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Circulating CD8 T cells from patients with mildto-moderate psoriasis are functionally impaired

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Background: Psoriasis is a systemic, immune-mediated, inflammatory skin disease in which T cells have been found to play a significant role. The phenotypic and functional properties of circulating CD8 T cells in the pathogenesis of the disease are still ill-defined.

Objective: This study aimed to assess changes in the phenotype, activation status and mediator release of CD8 T cells in the peripheral blood of patients with mild-to-moderate psoriasis.

Methods: Peripheral blood mononuclear cells from patients with mild-tomoderate psoriasis and healthy individuals were used to investigate the CD8 T cell immune phenotype and mediator release upon *in vitro* TCR-independent (phorbol 12-myristate 13-acetate (PMA) plus ionomycin (ION)) or TCRdependent (anti-CD3/CD28) activation by flow cytometry.

Results: Patients with psoriasis exhibited reduced circulating CD8 memory T cell frequency compared to healthy controls. Additionally, although CD8 T cell subsets showed similar levels of the skin homing marker CCR4, they demonstrated a significant upregulation of B- and T-lymphocyte attenuator (BTLA) expression compared to healthy individuals. Upon CD8 T cell activation, IL-17A and IL-17F were expressed at low and comparable levels in psoriasis patients and healthy controls. In contrast, CD69, IFN γ , and Granzyme B were significantly decreased in anti-CD3/CD28-activated CD8 T cell subsets. PASI scores positively correlated with IFN γ -producing CD8 T memory cells and negatively with TNF-producing CD8-T cell subsets.

Conclusion: Patients with mild-to-moderate psoriasis showed a significant decrease in CD8 T memory cells and reduced release of cytotoxic mediators by CD8 T cells. Thus, this indicates that psoriasis impacts the functionality of circulating CD8 T cells.

KEYWORDS

flow cytometry, CD8 T cells, multiparametric analysis, unsupervised clustering, immunophenotyping, peripheral blood mononuclear cells, psoriasis, PASI

1 Introduction

Psoriasis is a common, systemic and chronic immunemodulated skin disease affecting at least 60 million people worldwide (1). Its most frequent form, plaque psoriasis, is characterized by the presence of salmon-pink cutaneous plaques covered by silvery scales in white skin and grey in black skin (2-5). The Psoriasis Area and Severity Index (PASI) is commonly used to evaluate disease severity and clinical outcome (6, 7). The vast majority of patients with psoriasis have mild-to-moderate disease, with topical therapy considered as the first-line treatment (8, 9). There is broad consensus that a mixed interleukin (IL)-17 (T helper type 17: Th17) and interferon gamma (IFNy)/tumour necrosis factor (TNF) response (Th1/Tc1), driven by a vast network of cells, such as T and dendritic cells, plays a dominant role in promoting keratinocyte hyperproliferation and aberrant differentiation in psoriasis (10-12). Biologic agents targeting the IL-23/IL-17 immune axis and TNF signalling have revolutionized the treatment of moderate-to-severe psoriasis (11, 13, 14).

The role of CD8 T cells as significant sources of IL-17 (Type 17 CD8+T cells: Tc17) has also been extensively studied in the pathogenesis of psoriasis skin lesions (15–17). In psoriasis patients and mouse models, epidermal CD8 T cells exhibit an active Tc17 phenotype and blocking CD8 T cells prevents the development of psoriasis *in vivo* effectively (16, 18). Cheuk et al. reported that epidermal IL-17-producing CD8 T cells in psoriasis lesions co-express CD103 and are retained in resolved lesions after effective treatment, potentially contributing to local relapses (19).

Additionally, circulating CD8 T cells predominantly respond with IFN γ production in patients with the HLA-Cw6 allele, which is associated with early-onset psoriasis (20–22). Beyond conventional CD8 T cell subsets, circulating mucosa-associated invariant T-cells (MAIT) also contribute to the pathogenesis of the disease (23–25).

Over the past decades, the association between systemic inflammation and local immune activation has received increased recognition (26–28), suggesting the contribution of circulating T cells to the pathogenesis of psoriasis (29). Although multiple investigations have explored the characteristics of peripheral blood CD4 T cell populations in individuals with psoriasis, examining numerical, phenotypic, and functional profiles, the findings have been inconsistent (30–33). Hence, the features and roles of circulating CD8 T cells in psoriasis patients are still ill-defined.

Dysregulation of skin immune homeostasis led by loss of immune tolerance has been frequently reported in psoriasis (34, 35). Hence, inhibitors of T cell co-stimulation have been investigated as novel therapeutic targets for disease control (36-38) and in experimental human and animal psoriasis models, as summarised by Yao and Liu et al. (39, 40). For instance, the immune checkpoint protein programmed death protein-1 (PD-1) was found to be expressed by cutaneous IL-17A-producing T cells in psoriasis patients and in psoriasis-like mouse models (41). Kim et al. also found that blockade of the PD-1 axis leads to suppressed anti-CD3induced IL-17A production by $\gamma\delta$ T cells in imiquimod (IMQ)induced psoriasis-like skin inflammation (41). Additionally, Sgambelluri et al. reported a subset of blood CCR4/CD103expressing CD8+ effector T cells significantly correlating with disease severity in patients with moderate-to-severe psoriasis (42). Therefore, delineating the roles of skin homing chemokines and cosignalling molecules on peripheral blood CD8+T cell compartments suspected to play a role in psoriasis becomes paramount.

In this study, we aimed to provide a deeper understanding of the phenotype, activation status, and function of circulating CD8 T cells and their potential implications in mild-to-moderate psoriasis. We used flow cytometry to characterise peripheral blood CD8 T cell populations, their frequency, expression of skin-trafficking chemokine receptors and co-signalling molecules compared to healthy subjects. We then assessed the functionality of circulating CD8 T cells by examining the expression of activation markers and

Abbreviations: BTLA, B- and T-Lymphocyte Attenuator; CM, Central Memory; CTLA-4, Cytotoxic T Lymphocyte Antigen-4; DMSO, Dimethyl Sulfoxide; EM, Effector Memory; FBS, Fetal Bovine Serum; FCS, Flow Cytometry Standard; HVEM, Herpes Virus Entry Mediator; IFNγ, Interferon gamma; IL, Interleukin ION, Ionomycin; MAIT, Mucosal-Associated Invariant T-Cells; NK, Natural Killer; PASI, Psoriasis Area and Severity Index; PBMCs, Peripheral Blood Mononuclear Cells; PD-1, Programmed Cell Death Protein 1; PFA, Paraformaldehyde; PMA: Phorbol 12-Myristate 13-Acetate; PsA, Psoriatic Arthritis; Th, T helper; TIGIT, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain; TEMRA, Terminally Differentiated Effector Memory; TNF, Tumour Necrosis Factor; UMAP, Uniform Manifold Approximation and Projection.

the production of mediators, such as cytokines, upon activation. Multiparametric flow cytometry data was analysed using conventional manual gating and unsupervised clustering approaches.

2 Methods

2.1 Patients

This study was approved by the London Bridge Research Ethics Committee (REC number: 06/Q0704/18) and the University of Manchester (UREC ref: 2018-2696-5711). After obtaining informed consent, peripheral blood samples were collected from 7 psoriasis patients and 11 healthy subjects. Demographics and clinical characteristics of the enrolled subjects are summarised in Supplementary Table 1 and Supplementary Methods.

2.2 PBMC isolation

Human peripheral blood mononuclear cells (PBMCs) were freshly isolated from whole blood using density gradient centrifugation (Ficoll-Paque) as previously described (43). Cell viability and concentration were determined using trypan blue staining and a counting chamber. Subsequently, PBMCs were frozen in a freezing solution containing fetal bovine serum (FBS) or human serum albumin supplemented with 10% Dimethyl Sulfoxide (DMSO) and stored at -80°C until use.

2.3 Multiparametric flow cytometry analysis and intracellular cytokine staining

Cryopreserved PBMCs were rapidly thawed and washed in prewarmed RPMI medium (Sigma) supplemented with 10% FBS, 10% Penicillin-Streptomycin, and 2 mM L-glutamine. Thawed PBMCs were split across three staining panels for flow cytometry. For ex vivo immunophenotyping, chemokine and co-signalling molecule expression on CD8 T cells, PBMCs were plated in v-bottom 96well plates (2-5 x 10⁵ cells/well). For in vitro stimulation, PBMCs were seeded in 96-well flat-bottom plates (2-5 x 10⁵ cells/well). The cells were subsequently subjected to two distinct activation protocols. One underwent stimulation with phorbol 12-myristate 13-acetate (PMA, 25 ng/ml) and ionomycin (ION, 710 ng/ml) (Merck) for 6 hours, targeting protein kinase C and calcineurin pathways. The second was exposed to plate-bound anti-CD3 (1 µg/ml) and soluble anti-CD28 (1 µg/ml) (Biolegend) for 24 hours. Both simulations were conducted in culture medium incubated at 37°C with 5% CO2. Unstimulated cells were included as negative controls. Brefeldin A (Sigma-Aldrich) was incorporated into the culture medium during the final 6 hours of stimulation to inhibit intracellular vesicular transport.

Cell surface and intracellular staining were conducted on *ex vivo*, unstimulated and stimulated PBMCs. Detailed information regarding fluorochrome-conjugated antibodies utilised is provided in **Supplementary Table 2**. For surface staining, PBMCs were initially blocked with Human True StainFcX (Biolegend) and subsequently stained for cell surface markers for 30 min at 4°C in the dark, followed by live/dead staining (Thermo Fisher Scientific). Subsequently, cells were fixed with 2% paraformaldehyde (PFA) for 10 min at room temperature (Thermo Fisher).

In samples designated for cytokine production detection, following surface and live/dead staining, cells were fixed and permeabilised using 1x permeabilization buffer (Invitrogen), then stained with antibodies against intracellular markers for 1 h at room temperature. After washing, cells were resuspended in FACS buffer (PBS, 2 mM EDTA, 2% FBS), and analysis was performed using an LSR Fortessa flow cytometer (BD Biosciences). The complete experimental design is delineated in Supplementary Figure 1A.

2.4 Flow cytometry data analysis

Flow cytometry files (FCS) were exported and compensated using single-stained UltraComp Beads (Invitrogen). The compensated data were then processed using FlowJo Software (BD Biosciences) to eliminate debris, non-viable cells, and cellular aggregates (Supplementary Figure 1B). This preparatory step preceded both manual and unsupervised clustering analyses.

Manual gating was performed on flow cytometry data using FlowJo. CD8+ T cell populations (CD3+CD56-CD8+ cells) were identified based on the gating strategy provided in Supplementary Figure 1B and Supplementary Methods. The expression levels of different markers were analysed and subsequently plotted using Prism 10 (GraphPad software).

Unsupervised analyses were carried out using OMIQ (Dotmatics). For a detailed description, see Supplementary Methods.

2.5 Statistical analysis

Data are presented as percentage of total populations, mean \pm standard deviation (SD). In both manual gating and unbiased clustering, the Mann-Whitney U test was used to detect significant differences between groups in unpaired analyses. In unsupervised analyses, the raw p values were adjusted by the original Benjamini-Hochberg false discovery rate (FDR) method (44), using the *p.adjust* function in R (ver. 4.0.5), where applicable. EdgeR was used to screen FlowSOM-defined meta-clusters for significance based on cell counts in OMIQ and using a custom script in R for the analysis of unbiased clustering datasets (45). Spearman correlation and simple linear regression between PASI scores and identified subpopulations were calculated using Prism



FIGURE 1

CD8+ immune cell subpopulations in the blood of patients with mild-to-moderate psoriasis are comparable to those of healthy individuals. Peripheral blood mononuclear cells (PBMCs) from mild-to-moderate psoriasis patients (n=11) and healthy individuals (n=7) were labelled with different cell surface and chemokine markers and analysed using flow cytometry. (A) Representative plots of the chosen gating strategy. (B) Frequency of identified T, CD8+ T, CD8- T, NK, and NKT immune cell subsets in healthy and psoriasis subjects. (C) Representative plots of the chosen gating strategy (D) Frequency of CD8+ T cell subpopulations in mild-to-moderate psoriasis and healthy individuals. (E) Pie and bar charts of summarised results. Significant differences were analysed using the Mann-Whitney U test. p value < 0.05. Bar plots represent mean value \pm SD. CM, central memory T cells; EM, effector memory T cells; Effector, effector T cells; MAIT, mucosal-associated invariant T cells; Naïve, naïve T cells; NK, natural killer T cells; T, T cells. 10.3 (Graphpad). Adjusted p values < 0.05 were considered significant for all the analyses.

3 Results

3.1 Immuno-phenotyping reveals normal distribution of CD8 T cells, NK and NKT cells but reduced frequencies of CD8 memory T cells in psoriasis

Several studies have quantified the composition of immune cell populations in the peripheral blood of psoriasis patients, primarily focusing on those with moderate-to-severe phenotype (46–49). However, limited data exist on the distribution of CD8 T cell subsets in patients with mild-to-moderate psoriasis. To explore this, PBMCs were stained with a 14-color antibody panel to characterise lymphocytes and specific CD8 T cell subsets (Supplementary Table 2).

As shown in Figures 1A, B, the frequencies of total T cells, CD8⁻ T cells, natural killer (NK) and NKT cells were comparable between psoriasis patients and healthy controls, in line with previous reports (62-64). The overall frequency of CD8 T cells in psoriasis patients showed considerable variability but was also similar to healthy controls. We then investigated the distribution of CD8 T cell subsets within the total population by examining lineage cell surface markers, as summarised in Supplementary Table 3. The frequency of CD8 memory T cells was significantly decreased in psoriasis patients compared to healthy controls (p<0.05). However, we did not observe significant differences in the frequencies of central memory (CM) (CD45RA-CD45RO+CD27+), effector memory (EM) (CD45RA-CD45RO+CD27-), naïve (CD45RA +CD45RO-CD27+) and effector (CD45RA+CD45RO-CD27-) CD8 T cells, nor MAIT cells (TCRVa7.2+CD161+) between psoriasis patients and healthy controls (Figures 1C, D). A summary of the analysed immune cell and T cell subsets is presented in Figure 1E and Supplementary Table 4. No significant differences were found in the CD8+/CD8- ratio (data not shown).

In conclusion, these results indicate a normal distribution of circulating CD8 T cells in mild-to-moderate psoriasis.

3.2 Circulating CD8 T cells and MAIT CD8 cells from psoriasis patients display reduced CXCR3 and increased CCR4 expression compared to healthy controls

There is accumulating evidence that circulating memory T cells are associated with cutaneous manifestations in psoriasis (50). Since our data indicate a reduction in memory CD8 T cell frequency in psoriasis patients, we have next investigated the CD8 T cell phenotype, particularly the expression of receptors associated with tissue homing/trafficking chemokines.

As shown in Supplementary Figure 2, we observed no significant difference in CD69, CD103, CCR4 and CLA expression on CD8 T cells between psoriasis patients and healthy controls. However, CCR4 was expressed at a higher frequency in the CD8 MAIT cell compartment in psoriasis than in healthy subjects (Supplementary Figure 2B). CXCR3 expression was, instead, reduced in CD8 and CD8 memory T cell compartments in psoriasis compared to healthy controls (Supplementary Figure 2F). When analysing different CD8 T cell subpopulations, CXCR3 was particularly reduced in CM CD8 T cells. Conversely, the expression of the other homing/trafficking markers CD69, CD103, CLA and CCR4 was low across all CD8 T cell subsets in both cohorts studied (Supplementary Figure 3). Beyond CD8 T cells, the expression of CCR4, CD103 and CLA was significantly decreased in CD8-T cells, CXCR3 in NK cells and CD69 and CLA in NKT cells (Supplementary Figures 2B-E). However, unsupervised OMIQ analyses did not replicate these findings (see Supplementary Results).

In conclusion, these results hint at possible differences in the expression of receptors associated with tissue homing in CD8 T cells, namely CXCR3 and CCR4, from patients with mild-to-moderate psoriasis compared to healthy controls.

3.3 BTLA expression is increased in CD8 effector T cells

Co-signalling receptors play a role in modulating T cell activities. The expression of T-cell immunoreceptor with immunoglobulin andimmunoreceptor tyrosine-based inhibitory motif domains (TIGIT) on circulating CD4 T cells correlates with psoriasis severity (33, 51). Thus, to further dissect the phenotype of circulating CD8 T cells in psoriasis patients with mild-to-moderate disease severity, we examined the expression of co-signalling molecules (co-stimulatory: CD28, CD134 (OX40L), LIGHT; co-inhibitory: TIGIT, programmed cell death protein 1 (PD-1), BTLA) on circulating CD8 T cells.

As shown in Figure 2A, while CD28, TIGIT, BTLA and PD-1 are expressed by CD8 T cells, the costimulatory receptors LIGHT and CD134 are minimally expressed in both psoriasis patients and healthy controls (<2%). Although there was no difference in the expression of BTLA by CD8 T cells between the two cohorts (Figure 2B), upon examining CD8 T subsets, we found significant BTLA upregulation in CD8 effector T cells in psoriasis (p<0.005) (Figure 2D). BTLA expression was also increased in NK cells from psoriasis patients compared to healthy controls (p<0.05, Figure 2B).

As for the remaining markers, the expression of CD28 and TIGIT was similar between CD8 T cells and other cell subtypes in both healthy and psoriasis, similar to a previous report (52) (Supplementary Figure 4). Conversely, PD-1 expression was significantly decreased in CD8 T cells, CD8- T cells and NKT cells compared to healthy controls (Figure 2C). When performing



cells and other immune cell subsets, and **(D)** BTLA expression measured on circulating CD8 T cell subpopulations (CM, EM, Eff and Naïve) in psoriasis compared to healthy individuals. Differences were calculated using the Mann-Whitney U test. **p* value < 0.05. Bar plots represent mean value ± SD. CM, central memory T cells; EM, effector memory T cells; Eff, effector T cells; MAIT, mucosal-associated invariant T cells; Naïve, naïve T cells; NK, natural killer cells; NKT, natural killer T cells; T, T cells.

unsupervised analyses, however, we could not find significant changes in costimulatory/co-inhibitory marker frequencies in CD8 T cell sub-populations. Still, we identified differences in NKT cell subset frequencies between healthy and psoriasis patients, further discussed in Supplementary Results. In summary, our data demonstrate increased BTLA expression in CD8 effector T cells and reduced PD-1 expression in all CD3expressing subpopulations of patients with mild-to-moderate psoriasis, suggesting possible variation in T cell function if these co-inhibitory receptors are engaged.



Stimulated CD8 T cells from psoriasis patients display reduced CD69, Granzyme B and IFN γ expression compared to healthy individuals. (A) Peripheral blood mononuclear cells (PBMCs) isolated from psoriasis patients (n=7) and healthy controls (n=10) were stimulated with anti-CD3/CD28 or phorbol 12-myristate 13-acetate/ionomycin (PMA/ION) and membrane and intracellular expression of mediators was analysed in flow cytometry. Representative flow plots showing expression of CD69, Granzyme B, TNF, IFN γ , IL-17A and IL-17F on CD8 T cells. (B) Bar plots show the percentage of CD69+, Granzyme B+, TNF+, IFN γ +, IL-17A+ or IL-17F+ CD8 T cells when stimulated with antiCD3/CD28. (C) Bar plots show the percentage of CD69+, Granzyme B+ and IFN γ + cells within CM, EM, Eff and Naïve CD8 T cell subsets in psoriasis compared to healthy controls when stimulated with anti-CD3/CD28. Data are shown as mean \pm SD. Differences between groups were calculated using the Mann-Whitney U test. **p < 0.005, ***p < 0.0005. CM, central memory T cells; EM, effector memory T cells; Effector, effector T cells; Naïve, naïve T cells; ns, not significant.

3.4 Circulating CD8 T cells are functionally impaired in patients with psoriasis

It is widely recognised that the increased release of proinflammatory cytokines, such as IL-23, IL-17 and TNF, plays a critical role in developing psoriasis (53). To further investigate the changes in the effector function of circulating CD8 T cells in psoriasis, two *in vitro* stimulation methods, TCR-dependent (anti-CD3/CD28) and -independent (PMA/ION), were employed to assess the production of cytokines by T cells *in vitro*, as this varies significantly depending on the type of stimulus used (54–56). Following stimulation, the activation status (CD69) and the expression of mediators (Granzyme B, TNF, IFN γ and IL-17A/F) were assessed by surface and intracellular staining using flow cytometry.

As shown in the representative flow plots in Figure 3A, incubation with PMA/ION significantly increased the intracellular expression of IFN γ compared to anti-CD3/CD28 stimulation (p<0.005). In contrast, anti-CD3/CD28 exposure preferentially upregulated intracellular Granzyme B and membrane CD69 expression. IL-17A and IL-17F production by CD8 T cells was not significantly affected by either stimulation method.

The frequency of CD69+, Granzyme B+ and IFN γ + CD8 T cells in psoriasis patients was significantly lower following anti-CD3/CD28 stimulation (*p*<0.0005) (Figure 3B), suggesting downregulated TCRdependent activation and impaired Tc1 cytokine production in CD8 T cells from psoriasis patients. Conversely, no significant difference in mediator expression was detected between psoriasis patients and healthy controls when CD8 T cells were stimulated in a TCRindependent manner (PMA/ION) (Supplementary Figure 5).

Differences in CD69, Granzyme B and IFN γ expression were also assessed in CD8 T cell subsets. Total memory, central memory, effector memory, and naïve CD8 T cell subpopulations from psoriasis patients exhibited a significantly lower percentage of CD69+, Granzyme B+, and IFN γ + cells compared to healthy controls when stimulated with anti-CD3/CD28. Similarly, effector CD8 T cells showed a significantly lower CD69 and IFN γ expression in psoriasis patients. However, Granzyme B expression was not statistically different (Figure 3C). These results indicate that circulating CD8 T cells globally are functionally impaired in patients with mild-to-moderate psoriasis.

Finally, to investigate the differences in T cell activation and mediator expression using a more holistic approach, we analysed the entire dataset using OMIQ, selectively focusing on the CD3+CD56- cell population (Figures 4A–E). A total of 18 different meta-clusters, 4 of which CD8+ and 14 CD8- T cells, were identified (Figure 4E). Based on EdgeR analysis, three CD8 T cell clusters exhibited significant alterations in their count abundance across stimulations when comparing psoriasis patients with healthy individuals (Figures 4B–E).

Upon anti-CD3/CD28 stimulation, CD8+CD45RA+CD45RO-CD27+ T cells (Cluster 15) were significantly increased in psoriasis compared to the healthy cohort. Instead, CD8+CD45RA-CD45RO- T cells expressing Granzyme B (Cluster 17) and CD8+CD45RA +CD45RO-CD27+ naïve T cells expressing CD69 and Granzyme B (Cluster 18) were significantly downregulated (Figure 4D, right). Similarly, upon PMA/ION stimulation, CD8+CD45RA-CD45RO- T cells expressing Granzyme B (Cluster 17) were downregulated in psoriasis patients compared to healthy volunteers (Figure 4D, left). Of the identified CD8+ clusters using EdgeR, only Cluster 15 (including CD8+CD45RA+CD45RO-CD27+ T cells) was found significantly increased when analysing its frequency relative to total CD3+CD56- cells (FDR-adjusted p=0.016, Supplementary Table 5), but was not confirmed by manual gating analysis (Figures F, G). CD8populations found differentially expressed using OMIQ are summarised in Supplementary Table 5 and Supplementary Results.

In summary, supervised and unsupervised analyses of circulating CD8 T cell subsets demonstrate reduced TCRdependent functional responses in patients with mild-to-moderate psoriasis compared to healthy controls.

3.5 IFNγ-producing CD8 T memory subsets correlate with psoriasis severity

To define the impact of disease severity on T cell subpopulations, we performed correlation and linear regression

analysis between PASI scores and frequencies of found T cell subsets using supervised and unsupervised approaches.

Despite only in unadjusted analyses, we found that frequencies of CD45RA+CD45RO+ and IFN γ -producing memory CD8 T cells based on manual gating positively correlated with PASI scores. Conversely, CXCR3+ MAIT CD8- cells negatively correlated with disease severity (Table 1).

In unsupervised analyses, we found significant negative correlations between PASI scores and CD8- T subsets in both ex vivo and stimulated conditions, summarised in Supplementary Results and Supplementary Table 6.

These results suggest that, in mild-to-moderate psoriasis, memory CD8 T cells, in particular IFN γ -producing EM populations, and CD8- T cells, particularly TNF-producing cells, are modulated in function of the disease severity.

4 Discussion

This study characterised the peripheral blood CD8 T compartment in psoriasis patients using both manual and unsupervised analytical tools (57). While many published studies on CD8 T cells in psoriasis have primarily focused on moderate-to-severe cases (16, 42, 46–48, 58, 59), our research selectively examined the immunological features of subjects with mild-to-moderate psoriasis. This group represents more than 80% of individuals affected by the condition and is associated with lower odds of comorbidities compared to those with severe disease (60). Our findings demonstrate a normal distribution of circulating CD8 T cells with a significant decrease in CD8 memory T cells, higher expression of BTLA in CD8 effector T cells, and an impaired immune response in circulating CD8 T cell subsets.

Zecevic-Pasic et al. reported a negative correlation between NK lymphocyte numbers and PASI while T cells (including CD4+ and CD8+ subsets) showed no significant difference between psoriasis and control cohorts (61). Furthermore, significant differences in circulating T cells have been documented between mild and more severe forms of psoriasis, using a PASI score of 12 as a cut-off (62). Our study shows that patients with mild-to-moderate psoriasis have normal frequencies of circulating CD3, CD8, NK and NKT cells compared to controls. While this finding is consistent with published studies that investigate cohorts including patients with mixed disease severity, discrepancies remain with studies focusing on severe disease cohorts (62–64).

In our study, naïve T cells were found to be comparable between the two cohorts studied, yet highly variable among donors. Demographic factors, such as age, have been reported to influence lymphocyte subset frequency. This may help explain the increased variation in cell subset frequencies observed across different studies (65–67). Notably, our findings indicate that psoriasis patients have significantly decreased circulating memory CD8 T cells, particularly CCR7- EM phenotype (gating strategy refers to Supplementary Figure 1), compared to healthy controls, and cell subsets with effector functions expressing Granzyme B (68, 69). In contrast,



FIGURE 4

Unsupervised analysis reveals differences in mediator expression profile of T cell subsets following *in vitro* stimulation between psoriasis and healthy subjects. Peripheral blood mononuclear cells (PBMCs) isolated from psoriasis patients (n=7) and healthy controls (n=10) were stimulated with anti-CD3/CD28, phorbol 12-myristate 13-acetate/ionomycin (PMA/ION) or left unstimulated. Membrane and intracellular expression of mediators within manually gated CD3+CD56- T cells were analysed using OMIQ. (A) 18 identified FlowSOM clusters were projected onto two UMAP dimensions. The overlay plot shows concatenated event from all patient and healthy samples analysed (unstimulated, PMA/ION and anti-CD3/CD28) (B) from anti-CD3/CD28 or (C) PMA/ION stimulated PBMCs from psoriasis patients and healthy controls. Significant clusters identified clusters (gray) in (B–D) Volcano plot displaying the differential count abundance of identified clusters between psoriasis and healthy subjects calculated using EdgeR and plotted using EnhancedVolcano. (E) Heatmap of cell clusters identified by FlowSOM. Rows represent each identified cluster with a description of the phenotype on the right, and columns represent the markers of interest. Adjusted expression values were defined as higher than a specified threshold based on concatenated FMO controls for each marker in flow cytometry. (F) Manual validation of found significant clusters in PMA/ION and (G) anti-CD3/CD28 stimulations. Graphs represent mean \pm SD. Differences between groups were calculated using Mann-Whitney U. *p value < 0.05. FC, fold change; FDR, false discovery rate; FMO, fluorescence minus one; UMAP, uniform manifold approximation and projection.

Condition	Description	r	p value	FDR-adjusted p value	R ²	p value LR
Ex vivo	T CD8+CD45RA+CD45RO+ cells	0.8108	0.0349	0.3746	0.4833	0.0829
Ex vivo	CD8-CXCR3+CD161+TCRVα7.2+ MAIT cells	-0.8789	0.0167	0.3500	0.8532	0.0030
PMA/ION	T CD8+CD45RA-IFNγ+ Memory cells	0.8727	0.0167	0.5208	0.5170	0.0686
PMA/ION	T CD8+CD45RA-CD27-IFNγ+ EM cells	0.8469	0.0238	0.5208	0.7389	0.0131

TABLE 1 Correlation between psoriasis area and severity index (PASI) scores and manually-gated CD8 T cell subset frequencies in psoriasis patients (n=7).

In bold p values < 0.05. EM, effector memory; FDR, false discovery rate; IFNg, interferon gamma; LR, linear regression; MAIT, mucosal-associated invariant T cells; r, Spearman correlation.

Langewouters et al. demonstrated comparable levels of CD8 +CD45RO+ T cells between patients with PASI<12 and healthy individuals (62). The discrepancy between the current literature and our findings could stem from three factors. First, our study used double CD45RO and CD45RA labelling to identify memory or naïve T cell populations. Gating strategies using two different T cell subset markers, CD27 or CCR7, produced divergent results in identifying CD8 T cell sub-populations (Supplementary Figure 6), and this outcome aligns with previous research (66, 70, 71). Second, the definition of mild-to-moderate disease varies significantly between investigators. Currently, the "rule of 10" applies to PASI scores, which dictates access to biological treatment (72). However, in contrast to our study, some authors selected an arbitrary cut-off to include subjects with borderline scores (i.e., between 10 and 12) in the definition of moderate disease (33, 61, 62, 73, 74). Third, it remains uncertain whether the reduction in CD8+ memory T cells in the peripheral blood is due to the migration of these cells into skin lesions during an active phase of the disease. However, this would support the findings that CD8 T cells and memory T cells are increased in spreading psoriatic plaques (75).

CD8 T cell subsets at various stages of cell differentiation exhibit selective/shifted expression of CCR4 and other skin-tropic chemokines associated with systemic and/or cutaneous inflammation in psoriasis (42, 50, 76, 77). While a positive correlation between PASI and circulating CLA+ T cells in psoriasis patients has been previously observed, another study showed no difference in CLA expression in either circulating CD8 or CD4 T cells between patient and healthy cohorts (78-80). In our study, we could not show significant differences in the expression of CLA in CD8 T cells in mild-to-moderate psoriasis patients compared to healthy controls. However, our cohort did not discontinue topical treatment before sample collection, and we cannot exclude the possibility that treatment may have affected the expression of tissue-homing receptors such as CLA. Furthermore, Sigmundsdóttir et al. reported that systemic treatment can significantly influence the expression of the skin tropic marker CLA on blood T cells (80, 81)

We have investigated the expression of co-inhibitory and costimulatory receptors on circulating CD8 T cells of our target population. Our findings did not reveal any major dysregulation. However, we did observe a significant increase in BTLA expression on CD8 effector T cells. This possibly suggests suppressing functions of CD8 effector T cells in patients with mild-tomoderate psoriasis. Our results align with previous publications, pointing at no significant dysregulation of co-inhibitory BTLA on total CD8 T cells in mild psoriasis patients (PASI<10).

Several studies have investigated CD8 T cell function in psoriasis (18, 24, 82). Bose and colleagues demonstrated that patients with psoriasis exhibited no significant difference in cytokine production, including IFNy, TNF, and IL-2, compared to healthy donors using anti-CD3-activated PBMCs (83, 84). A recent study phenotyping circulating CD8 T cells revealed comparable production of selected cytokines in CD8 MAIT and recirculating memory CD8 T cell clusters (32). In our study, we applied two in vitro stimulation methods with different activation mechanisms (TCR-dependent and -independent). This allowed us to broadly assess the functionality of circulating CD8 T cells, specifically the production of pro-inflammatory mediators, to elucidate their contribution to the pathogenesis of psoriasis. We observed reduced functionality of peripheral blood CD8 T cells from psoriasis patients compared to healthy control, with a significant decrease in CD69, Granzyme B and IFNy expression across CD8 T cell subsets following TCR-dependent stimulation.

IL-17 has been considered as a hallmark cytokine of psoriasis pathogenesis (73). We studied circulating CD8 T cells producing IL-17A or IL-17F and observed low and comparable levels of IL-17A/F expression in both patient and control cohorts upon *in vitro* stimulation. This could be due to differences in stimulation protocols, as discussed by Manescu et al. (85). Previous studies have shown that activated CD4 T cells, rather than CD8 T cells, are considered the main source of IL-17 in the blood (84, 86) Significantly decreased Th17 cells and IL-17 expression were also found in peripheral blood and skin of psoriasis patients treated with topical therapy (87, 88). In an imiquimod-induced psoriasiform mouse model, treatment with topical steroids significantly decreased the levels of inflammatory cytokines TNF, IL-23 and IL-17 in psoriatic lesions (89).

IFN γ plays a significant role in the pathogenesis of psoriasis, as it is often found elevated in both serum and lesional skin of affected patients (90–93). Our results indicate a significant correlation between IFN γ -expressing, PMA/ION-activated CD8 memory T cell populations and PASI scores. This aligns with previous studies conducted on peripheral blood, proposing IFN γ as a biomarker to monitor disease progression (94), and response to successful anti-TNF treatment on skin transcriptomics data (93). In psoriatic skin lesions, tissue-resident CD8 T memory cells possess higher IFN γ - producing capacity than CD4 T cells (19, 95). However, further research is necessary to elucidate whether IFN γ -producing CD8 T memory cells in lesional skin result from the migration of circulating memory pools and activation *in situ* in patients with psoriasis.

We also found a positive correlation between double-positive CD45RO+CD45RA+ CD8 T cell frequency and disease activity. Prince et al. characterised circulating double-positive T cells as having an intermediate activation status (96). There is currently limited information on double-positive T cell subsets in the context of psoriasis. Cytometry by time-of-flight analysis of PBMCs from psoriasis patients and healthy controls showed comparable frequency of terminally differentiated effector memory (TEMRA) CD8 T cells, which are a subset of CD45RO-CD45RA double-positive T cells lacking CD27 expression (49). Interestingly, a recent paper investigating synovial tissues in patients with psoriatic arthritis found that T cell clusters co-expressing CD45RO and CD45RA show extensive interaction with vascular cells, macrophages, DCs and other T cells (97).

We also found that circulating CXCR3+CD8- MAIT cells in psoriasis negatively correlate with PASI scores, hinting at the possible recruitment of CD8- MAIT cells expressing the trafficking receptor CXCR3 from the circulation to inflamed skin in mild disease. As MAIT cells display an EM-like phenotype (98), a similar correlation between circulating CXCR3+CD4+ EM T cells and PASI scores was observed in patients with psoriasis (50).

While manual analysis is the current standard for analysing flow cytometry data, increasingly more applications of unsupervised clustering algorithms have been documented, paralleling the analyses done in mass cytometry (30, 32, 99-101). Unsupervised analyses present the advantage of being user-independent and capable of identifying minute populations that could be missed when analysing sets manually. Several pipelines have been proposed, differing in scaling and/or normalisation techniques, inclusion of FMO controls and clustering methods (102-104). However, some pitfalls are unique to this technique and especially relevant in the context of flow cytometrybased analyses. First, including all markers in an effort to capture populations of interest can lead to identifying multiple clusters with similar characteristics, a process known as over-clustering. This situation often necessitates manual intervention, as discussed by Baumgaertner et al. (57). Second, unlike mass cytometry datasets, flow cytometry data present unique challenges, such as donor autofluorescence, which complicates pooling samples together, as donor-dependent anomalies can produce clustering on outliers (105). In light of the methodological differences between the two strategies, we combined both manual and unsupervised analyses, offering a more comprehensive view of the results obtained.

5 Limitations

This study presents experimental design and analytical limitations worth addressing. The sample size, particularly of psoriasis patients (n=7), was small and unequal to healthy

subjects (n=11), with limited power to discriminate minute changes in cell subset frequencies or receptor expression.

All chosen patients underwent topical treatment with either steroids or calcipotriol at the time of sample collection. This could have contributed to the reduced activation observed compared to healthy subjects in functional assays and the lower representation of re-circulating cellular pools from the skin to the blood in psoriasis patients, as previously argued.

Other than PASI scores and treatment, clinical features, including HLA-Cw6 and HLA-DRB1*07 haplotype determination, were unavailable. These features could have helped determine the susceptibility to severe disease (20, 106) and further identify potential CD8 T subsets of interest. Similarly, our study only included Caucasian subjects, limiting the translation of the results to other ethnic groups.

In our study, we performed supervised and unsupervised analyses to capture different aspects of the same dataset. However, the mutual interpretability of manual and unsupervised analyses was limited by several factors. First, the unsupervised analyses were performed using arcsinh-transformed and batchcorrected files, which differed from the original files. Second, in some unsupervised analyses, we found markers below the positivity threshold when adjusting expression to FMO controls, which contrasted with manual analyses. We speculate that high donorspecific autofluorescence affects the possibility of investigating populations with very low marker expression. This is especially relevant when adjusting the expression to pooled fluorescence controls, as shifts in fluorescence from subjects with high backgrounds can mask donors with lower backgrounds.

Thus, discrepancies in the functional profiles of CD8 T cells compared to available studies can be attributed to multiple causes, including variations in experimental settings, data analysis techniques, size of the patient cohorts, and treatment, highlighting the need for further investigations.

6 Conclusion

Our findings highlight abnormalities in the phenotype and functionality of circulating CD8 T cells in patients with mild-tomoderate psoriasis. Whether the suppressed expression of CD8 T cell mediators contributes to the pathogenesis of mild-to-moderate psoriasis or results from topical treatment remains to be determined. Our study emphasises the need for a deeper understanding of CD8 T cell subsets in relation to psoriasis severity and treatment. Expanding the antibody panel to include markers for phenotype, skin homing chemokines, co-signalling molecules, and cytokines could more accurately identify relevant CD8 T cell subtypes. Furthermore, improved disease stratification within the patient cohort, considering demographic and clinical variables, could provide a clearer understanding of CD8 T cell alterations and their correlation with disease outcomes, potentially aiding therapy optimisation (107, 108).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by London Bridge and The University of Manchester Research Ethics Committees. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. CT: Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. IT: Resources, Writing – original draft, Writing – review & editing. JB: Resources, Writing – original draft, Writing – review & editing. PD: Resources, Writing – original draft, Writing – review & editing. CG: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. RB: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SB-P: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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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.2025. 1585378/full#supplementary-material

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