells in logarithmic growth were collected and transferred into a 96-well microplate. After 24 h, PSORI-CM02 was added to each well to make various concentrations (125, 250, 500, and 1,000 μg/mL, respectively) with six replicate wells *per* concentration. After further incubation for 24, 48, and 72 h, 10 μL of 5 mg/mL MTT was added to each well and incubated at 37°C for an additional 4 h. The supernatant then was removed and 100 μL of DMSO was added into each well. The absorbance (A value) was measured at the wavelength of 490 nm. The cell proliferation was presented as an OD value.

Cell Cycle Analysis

HaCaT cells were placed into six-well plates at 1.0×10^6 cells/well and treated with various concentrations of PSORI-CM02 (125, 250, and 500 μg/mL) for 72 h. The cells were collected, rinsed twice with ice cold PBS and then fixed in 70% ethanol at 4°C overnight. The cells then were subject to a 30-min incubation with 250 μl of RNase A (100 μg/ml) at 37°C and propidium iodide (50 μg/ml, 500 μl) staining for 1 h. Stained cells finally were analyzed *via* a FACS-Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Three independent experiments were carried out.

Administration of Drugs

BALB/c mice were randomly grouped into six groups ($n = 6$ *per* group). The control groups were normal mice that were totally untreated. Vehicle and treatment groups, but not control groups, were given topical administration of imiquimod cream to induce psoriasis. Vehicle groups were administrated with distilled water while treatment groups of the mice were orally given DXM (10 mg/kg) or PSORI-CM02 (3, 6, and 12 g/kg, respectively), which was dissolved in distilled water, for a period of seven days. For *in vitro* experiments, control groups were the untreated cells in the media.

Imiquimod-Induced Psoriasis-Like Mouse Model

According to our previous studies, mice were topically administrated with a dose of 62.5 mg of 5% imiquimod cream applied to a shaved area (3 cm × 2.5 cm) on their back for seven consecutive days. The psoriasis area and severity index (PASI), which comprised parameters for skin erythema, scaling, and thickness, was employed to assess the condition of the psoriasis-like lesion on day 7. Parameters were scored independently on a scale from 0 to 4 according to the clinical signs, in which "0" represents none; "1" denotes slight; "2" means moderate; "3" depicts marked; and "4" indicates very marked clinically.

Measurement of Mouse Temperature by Infrared Thermal Image

The temperature of mice in each group was determined by the infrared thermal imager (Fluke, USA) in a quiet-state mode and photographs were analyzed by Smart View 3.2 software. Mice were then euthanized and blood samples were collected in the absence of anticoagulants and subjected to centrifugation (3,000 rpm) for 10 min to obtain serum for biochemical analyses.

Histological Analysis

The dorsal skin of the mice was harvested, fixed in 4% paraformaldehyde in PBS, and embedded in paraffin. Sections (5 μm) were then made and stained with hematoxylin and eosin (H&E) for histological analysis.

Measurements of Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), and Malonaldehyde (MDA)

Skin tissues were homogenized in Tris-buffer (20 mM, pH 7.5) on ice using Ultra Turraks Homogenizer (IKA, Germany) and were subject to centrifugation (12, 000 rpm) at 4°C for 10 min. The supernatant was used to measure CAT and SOD activities and GSH and MDA levels. The protein concentration was measured using the Bradford method with BSA serving as a standard. The CAT and SOD activities and GSH and MDA levels were analyzed using commercial assay kits following the manufacturer's instructions (Jiancheng Company, Nanjing, China).

Measurements of TNF-α, IL-6, and IL-17 *via* Enzyme-linked Immunosorbent Assay (ELISA) and RT-PCR

To evaluate the serum levels of TNF-α, IL-6, and IL-17, commercially available ELISA kits (eBioscience, USA) were utilized based on the instructions. The absorbance was read at 450 nm with a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, USA). Total mRNA was extracted from skin tissue with Trizol reagents and mRNA was then transcribed to cDNA. The primer sequences were shown in **Table 3**. The relative mRNA expression levels of cytokines versus GAPDH were measured using an ABI 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA).

Western Blotting Analysis

Total protein samples from skin lesion tissues were acquired with RIPA lysis buffer followed by centrifugation (12,000 rpm and 5 min) in 4°C. Protein samples were subject to fractionation by SDS-PAGE and electro-transferred to PVD membranes. The

TABLE 3 | Primer sequences of target genes.

Target gene	Primer sequence (5′ → 3′)
TNF-α (forward)	ACTGATGAGAGGGAGGCCAT
TNF-α (reverse)	CCGTGGGTTGGACAGATGAA
IL-6 (forward)	TTCTTGGGACTGATGCTGGT
IL-6 (reverse)	CCTCCGACTTGTGAAGTGGT
IL-17 (forward)	TCAAAGCTCAGCGTGTCCAA
IL-17 (reverse)	TCTTCATTGCGGTGGAGAGTC
GAPDH (forward)	CAGGTTGTCTCCTGCGACTT
GAPDH (reverse)	TATGGGGGTCTGGGATGGAA

membranes were then blocked with 5% (w/v) skim milk in TBS-T containing 0.1% Tween-20 at room temperature for 2 h and subsequently incubated with primary antibody at 4°C overnight. Then, the membranes were washed three times using TBS-T and blotted with a corresponding secondary antibody conjugated with horseradish peroxidase for 1 h. Finally, the protein bands were detected using the enhanced chemiluminescence detection reagents. The band intensity was quantified using Image J software (NIH Image, Bethesda, MD, USA).

CD4+ Foxp3+ Treg Quantification

Lymph node and spleen cells from mice were prepared after treatments with PSORI-CM02. Cells were stained for surface markers with anti-CD4-PE/CD25-FITC Abs and then an intracellular marker with anti-Foxp3-APC using intracellular fixation/permeabilization kit (eBioscience, San Diego, CA, USA). CD4+ Foxp3 Tregs were analyzed using a FACSCalibur (BD, Biosciences). Since we found that only ~94% of CD4+ CD25+ T cells were FoxP3-positive (Figure S1 in Supplementary Material) in imiquimod-induced psoriatic mice, we quantified CD4+ FoxP3+ rather than CD4+ CD25+ cell population and defined the former as Tregs.

CD4+ CD25+ Treg Proliferation *In Vitro*

Splenic cells of C57BL/6J were harvested and CD4+ CD25+ T cells were purified by high-speed cell sorting *via* a FACSAria III (BD Biosciences). The purity of the cells was typically >96%. Then, the purified Tregs were labeled with 2 μM CFSE dye (Invitrogen, Karlsruhe, Germany) at 37°C for 10 min, and cultured in 96-well plates (2×10^5 cells/well) coated with anti-CD3/anti-CD28 Abs in complete RPMI-1640 media. PSORI-CM02 (125, 250, and 500 μg/mL) was also added to the medium. After culturing for 4 days, cell proliferation was analyzed *via* FACS analyses.

Statistical Analysis

The data were statistically evaluated by a one-way analysis of variance followed by Dunnett's test and denoted as mean values ± SDs. Statistically significant differences were identified as either $P < 0.05$ or $P < 0.01$. All analyses were carried out through SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Chemical Profiles of PSORI-CM02

In order to control the quality of PSORI-CM02, we mainly detected eighteen chemical compositions of PSORI-CM02 by UHPLC analysis: citric acid, gallic acid, 5-hydroxymethylfurfural, protocatechuic acid, and so on (**Table 2**). UHPLC profiling also showed that PSORI-CM02 did not contain rapamycin and cyclosporine (Figure S2 in Supplementary Material).

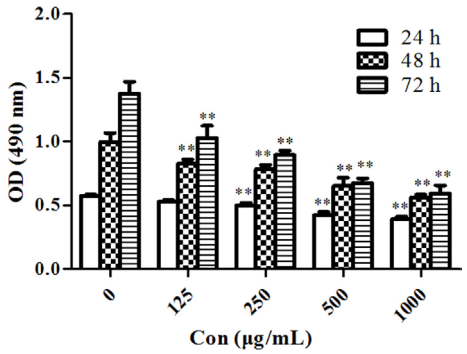

FIGURE 1 | PSORI-CM02 suppresses HaCaT cell proliferation. The proliferation of HaCaT cells *in vitro* was measured *via* MTT assays 24, 48, and 72 h after the cell culture. Data are shown as the mean values ± SDs ($n = 6$, $^*P < 0.05$ and $^{**}P < 0.01$ vs. the control group: concentration of 0 μg/ml). The results suggested that PSORI-CM02 suppressed HaCaT cell proliferation in a dose-dependent manner.

PSORI-CM02 Inhibits the Growth of HaCaT Cells

MTT assay was performed to evaluate the antiproliferative effects of PSORI-CM02 on HaCaT cells *in vitro*. As shown in **Figure 1**, PSORI-CM02 at various concentrations ranging from 125 to 1,000 μg/mL significantly suppressed HaCaT cell proliferation at all time points (24, 48, and 72 h) in a dose-dependent manner.

PSORI-CM02 Induces Cell Cycle Arrest in HaCaT Cells

Since cell cycle progression plays an essential role in cell proliferation, flow cytometry was employed to investigate cell cycle distribution in HaCaT cells after treatments of the cells with PSORI-CM02. As shown in **Figures 2A,B**, there was no significant difference in HaCaT cell cycle progression at all phases between the group treated with low concentration of PSORI-CM02 (L) and control group. Augmenting PSORI-CM02 concentration to 250 (M) or 500 (H) μg/mL increased the frequency of the cells at G1 phase while reducing their percentage at S phase. Taken together, these studies revealed that PSORI-CM02 formula induced HaCaT cell cycle arrest at G1 phase in a dose-dependent manner.

PSORI-CM02 Alleviates Clinical Symptoms and Reduces Skin Temperature of Imiquimod-Induced Psoriatic Mice

Imiquimod-induced psoriasis-like mouse models were utilized to evaluate the antipsoriatic effects of PSORI-CM02. After treatments with imiquimod cream for three days, the clinical signs of psoriasis-like lesions, including skin erythema, scaling,

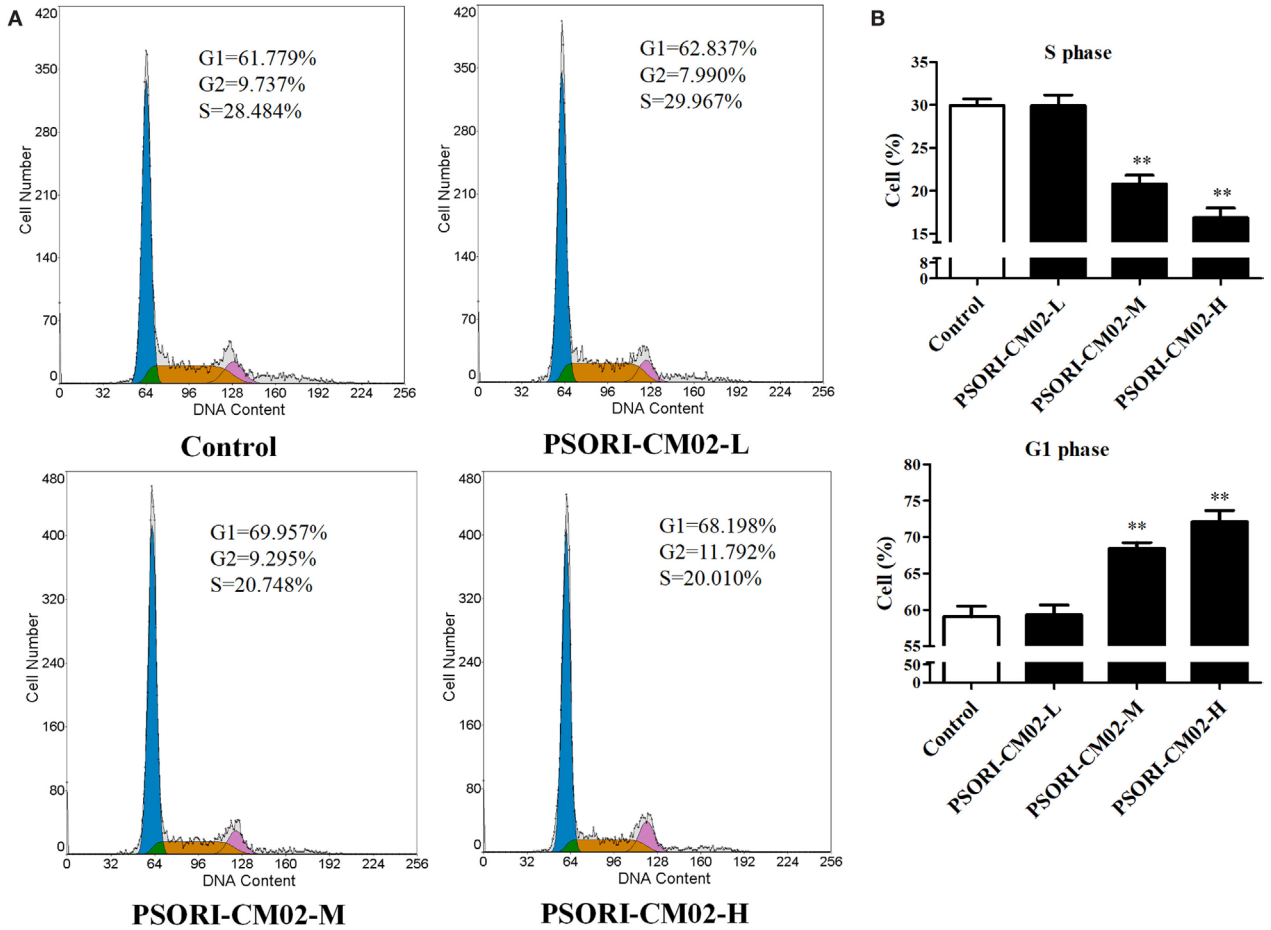

FIGURE 2 | PSORI-CM02 hinders HaCaT cell cycle progression. The cell cycles of HaCaT cells treated with PSORI-CM02 for 72 h were analyzed through flow cytometry using PI staining **(A)**. Histograms exhibited cell cycle distributions (%) in HaCaT cells **(B)**. Results represented the average proportions of S and G1 phases. Data are shown as the mean values ± SDs ($n = 3$, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control group).

and thickness, appeared on the shaved dorsal skin, indicating that imiquimod-induced psoriasis-like mouse models were established successfully. Seven days after treatments with PSORI-CM02 or DXM, overall skin lesions were reduced and average PASI scores were decreased significantly compared with the vehicle group, as shown in **Figure 3A**. Mice treated with high doses of PSORI-CM02 appeared to be healthy except for mild psoriatic skin lesions. Histology demonstrated no liver and kidney injury in these mice (data not shown), suggesting that PSORI-CM02 is not toxic.

Since temperature assessment is one of the important indexes in inflammation, lesion temperature was measured in this study. As shown in **Figure 3B**, the psoriasis-like lesion temperature was increased significantly ($P < 0.01$ vs. control group) seven days following administration of imiquimod cream while PSORI-CM02 treatments lowered the temperature remarkably compared to the vehicle group.

Histological Evaluations

Histological examinations *via* H&E staining were performed on the lesion skin seven days after treatments with PSORI-CM02. As depicted in **Figure 3C**, we found significant pathological changes characterized by increased acanthosis, hyperkeratosis of the epidermis, and abundant inflammatory infiltrates in the skin of imiquimod-induced psoriatic mice. Treatments with either DXM or PSORI-CM02 ameliorated the histological skin lesions, resulting in smoother epidermis, less parakeratosis, and reduced epidermal thickening.

Effects of PSORI-CM02 on Antioxidative/Oxidative Levels of SOD, GSH, CAT, and MDA

In this study, SOD, GSH, CAT, and MDA levels in the skin were measured by the corresponding assay kits seven days after treatments. As shown in **Figures 4A–D**, imiquimod-induced psoriatic mice produced lower levels of CAT, GSH, and SOD, but a higher level of MDA than did control mice. However, upon treatments with PSORI-CM02 at medium or high doses, the antioxidative activities of GSH, CAT, and SOD were elevated significantly ($P < 0.05$ and $P < 0.01$, respectively) compared to the vehicle group. Furthermore, PSORI-CM02 at the doses of 6 and 12 g/kg were shown to obviously reduce an MDA level in the skin

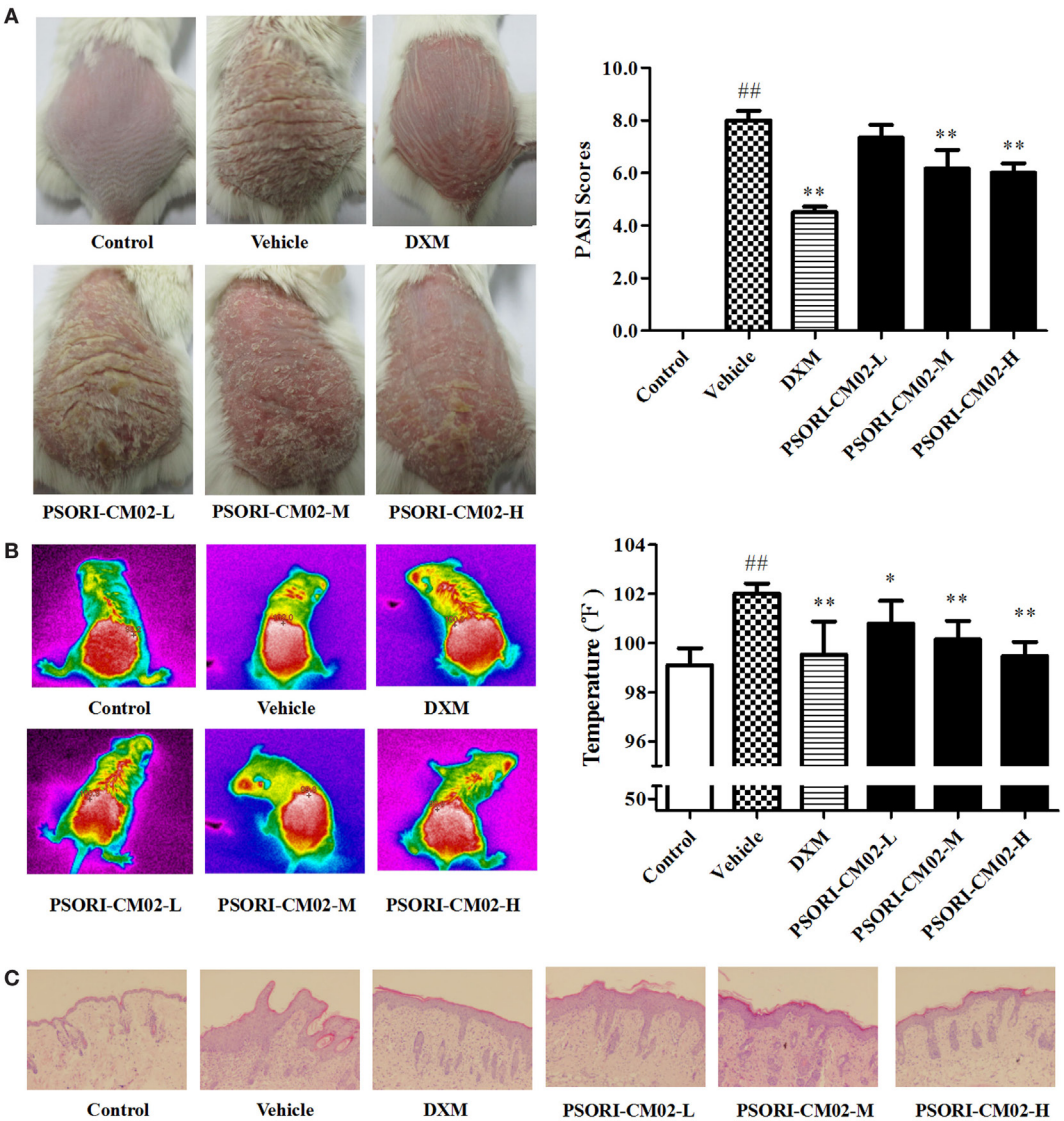

FIGURE 3 | PSORI-CM02 ameliorates murine psoriasis. Shown are macroscopic appearance and the psoriasis area and severity index (PASI) scores of the skin lesions **(A)**, the infrared thermal image of the skin tissue **(B)**, and histological evaluation of the skin tissue *via* H&E staining (magnification 100×) **(C)** in imiquimod-induced psoriasis-like mice treated without or with PSORI-CM02. Data are presented as the mean values ± SDs ($n = 6$, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle group).

tissue (both $P < 0.01$). These results suggested that PSORI-CM02 effectively regulated the oxidative/antioxidative balance toward a more favorable physiological equilibrium in imiquimod-induced psoriatic mice.

PSORI-CM02 Suppresses mRNA and Protein Expressions of Proinflammatory Cytokines in Imiquimod-Treated Psoriatic Mice

Effects of PSORI-CM02 on proinflammatory cytokine expression were also observed *via* ELISA and RT-PCR seven days after PSORI-CM02 treatment. As shown in **Figures 4E–G**, the levels of TNF-α, IL-6, and IL-17 in serum in imiquimod-induced psoriatic mice (vehicle) were increased compared to the control group. Although low doses of PSORI-CM02 did not achieve statistical significance, PSORI-CM02 at medium and high doses significantly reduced the levels of proinflammatory cytokines TNF-α, IL-6, and IL-17 in the serum compared to the vehicle group.

The mRNA expressions of TNF-α, IL-6, and IL-17 in skin tissue were also determined using RT-PCR. As shown in **Figures 4H–J**, the mRNA expressions of TNF-α, IL-6, and IL-17 were augmented after treatments with imiquimod (vehicle) while administration

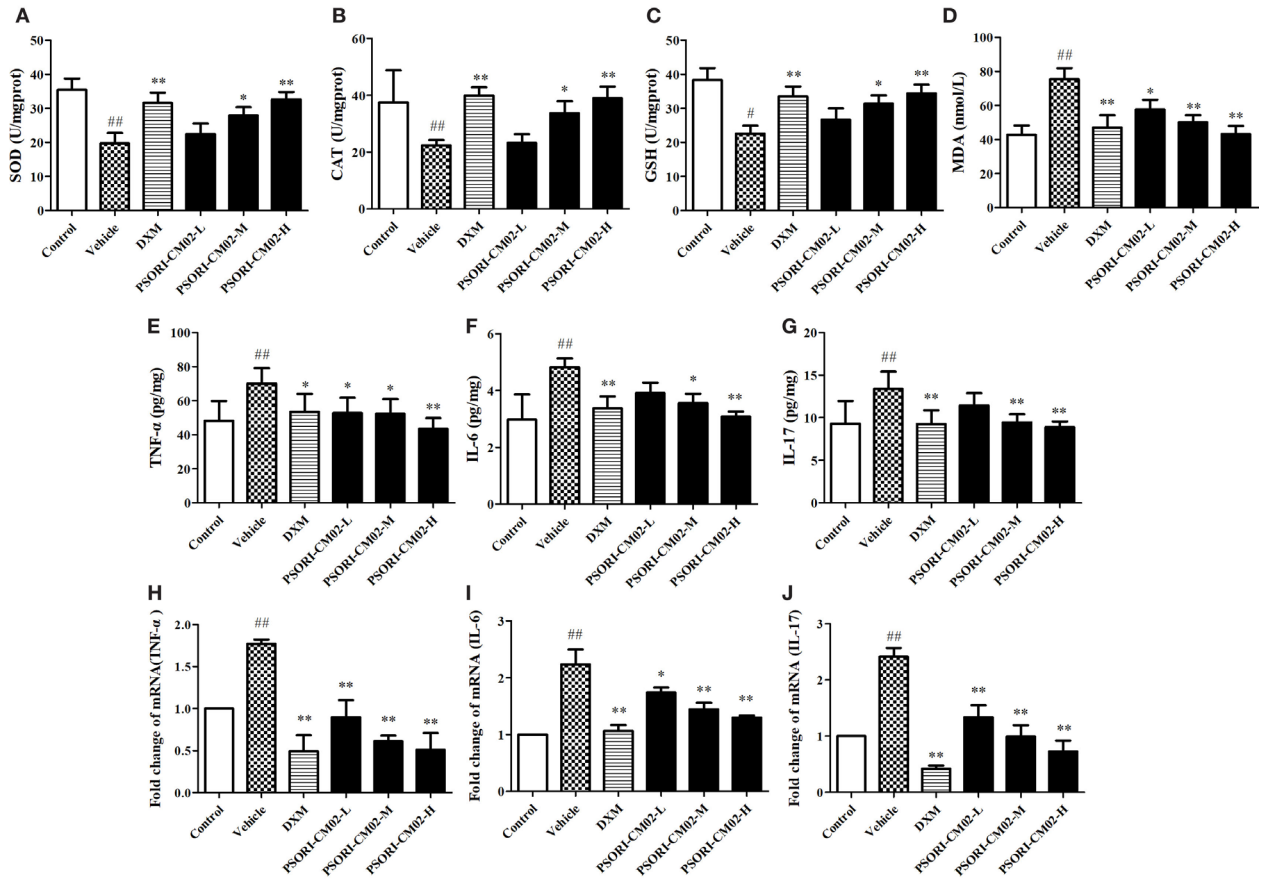

FIGURE 4 | PSORI-CM02 alters oxidative and antioxidative balance and suppresses proinflammatory cytokine expression. Effects of PSORI-CM02 on the activities of superoxide dismutase **(A)**, catalase **(B)**, glutathione **(C)**, and malonaldehyde **(D)** in homogenized skin were determined using enzymatic activity assay kits while the levels of TNF-α **(E)**, interleukin (IL)-6 **(F)**, and IL-17 **(G)** in the serum of imiquimod-induced psoriasis-like mice were evaluated by enzyme-linked immunosorbent assay kits 7 days after PSORI-CM02 treatments. The mRNA levels of TNF-α **(H)**, IL-6 **(I)**, and IL-17 **(J)** in the skin were also determined using RT-PCR. Data shown are the mean values ± SDs ($n = 6$, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle group).

of PSORI-CM02 at all three doses downregulated the mRNA levels of these cytokines compared with imiquimod-treated group without PSORI-CM02 (vehicle).

PSORI-CM02 Inhibits NF-κB Signaling in the Skin with Psoriasis-Like Lesions

Since PSORI-CM02 suppressed proinflammatory cytokine expression, we further explored the mechanisms underlying its antipsoriatic or anti-inflammatory effects. NF-κB signaling is a prominent therapeutic target for treating inflammatory diseases since aberrantly activated NF-κB signaling pathway contributes to inflammatory skin disorders. Thus, western blotting analysis was performed to evaluate the effects of PSORI-CM02 on NF-κB signaling pathway involving protein expression of NF-κB (P65) and IKKα. As shown in **Figure 5**, the expression of NF-κB and IKKα markedly increased after treatments with imiquimod compared to control group. In contrast, treatments with PSORI-CM02 markedly suppressed the expression of NF-κB and IKKα ($P < 0.05$ and $P < 0.01$, respectively) compared to the vehicle group.

PSORI-CM02 Upregulates CD4+ Foxp3+ Tregs in Psoriatic Mice

Regulatory T cells, a small subset of T cells, play an important role in preventing autoimmune diseases, including psoriasis. Therefore, we determined the frequency of Tregs in lymph nodes and spleens of psoriasis-like mice using FACS analyses seven days after treatments with PSORI-CM02, and the results were displayed in **Figure 6**. Treatments with either medium or high doses of PSORI-CM02 significantly augmented the frequency of CD4+ Foxp3+ Tregs in both lymph nodes and spleens compared with the vehicle group, although low doses of PSORI-CM02 only slightly increased the proportion of the Tregs.

PSORI-CM02 Promotes CD4+ CD25+ Treg Cell Proliferation *In Vitro*

Given that PSORI-CM02 could upregulate the frequency of CD4+ Foxp3+ Tregs in imuquimod-induced psoriatic mice *in vivo*, we determined its effects on Treg cell proliferation *in vitro*. FACS-sorted CD4+ CD25+ Tregs derived from naïve mice were labeled

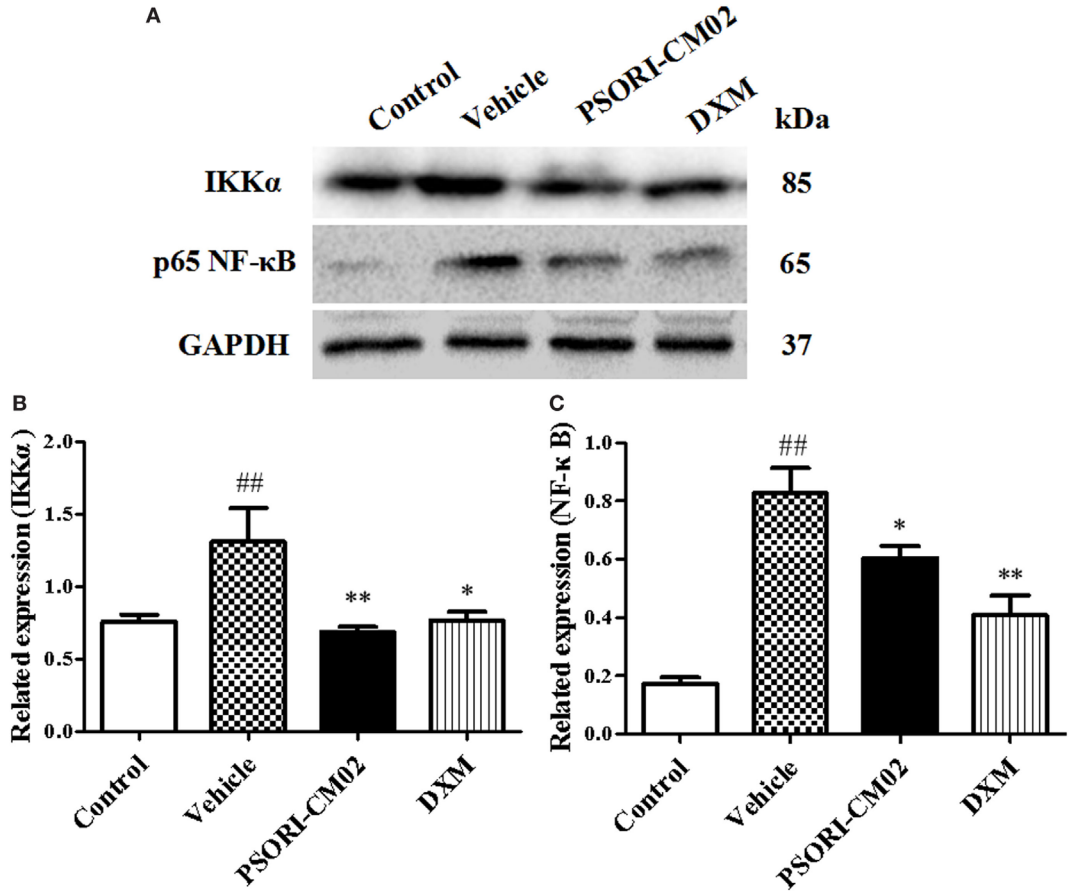

FIGURE 5 | PSORI-CM02 inhibits NF-κB expression in the skin of imiquimod (IMQ)-induced psoriasis-like mice. Impacts of PSORI-CM02 on protein expressions of IKKα and p-65 NF-κB in skin tissue of IMQ-induced psoriasis-like mice were determined using Western blotting analyses seven days after PSORI-CM02 treatments. The expression of IKKα or NF-κB was detected using Western blotting **(A)**. The densitometry analyses of the immunoblotting are shown for IKK **(B)** and NF-κB **(C)**. Data shown are the mean values ± SDs ($n = 3$, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle group).

with CFSE and stimulated with anti-CD3 and anti-CD28 Abs in the absence or presence of PSORI-CM02 for 4 days. As shown in **Figure 7**, PSORI-CM02 significantly promoted CD4+ CD25+ Treg cell proliferation compared to the control group, suggesting that it can also expand Tregs *in vitro*.

DISCUSSION

Psoriasis is known as a chronic inflammatory dermatologic disease that affects around 2–3% of the general population (11). Although the causes for psoriasis are not fully understood, its most important pathological features include irregular epidermal hyperplasia, inflammatory infiltration and hyperplastic dermal blood vessels (12). Natural herbal medicine has been verified to be effective in preventing and treating psoriasis by attenuating aberrant proliferation and differentiation of keratinocytes (13, 14) and exerting anti-inflammatory, immunoregulatory (15, 16) and antiangiogenic effects (17). PSORI-CM02 formula is generally used for treating psoriasis in Guangdong Provincial Hospital of Chinese Medicine and has been registered (registration number: ChiCTR-IOR-15006768) for a randomized, double-blinded and placebo-controlled clinical trial. In this study, we endeavored to confirm the therapeutic effects of PSORI-CM02 formula in imiquimod-induced psoriasis-like mice and unravel its possible mechanisms of action *in vivo* as well as its antiproliferative property in HaCaT cells *in vitro*.

It is well known that cell cycle progression mediates cell growth and proliferation and that cell cycle arrest can trigger inhibition of cell proliferation and growth (18, 19). In the current study, the effects of PSORI-CM02 on cell cycle progression was analyzed using flow cytometry, and our results indicated that PSORI-CM02 suppressed HaCaT cell growth by arresting them at the G1 phase (**Figure 2B**), suggesting that PSORI-CM02 formula may attenuate imiquimod-induced murine psoriasis by inhibiting HaCaT cell growth.

It has been reported that repeatedly topical use of imiquimod in mice results in the influx of various immune cells and hyperplasia of the epidermis, generating a widely used murine model of psoriasis (20). In our study, we established the same model of imiquimod-induced psoriasis and observed that PSORI-CM02

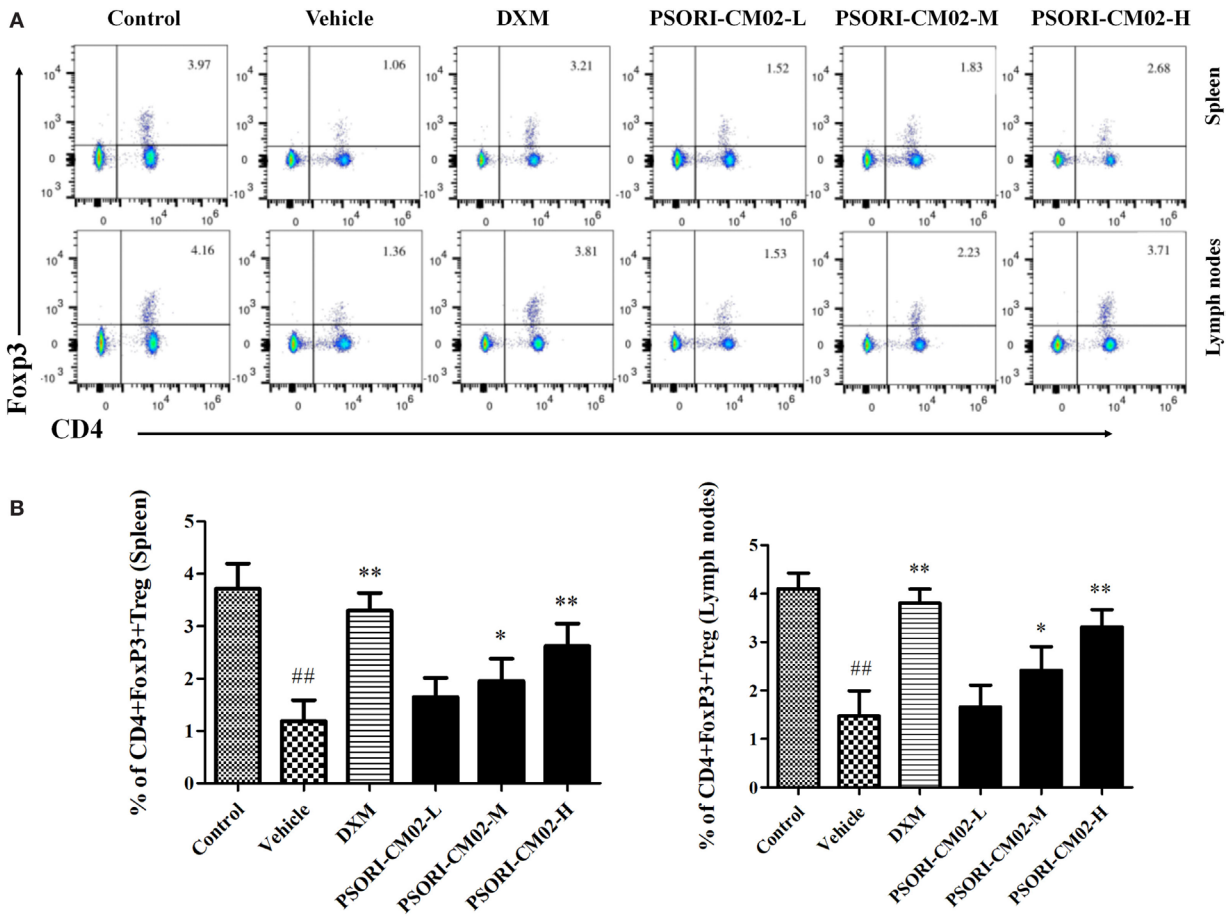

FIGURE 6 | PSORI-CM02 induces CD4+ Foxp3+ regulatory T cells (Tregs) in imiquimod (IMQ)-induced psoriasis-like mice. Effects of PSORI-CM02 on CD4+ Foxp3+ Treg frequency in spleens and lymph nodes of IMQ-induced psoriasis-like mice were observed. Spleen and lymph node cells were isolated from IMQ-induced psoriasis-like mice seven days after treatments with PSORI-CM02 or dexamethasone acetate (DXM). To quantify CD4+ Foxp3+ Tregs, cells were stained for CD4 surface and intracellular Foxp3 makers **(A)** and CD4+ Foxp3+ Treg frequency in spleen and lymph node were shown **(B)**. Data shown are the mean values ± SDs ($n = 3$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle group).

significantly lowered the PASI scores, reduced inflammatory skin temperature, and decreased the epidermal hyperplasia and epidermal thickening compared to the vehicle group, indicating that PSORI-CM02 ameliorates imiquimod-induced murine psoriasis.

We also attempted to reveal the mechanisms underlying the therapeutic effects of PSORI-CM02 on psoriasis. Production of reactive oxygen species (ROS) and weakened antioxidant system take a vital part in the pathogenesis and development of this intractable disease (21). Increased ROS production primarily affects the essential molecules in cells, such as DNA, lipid, protein, and carbohydrate. A balance between antioxidants, including SOD, CAT, and GSH, and oxidants, such as MDA, in normal skin is maintained. Malfunction of the antioxidative system and an increased production of ROS may result in skin diseases, including psoriasis (22–24). In our study, the antioxidative activities of SOD, CAT, and GSH were much lower in imiquimod-treated psoriatic skin than in normal skin whereas the level of oxidative MDA in the psoriatic skin was increased compared to that in the normal skin, which was consistent with the oxidative/antioxidative case of patients with psoriasis (25, 26). Treatments with PSORI-CM02 significantly increased the activities of these antioxidants, including SOD, CAT, and GSH, while decreasing the level of oxidant MDA. Therefore, PSORI-CM02 likely possesses a significant antioxidative feature, resulting in reduced free radical stress and subdued oxidative damage.

Excessive proinflammatory cytokines, such as TNF-α and IL-6, play a key role in the pathogenesis and progress of psoriasis. TNF-α released by multiple types of cells, including activated macrophages, dendritic cells, Th1/Th17 cells, cytotoxic T cells and adipocytes (27, 28), in the serum of patients with psoriasis is essential for the progression of psoriasis (29). It not only activates dendritic cells *via* NF-κB signaling, but also induces expression of adhesion molecules, angiogenic VEGF and other proinflammatory cytokines (30, 31), leading to cell proliferation and inflammation. On the other hand, IL-6 recruits neutrophils (32) and promotes the proliferation of keratinocytes in

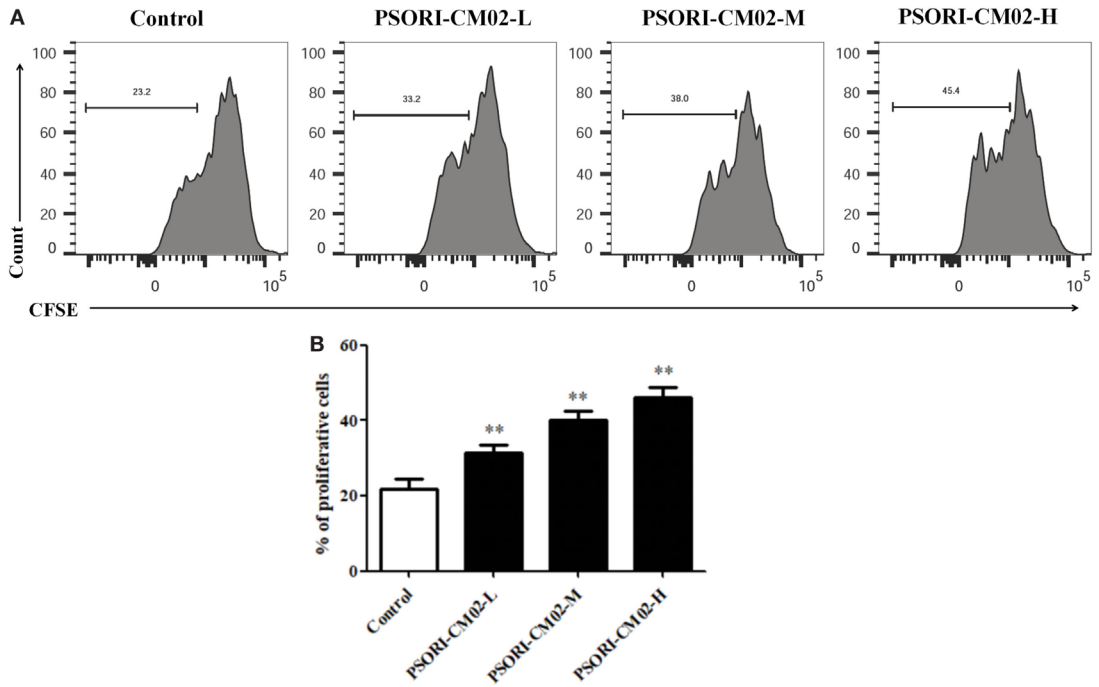

FIGURE 7 | PSORI-CM02 promotes CD4+ CD25+ regulatory T cell (Treg) cell proliferation *in vitro*. Effects of PSORI-CM02 on CD4+ CD25+ Treg cell proliferation *in vitro* were observed. FACS-sorted CD4+ CD25+ Tregs were labeled with CFSE dye and cultured in 96-well plates coated with anti-CD3/anti-CD28 Abs in complete RPMI-1640 media in the absence or presence of PSORI-CM02 (125, 250, and 500 μg/mL). After culturing for 4 days, cell proliferation was analyzed *via* FACS analyses **(A)** and cell proliferation were analyzed **(B)**. Data shown in the bar graph are the mean values ± SDs from three separate experiments ($n = 3$. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control group).

imiquimod-induced psoriasis-like murine skin (33). In the serum of patients with psoriasis, the level of IL-6 is highly elevated (34). Thus, anti-IL-6 mAb has been proposed to be a novel therapeutic option for the treatment of psoriasis (35).

IL-23/IL-17 axis plays a central role in the development of various autoimmune diseases, including human psoriasis and imiquimod-induced murine psoriasis (20, 36). The number of IL-17-producing cells is elevated in mice topically administered with imiquimod (20, 37, 38). Th17 cells are proposed to be a cardinal source of IL-17 family cytokines, and IL-17 has been deemed as a critical cytokine for the establishment and maintenance of the psoriatic phenotype (39). It promotes inflammation *via* binding the receptor located on keratinocytes, dendritic cells, dermal fibroblasts, and endothelial cells (40). Our results also indicate that PSORI-CM02 ameliorates murine psoriasis by reducing IL-17 production.

NF-κB is a key inflammatory signaling pathway and a critical contributor mediating the pathogenesis and progression of psoriasis (41, 42). NF-κB signaling alters functional states of keratinocytes and immune cells *via* exerting its effects on cellular proliferation, differentiation and apoptosis as well as cytokine and chemokine production (39). When keratinocytes are stimulated with TNF-α, their NF-κB signaling pathway is activated, resulting in the overexpression and release of various proinflammatory cytokines, chemokines and enzymes (43). In our present work, we found that PSORI-CM02 significantly suppressed the expression of NF-κB and IKKα in the skin of imiquimod-simulated psoriatic mice. Thus, PSORI-CM02 exerted its therapeutic effects on murine psoriasis by inhibiting NF-κB signaling pathway.

It has been reported that the imbalance between Tregs and Th17 cells is critical for the pathogenesis and development of psoriasis (44). Tregs are essential for the immune homeostasis and tolerance because of their capability of inhibiting the function of other immune cells and inflammatory responses (45). Therefore, we quantified CD4+ Foxp3+ Tregs in spleens and lymph nodes of psoriasis-like mice *in vivo* and determined the effects of PSORI-CM02 on CD4+ CD25+ Treg proliferation *in vitro*. Our results demonstrated that PSORI-CM02 upregulated the frequency of CD4+ Foxp3+ Tregs *in vivo* and promoted *in vitro* proliferation of CD4+ CD25+ Tregs as well, indicating that these Tregs play a role in attenuation of murine psoriasis by PSORI-CM02. On the other hand, previous studies by Di et al. demonstrated that astilbin ameliorated the inflammation in imiquimod-induced psoriasis-like mice *via* suppressing Th17 cell differentiation and IL-17 secretion (16). In our study, we found that astilbin was one of the main ingredients in PSORI-CM02 formula, and that PSORI-CM02 could inhibit the expression and production of IL-17, which was partially consistent with their studies on astilbin. However, we found that PSORI-CM02 also increased Tregs *in vitro* and *in vivo* and suppressed the expression of NF-κB and IKKα. PSORI-CM02 formula was composed of numerous chemical compositions with 18 major compositions identified already. The therapeutic

effect of PSORI-CM02 could be stronger than that of astilbin alone.

In conclusion, we demonstrated that PSORI-CM02 inhibited the keratinocyte proliferation through arresting HaCaT cells at G1 phase. In addition, we found that PSORI-CM02 effectively protected imiquimod-induced psoriasis-like mice from skin lesions by decreasing TNF-α, IL-6, and IL-17 levels, regulating the oxidant/antioxidant status, altering the balance between Th17 response and CD4+ Foxp3+ Treg generation, and inhibiting NF-κB signaling pathway.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Chinese national guidelines and institutional review board of Guangdong Provincial Academy of Chinese Medical Sciences. The protocol was approved by the Institutional Animal Care and Use Committee of Guangdong Provincial Academy of Chinese Medical Sciences.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CL, ZD, and LH. Performed the experiments: HC, HL, MW, WY, and XL. Analyzed and interpreted the data: HC, YY, and HZ. Revised the data analysis and interpretation: HL and ZD. Wrote the article: HC, HL, and ZD. All authors have read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fimmu.2017.01767/full#supplementary-material.

FIGURE S1 | FoxP3+ regulatory T cell phenotypes. Spleen cells from imiquimod-induced psoriatic mice were stained for CD4, CD25 and FoxP3 and analyzed *via* FACS. Only ~94% of CD4+ CD25+ T cells were FoxP3-positive in imiquimod-induced psoriatic mice.

FIGURE S2 | UHPLC profiling of PSORI-CM02. Eighteen major chemical compositions in PSORI-CM02 formula, including citric acid, gallic acid, 5-hydroxymethylfurfural and protocatechuic acid etc., were detected *via* UHPLC analysis, The results also showed that PSORI-CM02 did not contain conventional immunosuppressive agents cyclosporine and rapamycin.

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REVIEW
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Epidermal mTORC1 Signaling Contributes to the Pathogenesis of Psoriasis and Could Serve as a Therapeutic Target

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Although modern biologics targeting different inflammatory mediators show promising therapeutic success, comprehensive knowledge about the molecular events in psoriatic keratinocytes that contribute to the pathogenesis and could serve as therapeutic targets is still scarce. However, recent efforts to understand the deregulated signal transduction pathways have led to the development of small molecule inhibitors e.g., tofacitinib targeting the Jak/Stat cascade that opens additional therapeutic options. Recently, the PI3-K/Akt/mTOR signaling pathway has emerged as an important player in the control of epidermal homeostasis. This review summarizes the current knowledge on the role of this pathway in the pathogenesis of psoriasis, especially the epidermal manifestation of the disease and discusses current approaches to target the pathway therapeutically.

Keywords: psoriasis, mTORC1, keratinocytes, rapamycin, topical agent

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INTRODUCTION

Psoriasis is a common, chronic inflammatory skin disease that affects 2–3% of the population and is associated with a reduced quality of life and a shortened life expectancy due to the association with the metabolic syndrome and cardiovascular pathologies (1). Clinically psoriasis presents with red, scaly plaques, which mostly affect predilection sites such as extensor surfaces of forearms and shins, umbilical, perianal, retro-auricular regions, and scalp (2). These plaques are characterized by epidermal hyperproliferation with impaired keratinocyte differentiation, extravasation of lymphocytes, and angio(neo)genesis. Currently it is assumed that sustained activation of plasmacytoid dendritic cells by epidermal antigens due to skin trauma or infection is the first step in the pathogenesis of psoriasis (3). This induces the maturation of myeloid dendritic cells, which in turn promote via secretion of IL-6, IL-12, and IL-23 the differentiation of T cells into Th1 and Th17 cells (4). Their effector cytokines such as IL-17, IL-22, and TNF-α induce and maintain hallmarks of psoriasis such as keratinocyte proliferation, and disturbed differentiation, leading to epidermal acanthosis, hyperkeratosis, and parakeratosis (5). Activated keratinocytes in turn produce important proinflammatory cytokines and chemokines that are able to recruit a broad spectrum of inflammatory cells from the vascular system. Thus, a "vicious circle" of excessive immune response, epidermal hyperproliferation, and neovascularization is initiated, which leads to the complex clinical appearance of psoriasis (6). The immunological events leading to the described epidermal changes are well understood and various "biologics" against different inflammatory cytokines such as TNF-α, IL-17A, or IL-12/IL-23 show promising results in the therapy of psoriasis (7). However, comprehensive knowledge about the intracellular

epidermal processes induced by the immunological network, and which could serve as potential therapeutic targets, is still missing. There is some evidence that signaling pathways such as Stat1, Stat2, and Stat3 (8–10), MAPK family kinases (11–14), Wnt5a (15), or NF-kB (16–20) are dysregulated in the psoriatic epidermis and some of them have been targeted by molecular inhibitors (21, 22).

THE PI3-K/Akt/mTOR SIGNALING CASCADE

The serine/threonine kinase Akt, also known as protein kinase B (PKB), represents a crucial signaling point in eukaryotic cells and plays a central role in the regulation of cellular processes such as growth, proliferation, and metabolism (23). One of the main downstream mediators of Akt is the mTOR signaling pathway. mTOR (mechanistic target of rapamycin) occurs in two different multiprotein complexes, both of which possess the mTOR kinase as a catalytic subunit and share some regulatory proteins (mLST8, Deptor), while other proteins are complex-specific. Specific to the mTOR complex 1 (mTORC1) is the scaffold protein Raptor, which regulates the assembly and localization of the complex. This complex can be inhibited by rapamycin (24). The rapamycin-insensitive mTOR complex 2 (mTORC2), on the other hand, additionally consists of the scaffold proteins Rictor and Protor1/2 and phosphorylates Akt on Ser473 (25) and thus regulates proliferation and cell growth.

After ligand binding to cognate receptors such as tyrosine kinase receptors (RTK) or G-protein-coupled receptors (GPCR) phosphatidylinositol 3-kinase (PI3-K) becomes activated either directly or via adaptor proteins like the insulin receptor substrate 1 (IRS-1) (**Figure 1**). PI3-K mediates the synthesis of $3'$-phosphoinositides (PIP_3) at the plasma membrane, which act as lipid-second messengers and recruit Akt and the phosphoinositol-dependent-kinase (PDK1) to the membrane. PDK1 can then activate Akt by phosphorylation on Thr308. For complete activation of Akt, phosphorylation of Ser473 by mTORC2 is required. Fully activated Akt is then able to phosphorylate a large number of signal molecules with different functions in the control of growth, proliferation, metabolism, or apoptosis (26). Akt and other signal molecules regulate the TSC complex, consisting of TSC1 and 2 (**Figure 1**). This complex is an important regulator of mTOR by acting as a GTPase-activating protein for Rheb (Ras homolog enriched in brain). The GTP-bound form of Rheb interacts directly with mTOR and activates the complex (27, 28). Furthermore, Akt phosphorylates the proline-rich Akt substrate of 40 kDa (PRAS40), whose inhibitory interaction with mTOR is then dissolved (29), so that the mTOR kinase is fully activated.

By phosphorylation of downstream molecules, mTORC1 regulates the biosynthesis of macromolecules necessary for cellular growth and proliferation. By phosphorylating two key proteins of translation initiation S6 kinase-1 (S6K-1) and eukaryotic initiation factor 4E (eIF-4E) binding protein-1 (4E-BP1), mTORC1 controls the rate of protein biosynthesis (30). In particular, mTORC1 regulates the translation of mRNAs with a so-called $5'$TOP (5'terminal oligopyrimidine) motifs. These mRNAs mainly code for ribosomal proteins and components of the translation machinery (31), so that mTORC1 activity also contributes to the general synthesis of proteins in this way. Furthermore, mTORC1 controls the synthesis of lipids through regulation of the transcription factor SREBP (32), the production of nucleotides (33), and inhibits catabolic processes such as autophagy (34).

mTOR SIGNALING IN PSORIASIS

Recently attention has been drawn to the PI3-K/Akt/mTORC1 cascade as a regulator of epidermal homeostasis and its putative role in inflammatory skin diseases. Akt is highly activated in all epidermal layers of psoriatic lesions (35), except the basal Ki-67 positive layer that represents dividing cells (36). This may be explained either by psoriatic keratinocytes that keep their proliferative pathways turned on, even after leaving the basal layer. Alternatively, Akt could prevent cellular apoptosis, which also contributes to the fast maturation process of psoriatic keratinocytes (37). Inhibition of PI-3K/Akt could be a promising therapeutic strategy as the Vitamin D analog 1α, 25-dihydroxyvitamin D3-3-bromoacetate (BE) reversed IL-22-induced psoriasiform changes *in vitro* (38).

Our group showed for the first time that the central mediator of Akt signaling, the kinase mTOR, is hyperactivated in lesional and nonlesional skin of psoriasis patients, while downstream signaling molecules such as S6K-1, the ribosomal protein S6, and 4E-BP1 are only activated in suprabasal layers in lesional skin (39, 40). Furthermore, it was shown that additional components of mTORC1 such as Rheb and Raptor are overexpressed in psoriatic skin and others such as PRAS40 are hyperactivated (40). That hyperactivated mTORC1 signaling is indeed an important aspect in psoriasis, showed the work by Shirsath et al.: In a genetic mouse model of psoriasis, PUVA treatment not only ameliorated the histological psoriasis score, but also normalized mTORC1 signaling (41). The divergent localization of the activated signal components in the epidermis points toward a pathophysiological contribution of deregulated mTORC1 signaling in psoriasis. For example, the hyperactivation of mTORC1 in the basal layer may indicate a role during the enhanced proliferation of psoriatic keratinocytes, while the suprabasal hyperactivation points toward a role in aberrant differentiation. Using different *in vitro* approaches our group showed that healthy keratinocytes switch off Akt/mTORC1 signaling as soon as differentiation is initiated. This appears to be associated with proliferation control, as Ki-67 positive cells in the basal layer of healthy skin also showed mTOR activity. Thus, inactivation of mTOR seems to be a prerequisite for keratinocytes to initiate terminal differentiation. In contrast, in an inflammatory environment such as psoriasis, the mTORC1 cascade is aberrantly activated in all epidermal layers. We were able to show that IL-1β, IL-17A, TNF-α, and in particular a mix of these cytokines leads to activation of the mTORC1 signaling cascade. In addition, the pathway might be activated by miRNAs that are deregulated in psoriatic skin (42, 43), or by mechanosensitive molecules such as polycysteins (44).

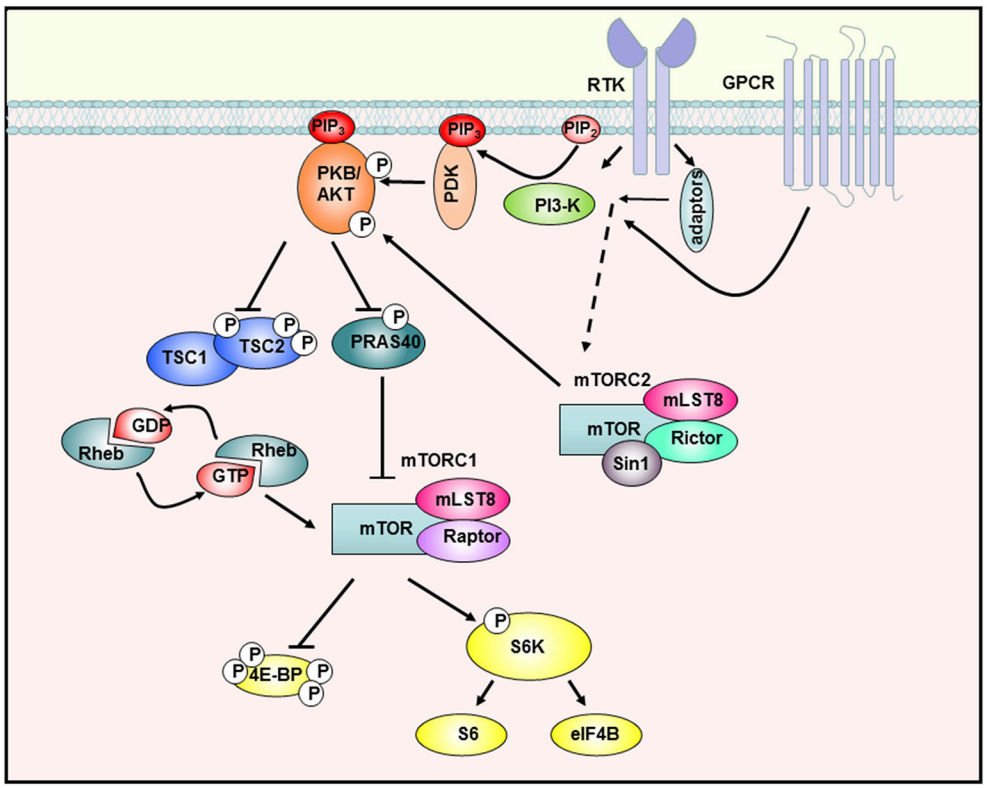

FIGURE 1 | The PI3-K/Akt/mTOR signaling cascade. Stimulation of receptor tyrosine kinases (RTK) and G-protein-coupled receptors (GPCR) leads to activation of PI3-K, which then synthesizes PIP_3 in the membrane. Subsequently, Akt is recruited to the membrane and phosphorylated by PDK-1 and mTORC2. The activated Akt kinase phosphorylates various substrates such as PRAS40, which is then inactivated and releases mTORC1. In addition, TSC2 is inhibited so that the downstream GTPase Rheb remains GTP-bound and can activate mTORC1. The fully activated mTORC1 complex then activates proteins of the translation machinery by phosphorylating S6K-1 or 4E-BP1 [adapted from Manning et al. (23)].

The latter could explain the predilection of psoriatic plaques to sites of increased mechanical stress such as elbows and knees. Our group could prove that continuous mTORC1 activity contributes to the proliferation of keratinocytes and simultaneously inhibits proper keratinocyte maturation. Thus, we suggest a model where mTORC1 signal transduction functions as a central switch between keratinocyte proliferation and differentiation (**Figure 2**). This model is supported by findings from Mitra et al. showing that IL-22 regulates keratinocyte proliferation via the Akt/mTOR cascade (45).

Apart from this model also other mechanisms, how epidermal mTORC hyperactivation can contribute to the pathogenesis of psoriasis are being discussed. Patel et al. could show that the release of pro-inflammatory mediators such as IL-6, CXCL8, or VEGF by keratinocytes is mediated via mTORC (46). Another mechanism by which hyperactive PI3-K/Akt/mTORC1 signaling might contribute to the pathogenesis of psoriasis could be through inhibiting autophagy (47). Autophagy and more specifically nucleophagy is an important mechanism during keratinocyte differentiation and maturation into corneocytes. Thus, high mTORC1 activity inhibits nuclear degradation and contributes to parakeratosis (retention of nuclei), one of the hallmarks of psoriasis (48).

Although not the main focus of this review, it has to be mentioned that mTORC1 signaling also has important functions in the innate (49) and adaptive immune system (50–52). Specifically a role for mTORC1 and 2 has been attributed to the regulation of immune cell energy metabolism and thereby to the control of their function and differentiation (53). Deregulated mTORC1 signaling was found in peripheral blood mononuclear cells (PBMCs) of psoriasis patients (54), which seems to contribute to their pathological behavior (55). Regulatory T-cells from psoriasis patients show increased mTOR phosphorylation and treatment with methotrexate reduces mTOR activation (56). In addition, a novel vitamin D analog reduced mTORC1 activity in activated memory T cells form psoriasis patients and thus contributed to the immunosuppressive effect of the drug (57).

mTOR SIGNALING AS A THERAPEUTIC TARGET IN PSORIASIS

The mTOR complex is also interesting because of its inhibitor rapamycin (sirolimus), which was isolated from Streptomyces hygroscopicus in 1975 (58). This bacterial strain was first found in the soil of Rapa Nui Island (Easter Island), after

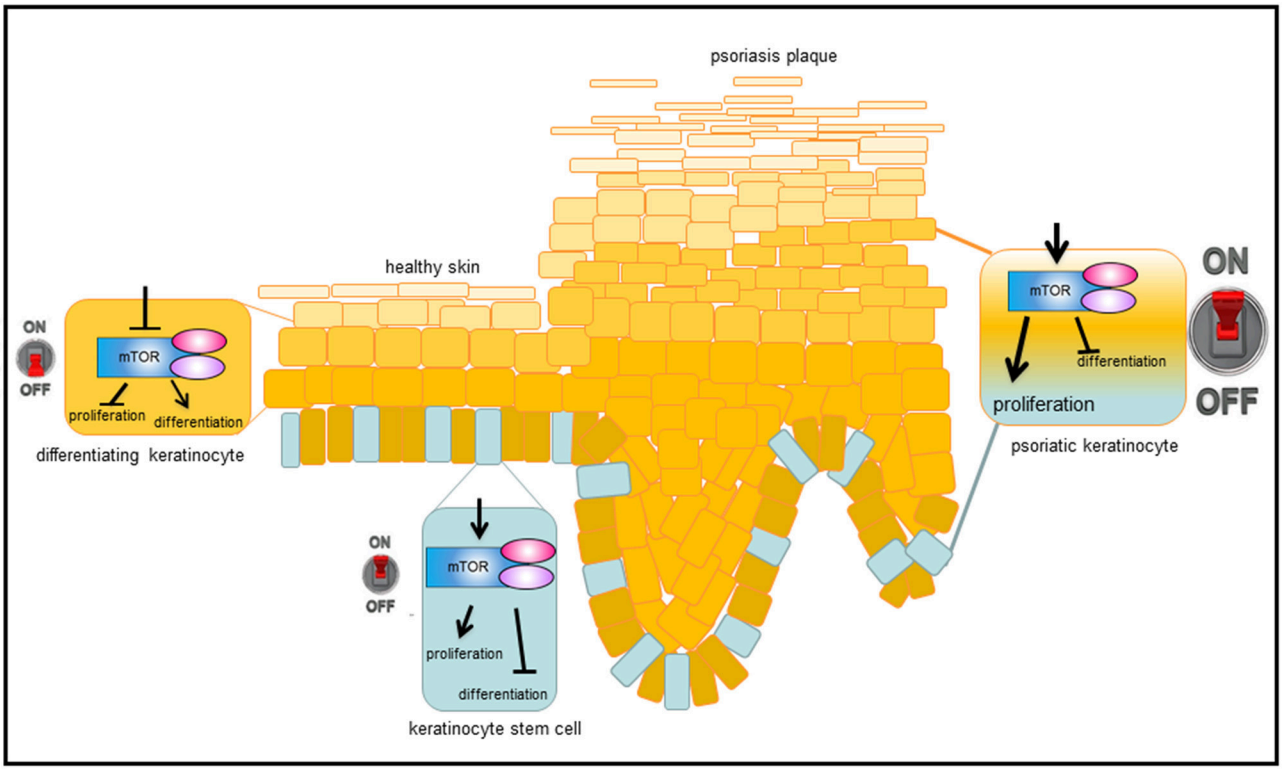

FIGURE 2 | Model, how mTORC1 signaling controls epidermal homeostasis and contributes to psoriasis. In healthy, basal keratinocytes, mTORC1 signaling pathway is active and controls proliferation while blocking differentiation. When cells leave the proliferative, basal compartment, mTORC1 is switched off and differentiation is enabled. Under inflammatory conditions, such as in psoriasis, mTORC1 is permanently activated, which leads to massive proliferation in the basal layer and disturbed keratinocyte differentiation in suprabasal layers, resulting in the phenotypic changes typical of psoriasis.

which the substance was named. Even before the kinase mTOR was identified as a target protein of rapamycin in 1994 (59), rapamycin was known for its anti-proliferative properties on lymphoid cells and associated immunosuppressive properties (60). Rapamycin is therefore still used to prevent transplant rejection (61) and restenosis after implantation of stents in coronary vessels (62). In addition, anti-tumor effects of rapamycin and its analogs (rapalogs) have been under investigation (63, 64).

It is particularly interesting that rapamycin has also been tested for its antiproliferative and immunosuppressive properties in a few small studies in psoriasis patients. Systemic administration of everolimus (a derivative of sirolimus) was successful in a single patient (65), whereas a larger study showed good results for sirolimus in combination with cylosporin therapy (66). In addition, in a renal transplant patient with refractory psoriasis, everolimus ameliorated skin lesions (67). Remarkably, only limited new substances for topical anti-psoriatic therapy have been developed in recent years and new product launches mostly consisted of derivatives or further developments of established agents (68). Thus, the establishment of new substances for topical application is desirable. In one small trial topical treatment with rapamycin led to a significant improvement of the clinical score, while the thickness of the plaques was unchanged (69). To further explore this therapeutic option, the effectiveness of topical rapamycin was investigated in the imiquimod-induced psoriasis mouse model, which showed activation mTORC1 signaling similar to human psoriasis (70, 71). Mice treated with rapamycin showed a significant improvement in clinical appearance (redness, swelling, and flaking), reduced angioneogenesis and normalization of epidermal thickness compared to the control group. While the imiquimod-treated mice showed a clear activation of mTORC1 and downstream molecules, rapamycin reduced the activity to the level of untreated mice. Rapamycin normalized the expression and distribution of differentiation markers such as keratins, involucrin, and loricrin. In addition, the influx of innate immune cells into the draining lymph nodes was partially reduced by rapamycin treatment (71). In the same mouse model rapamycin treatment also restored the expression of tropomyosins, which are downregulated in psoriatic lesion and could also contribute to the disease (72).

Rapamycin is an allosteric inhibitor, that requires binding to its intracellular receptor, FKBP12, to selectively inhibit some, but not all functions of mTORC1 (73). mTORC2 is considered rapamycin-insensitive, although it can be inhibited by chronic rapamycin treatment in some cell types (74).

To inhibit all functions of both complexes, selective ATP-competitive inhibitors of mTOR were developed (75, 76). As they are efficiently inhibiting both mTOR complexes and thus inhibit Akt signaling, they could be interesting therapeutic compounds in psoriasis. The same rationale was applied, by Chamcheu et al., that showed efficient inhibition of PI3-K, mTOR, and S6K-1 by Delphinidin, an antioxidant plant pigment (77). Topical Delphinidin was able to ameliorate symptoms in two different psoriasiform mouse models (77, 78).

In summary, there is increasing evidence that specifically topical application of mTORC inhibitors can be a successful strategy for anti-psoriatic therapies and underline the need to further explore the mTORC1 signaling pathway as a therapeutic target in psoriasis.

AUTHOR CONTRIBUTIONS

CB conceptualized and wrote the manuscript and created the figures.

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