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# CCR4$^+$CD8$^+$ T cells clonally expand to differentiated effectors in murine psoriasis and in human psoriatic arthritis

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**Psoriasis is a chronic inflammatory skin disease with an autoimmune component and associated with joint inflammation in up to 30% of cases. To investigate autoreactive T cells, we developed an imiquimod-induced psoriasis-like inflammation model in K5-mOVA.tg C57BL/6 mice expressing ovalbumin (OVA) on the keratinocyte membrane, adoptively transferred with OT-I OVA-specific CD8$^+$ T cells. We evaluated the expansion of OT-I CD8$^+$ T cells and their localization in skin, blood, and spleen. scRNA-seq and TCR sequencing data from patients with psoriatic arthritis were also analyzed. In the imiquimod-treated K5-mOVA.tg mouse model, OT-I T cells were markedly expanded in the skin and blood at early time points. OT-I T cells in the skin showed mainly CXCR3$^+$ effector memory phenotype, whereas in peripheral blood there was an expansion of CCR4$^+$CXCR3$^+$ OT-I cells. At a later time point, expanded OVA-specific T-cell population was found in the spleen. In patients with psoriatic arthritis, scRNA-seq and TCR sequencing data showed clonal expansion of CCR4$^+$ $T_{CM}$ cells in the circulation and further expansion in the synovial fluid. Importantly, there was a clonotype overlap between CCR4$^+$ $T_{CM}$ in the peripheral blood and CD8$^+$ T-cell effectors in the synovial fluid. This mechanism could play a role in the generation and spreading of autoreactive T cells to the synovioentheseal tissues in psoriasis patients at risk of developing psoriatic arthritis.**

**Keywords:** Chemokine receptors · Clonal expansion · Psoriasis and psoriatic arthritis · Self-reactive CD8 T cells · T-cell recirculation

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

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## Introduction

Psoriasis is a chronically relapsing skin inflammation affecting about 2% of the population worldwide. Plaque psoriasis is the most common form of the disease. It is associated with important systemic manifestations and in up to 30% of cases with psoriatic arthritis [1–4].

The disease arises from a complex interplay between keratinocytes, microvascular endothelium, dendritic cells (DC), and T cells, resulting in a self-sustaining inflammatory cycle that develops around the IL-23/IL-17 axis [5–9]. In patients with psoriasis, we previously evidenced the role of the axis CCR7/CCL19 in the formation of dermal DC–T cells clusters, which therefore emerge as central structures in psoriasis pathogenesis [10].

An autoimmune component of psoriasis has been described, and T cells reactive to autoantigens cathelicidin LL-37, melanocytic ADAMTSL5, lipid antigen PLA2G4D, and keratin 17 have been identified in patients' peripheral blood [11–15]. Nevertheless, the role of this component in the development of psoriasis and its extracutaneous manifestations remains elusive.

Our previous work showed that in patients with cutaneous psoriasis, CCR4$^+$ cells were expanded in the circulating memory CD4$^+$ and CD8$^+$ compartments and their percentage positively correlated with the severity of the disease, pointing out CCR4$^+$ memory T cells as a candidate skin-to-blood recirculating subset [16]. In patients with psoriatic arthritis, analysis of memory CD4$^+$ and CD8$^+$ T cells of blood and synovial fluid indicates a shift from the CCR4$^+$CXCR3$^-$ phenotype in peripheral blood to CCR4$^+$CXCR3$^+$and CXCR3$^+$CCR4$^-$ that predominate in synovial fluid [17–19]. Importantly, a parallel study of scRNA-seq analysis in patients with psoriatic arthritis confirmed an accumulation of CXCR3$^+$ CD8$^+$ T cells in the synovial fluid [20].

We hypothesized that in immunopathological skin conditions such as psoriasis, the CCR4$^+$ subset could be involved in spreading self-reactive T cells in the circulation and could play a role in disease recurrence or redistribution to distant sites. This could be accompanied by a shift of the phenotype upon antigen re-encounter from CCR4$^+$ $T_{CM}$ to the CXCR3 $T_{EM}$ phenotype.

To test this hypothesis, we used the imiquimod-induced psoriasis-like inflammation model in K5-mOVA.tg C57BL/6 mice adoptively transferred with ovalbumin-specific OT-I.tg naive T cells to add the antigen-specific T-cell component to psoriasis-like inflammation [21–23].

In this mouse model, we evaluated whether prolonged skin inflammation induced by imiquimod treatment favors the activation of OT-I CD8$^+$ T cells specific for the cutaneous self-antigen. We also evaluated the recirculation of antigen-specific memory T cells from the skin to the blood and spleen, and we analyzed the changes in their memory phenotype and chemokine receptor profile. Finally, we analyzed scRNA-seq data paired with TCR $\alpha/\beta$ chain sequencing from patients with psoriatic arthritis to evaluate the clonal expansion and clonotype sharing in the relevant CD8$^+$ T-cell subsets in peripheral blood and in synovial fluid.

## Results

### Skin expansion of OT-I CD8$^+$ T cells in K5-mOVA.tg mice with psoriasis-like inflammation at day 9

To determine the number, the phenotype, and the localization of adoptively transferred T cells in K5-mOVA.tg mice with psoriasis-like inflammation, we traced OT-1 CD8$^+$ T cells using the CD45.1/CD45.2 donor–recipient discrimination method. We transferred OT-I OVA-specific T cells CD45.1$^+$CD45.2$^+$ in CD45.2 recipient, and we analyzed the percentage of CD45.1$^+$CD45.2$^+$ CD8$^+$ T cells in the skin, blood, and spleen at day 9 (Fig. 1A). As shown in Fig. 1B, at this time point, the percentage of adoptively transferred OT-I CD45.1$^+$CD45.2$^+$ T cells was markedly increased in the skin CD8$^+$ population. Differently, the percentage of CD45.1$^+$CD45.2$^+$ T cells in CD8$^+$ T cells was minimal in the spleen. In peripheral blood CD8$^+$ T cells, the percentage of OT-I cells was not significantly different in the two groups. Nevertheless, analysis of the percentage of the total CD8$^+$ T cells (comprising both OT-I and recipient CD8$^+$ T cells) in CD45.2$^+$ leukocyte population indicates increased migration toward the imiquimod-treaded skin of the entire CD8$^+$ population (Fig. 1C). The percentage of total CD8$^+$ T cells was also increased within the blood CD45.2$^+$ leukocyte population, whereas a small but significant decrease was observed in the spleen. Therefore, we calculated the number of CD45.1$^+$CD45.2$^+$ OT-I CD8$^+$ T cells in $10^5$ CD45.2$^+$ leukocytes in the skin, blood, and spleen. The number takes into account the overall migration of CD8$^+$ T cells (OT-I and recipient T cells) to the inflamed tissue. Data in Fig. 1D clearly show a marked increase of the OT-I CD8$^+$ T-cell number in CD45.2$^+$ cells in the skin of imiquimod-treated mice. At this time point, the increase was evident also in peripheral blood, whereas no significant differences were observed in the spleen.

### Memory T-cell phenotype and chemokine receptor profile of OT-I CD8 T cells expanded by skin inflammation in K5-mOVA.tg mice

Analysis of the memory phenotype of CD45.1$^+$CD45.2$^+$ OT-I CD8$^+$ T cells revealed that in the inflamed skin of mice with psoriasis-like inflammation OT-I T cells had almost entirely an effector memory phenotype (CD44$^{hi}$CD62$^{low}$) (Fig. 2A and B and Supporting information Fig. S1).

This result confirms the strong expansion of OVA-specific CD8$^+$ T cells with effector memory phenotype in the skin of mice developing psoriasis-like inflammation. Importantly, OT-I T cells with an effector memory phenotype were increased in peripheral blood of imiquimod-treated mice. Conversely, no significant differences were observed in the spleen.

Finally, we examined the chemokine receptors profile of central memory and effector memory T cells in the skin, blood, and spleen of imiquimod-treated and untreated mice. Results in Fig. 2C and D show the percentage of CCR4 and CXCR3

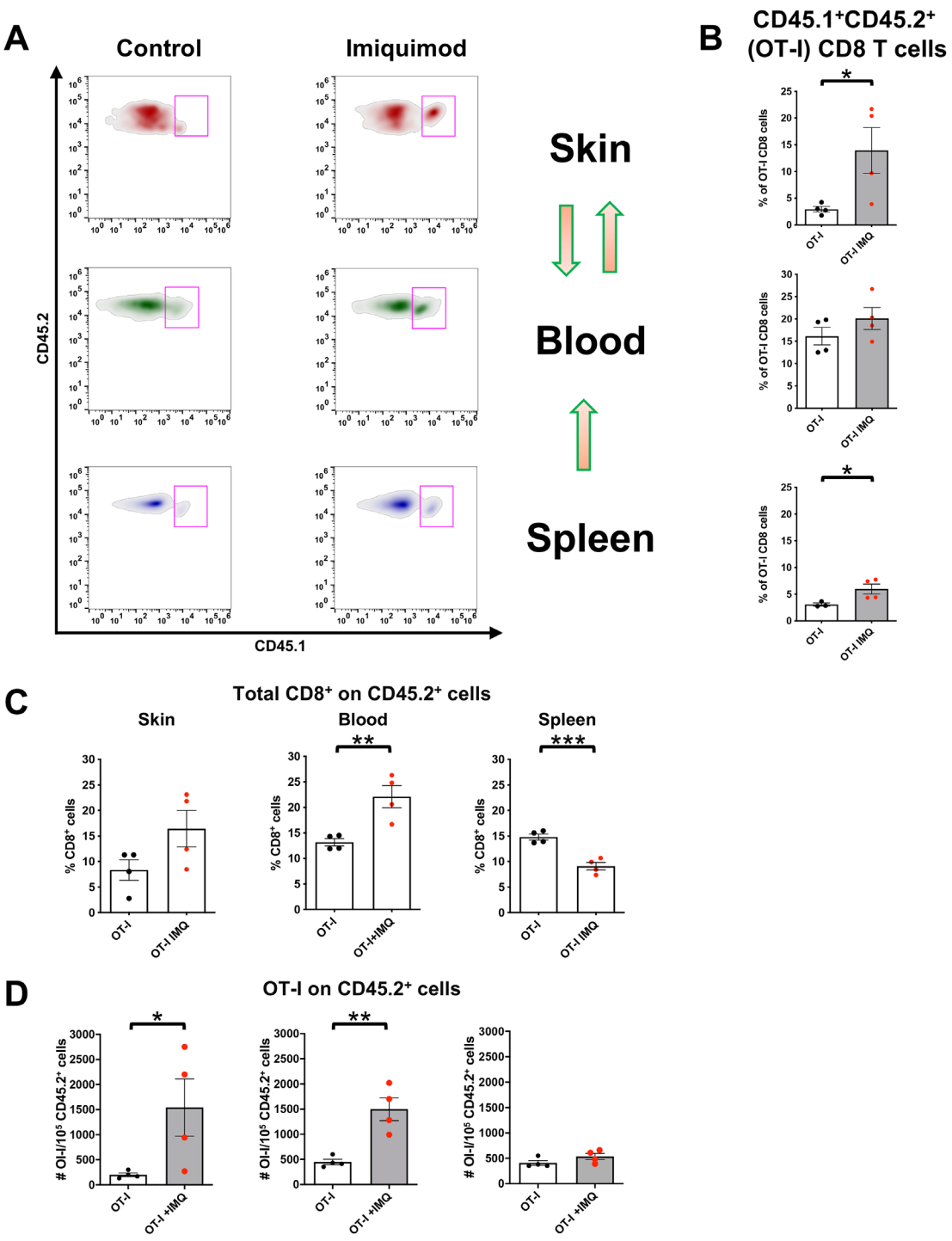


**Figure 1.** Skin expansion of OT-I CD8$^+$ T cells in K5-mOVA.tg mice with psoriasis-like inflammation at day 9. K5-mOVA.tg mice were treated for 7 days with imiquimod by applying imiquimod cream in the ears and on shaved back skin. At day +1, mice were adoptively transferred with congenitally marked CD45.1/CD45.2 OT-I CD8$^+$ T cells ($1 \times 10^6$ cells/mouse). At day 9, mice were sacrificed and cells were isolated from the skin, blood, and spleen ($n = 4$ mice/treatment group). (A) Representative flow cytometry plots showing CD45.1$^+$CD45.2$^+$ OT-I T cells within the total CD8$^+$ T-cell population (CD45.2) in the three compartments. (B) The percentages of CD45.1$^+$CD45.2$^+$ OT-I T cells within the CD45.2$^+$ CD8$^+$ T cells in the skin, blood, and spleen is reported. The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann–Whitney test and were considered significant for $p$ values ≤ 0.05. (C) Percentage of the total CD8 T cells in the CD45.2 leucocyte gate in the three compartments is reported for the untreated (OT-I) and imiquimod-treated (OT-I IMQ) transferred group of mice. The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann–Whitney test. Differences were considered significant for $p$ values ≤ 0.05. (D) The number of CD45.1$^+$CD45.2 OT-I T cells in $10^5$ CD45.2$^+$ leucocytes in skin, blood, and spleen is reported in the figure. Differences between the untreated (OT-I) imiquimod-treated (OT-I IMQ) groups are shown in the figure. The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann-Whitney test. Differences were considered significant for $p$ values ≤ 0.05.

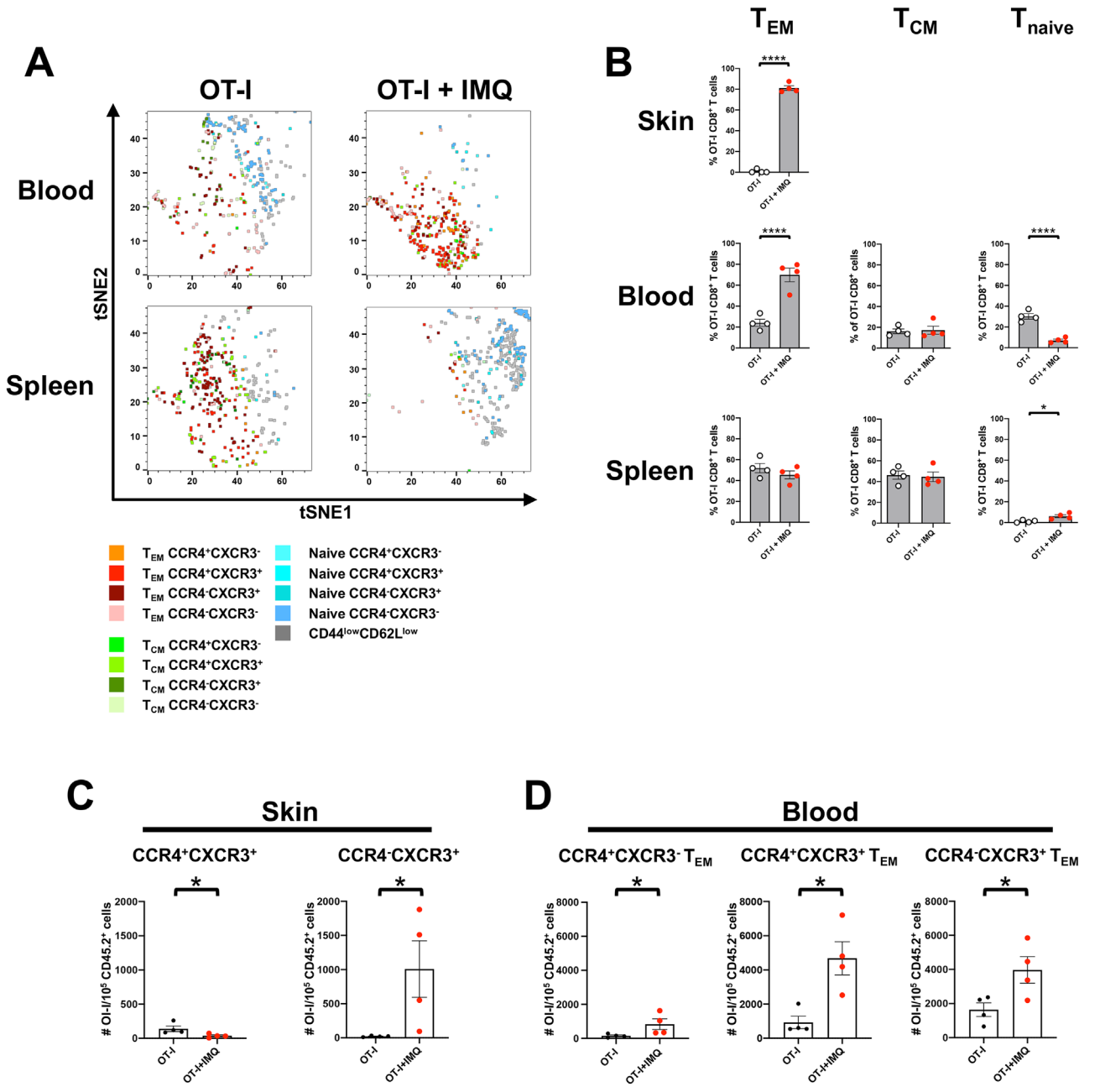


**Figure 2.** Memory T-cell phenotype and chemokine receptor profile of OT-I T cells primed by skin inflammation in K5-mOVA.tg mice at day 9. (A) Lymphocyte subpopulations within the OT-I $CD8^+$ T cells identified by Boolean gating were concatenated from all tissue samples after down-sampling and analyzed with t-SNE. Manual gating was performed on t-SNE clusters after annotation. Gated clusters were then overlaid onto the total t-SNE map (right, colored clusters) after arbitrary assignment of cluster numbers. (B) The percentage of $T_{EM}$, $T_{CM}$, and $T_{naive}$ in the congenitally marked OT-I gate is reported for the imiquimod treated and untreated OT-I T-cell transferred groups of mice in the skin blood and spleen ($n$ = 4 mice/treatment group). The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann-Whitney test. Differences were considered significant for $p$ values ≤ 0.05. (C) The number of $CCR4^+CXCR3^-$, $CCR4^+CXCR3^+$, or $CCR4^-CXCR3^+$ skin OT-I T cells or OT-I $T_{EM}$ cells was calculated on $10^5$ CD45.2 gated leucocytes in the skin blood and spleen ($n$ = 4 mice/treatment group). The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann-Whitney test. Differences were considered significant for $p$ values ≤ 0.05.

positive cells in the skin and peripheral blood of the two groups of mice, evidencing that, in the skin of imiquimod-treated mice, OT-I cells were mainly $CXCR3^+$, whereas in peripheral blood there was a significant enhancement of $CCR4^+$ OT-I T cells (Fig. 2D and Supporting information Fig. S2). This enhancement in peripheral blood is mainly evident in the effector memory compartment and has a similar trend in central memory cells (not shown) [24–27].

### Accumulation of OT-I CD8$^+$ T cells in the spleen of imiquimod-treated K5-mOVA.tg mice at day 15

Mice were treated according to the schedule in Fig. 3A. At day 15, a marked increase in the percentage of SIINFEKL-OVA-specific CD8$^+$ T cells was observed in the spleen of imiquimod-treated OT-I transferred K5-mOVA.tg mice as compared to their OT-I transferred untreated counterpart. We did not observe any significant increase in SIINFEKL-OVA-specific CD8$^+$ T cells in mice without transfer of OT-I T cells treated with imiquimod compared to their untreated counterpart (Fig. 3B and C). At this time point, psoriasis-like inflammation had resolved and we could detect in the skin only minimal levels of SIINFEKL-Tetr$^+$ T cells in all groups of mice.

We then wanted to confirm that the observed expansion of OVA-specific CD8$^+$ OT-I cells was dependent on the presence of the cutaneous self-antigen ovalbumin, presented in the context of psoriasis-like inflammation. To this end, OT-I T-cell adoptive transfer and imiquimod treatment was performed in both wild-type (WT) and K5-mOVA.tg mice. The results reported in Fig. 3C clearly show that expansion of OVA-specific T cells upon treatment with imiquimod only occurs in mice expressing cutaneous OVA (K5-mOVA.tg mice). Adoptive transfer of naive OT-I CD8$^+$ T cells in WT mice treated with imiquimod did not induce spleen expansion of adoptively transferred OVA-specific T cells. The result was even more pronounced when we measured the percentage of SIINFEKL-Tetr$^+$ cells on Vβ5 (Fig. 4D).

Supporting information Fig. S3A and B shows that in K5-mOVA.tg imiquimod-treated mice adoptively transferred with OT-I T cells, there was a significant expansion of spleen Vβ5 CD8$^+$ $T_{EM}$ cells. The difference was significant in comparison to either the OT-I T cell adoptively transferred K5-mOVA.tg mice without treatment or the OT-I T cell adoptively transferred and imiquimod-treated WT mice.

This set of experiments provides the evidence that psoriasis-like inflammation induces the activation and the expansion of T cells specific for cutaneous self-antigens and that these cells can localize to the spleen.

### Expanded CCR4$^+$CXCR3$^+$ $T_{CM}$ cells in the circulating CD8$^+$ compartment in patients with psoriatic disease

To confirm that the expansion of CCR4$^+$ CD8$^+$ T cells can also occur in the human pathology, we analyzed flow cytometry data obtained from a cohort of patients with psoriatic disease. The phenotype of peripheral blood mononuclear cells (PBMC) from 12 patients with cutaneous psoriasis and 11 patients with psoriatic arthritis was compared with the phenotype of 12 healthy subjects in the same age range. The data show that patients with cutaneous psoriasis have an expanded subpopulation of cells with CCR4$^+$ $T_{CM}$ phenotype within the total CD8$^+$ T-cell population in peripheral blood as compared to the healthy control group (Supporting information Figs. S4 and S5). Expansion of CCR4$^+$CXCR3$^+$ $T_{CM}$ cells was observed for both psoriasis and PsA patients, whereas there was no difference in the percentage of CXCR3$^+$ $T_{CM}$ cells between patients' and healthy control groups. No significant differences were observed in the $T_{EM}$ compartment, where the majority of the cells had a CXCR3$^+$ phenotype. The prevalence of CCR4 expression in $T_{CM}$ cells agrees with previous observation in healthy subjects and patients with psoriasis [28]. However, there is an apparent discrepancy with the mouse data (Fig. 2C) showing an increase in CCR4$^+$CXCR3$^+$ phenotype in the $T_{EM}$ compartment that could be due to the imiquimod model that rapidly induces intense skin inflammation, thus shifting the T-cell phenotype to $T_{EM}$.

Data are represented both as a representative tSNE (Supporting information Fig. S4A) and as plots showing the difference between individual $T_{CM}$ and $T_{EM}$ subsets, namely CCR4$^+$CXCR3$^-$, CCR4$^+$CXCR3$^+$, and CCR4$^-$CXCR3$^+$ within the total population of CD8$^+$ T cells in peripheral blood from patients and control subjects (Supporting information Figs. S4B and S5).

We assessed the possibility that CCR4$^+$ $T_{CM}$ cells can represent a subset with high plasticity that, upon antigen encounter, can progressively shift their phenotype toward CXCR3$^+$ $T_{EM}$. We therefore sorted CCR7$^+$CD45RA$^-$CXCR3$^-$ cells from PBMCs from one healthy subject and we stimulated them with αCD3/αCD28 beads. After 3 and 6 days, we analyzed whether a shift in their phenotype occurred. Data in Supporting information Fig. S6 show that, at day 3, after TCR stimulation, all CCR4$^-$-enriched CXCR3-depleted CD8$^+$ $T_{CM}$ cells shift their phenotype to double positive CCR4$^+$CXCR3$^+$ $T_{CM}$. Interestingly, at day 9 the majority of the cells still have a CCR4$^+$CXCR3$^+$ phenotype, however a small fraction of cells single positive for CXCR3 and a fraction of cells with $T_{EM}$ phenotype were detected.

These results together reinforce the evidence of an increased percentage of CCR4$^+$ $T_{CM}$ cells in the circulating CD8$^+$ T-cell compartment of patients with psoriatic disease and support the possibility that this subset upon antigen encounter can shift its phenotype and give rise to more differentiated and inflammatory subsets.

### Circulating CCR4$^+$ CD8$^+$ $T_{CM}$ cells share the clonotype with synovial fluid effectors in patients with psoriatic arthritis

To explore whether the CCR4$^+$ $T_{CM}$ subset in patients with psoriatic arthritis could represent a subpopulation of clonally expanded cells linked with those cells in the synovial fluid, we analyzed a publicly available scRNA-seq and single-cell TCR sequencing data of memory-enriched (CD45RA$^-$) CD3$^+$CD8$^+$CD4$^-$ cells isolated from peripheral blood and synovial fluid of three patients with psoriatic arthritis [20]. Clusters corresponding to CD8$^+$ T cells were subclustered to identify subpopulations. A total of 13 clusters were identified (Supporting information Fig. S7A and B) and annotated based on the expression of canonical markers. Specifically, the following CD8$^+$ T-cell subsets were identified: central memory ($T_{CM}$, corresponding to clusters 1, 5, 7, 9, 10) expressing *CCR7* and *SELL* (CD62L), CCR4$^+$ $T_{CM}$ (cluster 4),

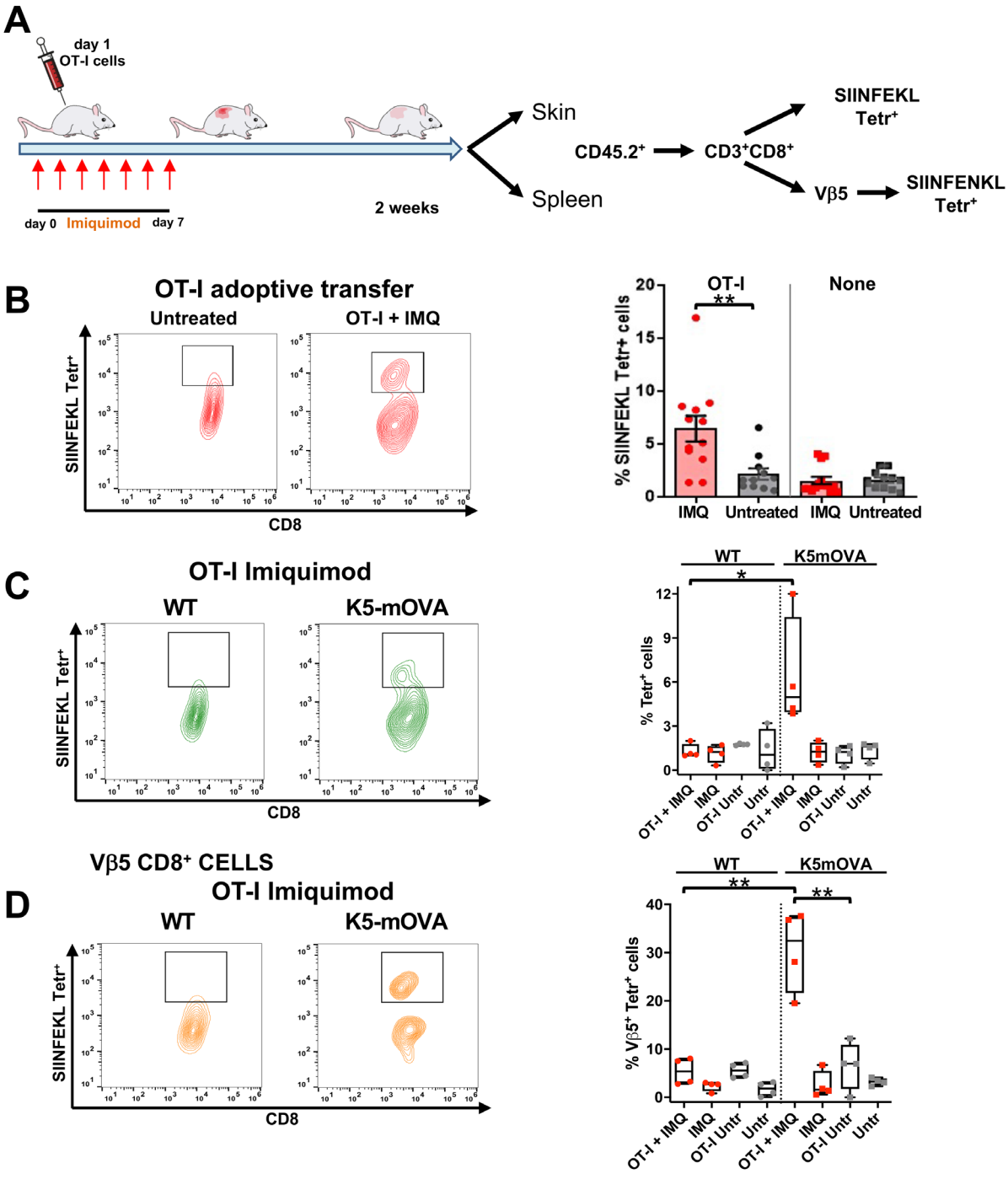


**Figure 3.** Accumulation of SIINFEKL/OVA-specific OT-I CD8$^+$ T cells in the spleen of imiquimod-treated K5-mOVA.tg mice at day 15. (A) Schematic representation of the experiment. K5-mOVA.tg mice were treated for 7 days with imiquimod and at day +1 from the first imiquimod application were adoptively transferred with OT-I T cells ($1 \times 10^6$ cells/mouse). At day 15, mice were sacrificed and cells were isolated from the skin blood and spleen ($n = 11$ mice/treatment group derived from three different experiments). (B) Representative flow cytometry plots showing the percentage of SIINFEKL-Tetr$^+$ OT-I cells within the total CD8$^+$ T-cell population in the spleen of adoptively transferred K5-mOVA.tg mice either untreated or treated with imiquimod. The percentage of SIINFELKL-Tetr$^+$ Vβ5$^+$ cells on CD8$^+$ gated T cells in the spleen is reported in the figure. The bars represent the mean ± SEM. Significance levels of the differences were calculated by Mann-Whitney test and were considered significant for $p$ values $\leq 0.05$. (C) Representative flow cytometry plots show the percentage of SIINFEKL-Tetr$^+$ OT-I cells within the total CD8 T-cell population in the spleen of adoptively transferred WT and K5-mOVA.tg mice untreated and treated with imiquimod (n = 4 mice/treatment group from two experiments). The percentage of positive cells for each group is represented as Box and Whiskers plot. Significance levels of the differences were calculated by Mann–Whitney test and were considered significant for $p$ values $\leq 0.05$. (D) Representative flow cytometry plots showing the percentage of SIINFEKL-Tetr$^+$ Vβ5$^+$ OT-I cells within the total CD8 T-cell population in the spleen of adoptively transferred WT and K5-mOVA.tg mice untreated treated with imiquimod ($n = 4$ mice/treatment group from two experiments). The percentage of positive cells for each group is represented as Box and Whiskers plot. Significance levels of the differences were calculated by Mann-Whitney test and were considered significant for $p$ values $\leq 0.05$.

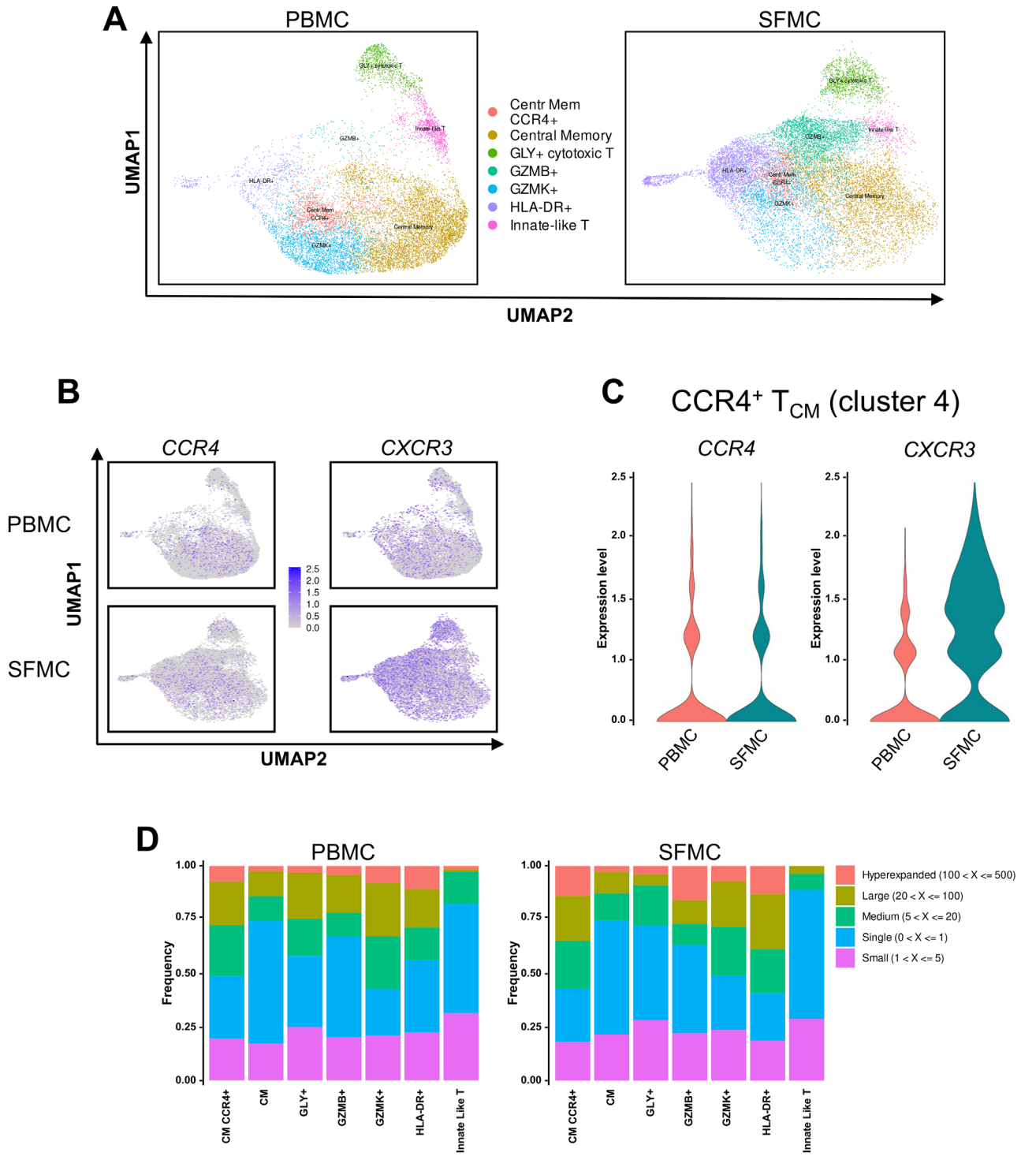


**Figure 4.** Clonal expansion of CD8$^+$ T-cell subsets in PsA patients. (A) UMAP representing CD8$^+$ T-cell subsets identified by scRNAseq analysis in peripheral blood (PBMC) and in synovial fluid (SFMC) from three PsA patients. (B) Feature plots showing the expression levels of *CCR4* and *CXCR3* in peripheral blood (PBMC) and in synovial fluid (SFMC) CD8$^+$ T cells. (C) *CCR4* and *CXCR3* expression in the CCR4$^+$ T$_{CM}$ subsets of peripheral blood (PBMC) and in synovial fluid (SFMC). (D) Stacked bar chart with the proportions of clonal expansion ranges in the CD8$^+$ T-cell subsets from peripheral blood and synovial fluid.

innate-like T cells expressing *IL7R*, *CCR6, GZMK*, and *KLRB1* (cluster 8), a subset characterized by high expression of *HLA-DR* genes (HLA-DR$^+$, cluster 2 and 12), and three effector subsets characterized by the expression of cytotoxic molecules (*GZMK, GZMB*, and *GNLY*) (Fig. 5A and Supporting information Fig. S7A and B). As previously evidenced by Penkava et al. [20], there is a marked expansion of *GZMB*$^+$ and *HLADR*$^+$ CD8$^+$ T-cell effectors in the synovial fluid (Fig. 4A).

The expression *CCR4* was restricted to cluster 4, both in peripheral blood and synovial fluid, whereas *CXCR3* was more broadly distributed across the clusters (Fig. 4B). Importantly, by analyzing CCR4 and CXCR3 expression in CCR4$^+$ $T_{CM}$, we observed a marked increase in CXCR3 in the synovial fluid, with unchanged levels of CCR4 (Fig. 4C).

Moreover, clonal expansion analysis in the different clusters indicated higher frequency of hyperexpanded and largely expanded clones in the circulating *CCR4*$^+$ $T_{CM}$ subset compared with the $T_{CM}$ negative for CCR4 (Fig. 4D and Supporting information Fig. S8). A further expansion was observed in the synovial fluid CCR4$^+$ $T_{CM}$. Highly expanded clones were also observed in differentiated effectors, such as *GZMB*$^+$ and *HLADR*$^+$ CD8$^+$ T cells in the synovial fluid.

We then investigated whether the expanded effector population (*GZMK*$^+$*, HLADR*$^+$, and *GZMB*$^+$) in the synovial fluid have a common origin with the circulating CCR4$^+$ $T_{CM}$. For this purpose, we evaluated the clonotype sharing between the circulating CCR4$^+$ $T_{CM}$ and the cell subpopulations in the synovial fluid.

Clonal overlap analysis showed that synovial fluid T-cell clones, with *HLADR*$^+$, and *GZMK*$^+$ phenotypes, shared multiple clonotypes with the circulating CCR4$^+$ $T_{CM}$ (Fig. 5A). Importantly, high level of clonotype sharing was observed also between CCR4$^+$ $T_{CM}$ and *HLADR*$^+$ in the synovial fluid. Quantitative analysis of the TCR repertoire overlap, using the Morisita index, revealed that the circulating *CCR4*$^+$ $T_{CM}$ cells shares the TCR repertoire with both CCR4$^+$$T_{CM}$ and *GLY*$^+$*, HLADR*$^+$, and *GZMK*$^+$ subsets in the synovial fluid (Fig. 5B). In contrast, the circulating $T_{CM}$ subset negative for *CCR4* showed little clonal similarity with the subsets in the synovial fluid. Together, these data evidence that the CD8$^+$ *CCR4*$^+$ $T_{CM}$ cells in the circulation selectively share clonality with the expanded CD8$^+$ T-cell effectors in the synovial fluid. This in turn indicates that *CCR4*$^+$ cells in the blood can represent the circulating counterpart of the synovial CD8$^+$ T-cell effectors. It also points out a phenotypic and functional link between recirculating skin-primed CCR4$^+$ $T_{CM}$ cells and T-cell effector in the joints of patients with psoriatic arthritis.

## Discussion

The results of this study indicate that the induction of psoriasis-like inflammation in K5-mOVA transgenic mice adoptively transferred with naïve OT-I CD8$^+$ T cells leads to selective expansion of CD8$^+$ OT-I T cells. At early time points such as day 9, the expansion is marked in the skin and starts to be evident in peripheral blood. Skin OT-I T cells showed an effector memory phenotype and expression of CXCR3, whereas in peripheral blood we also observed a significant increase in CCR4$^+$ and CCR4$^+$CXCR3$^+$ memory T cells.

At day 15, OVA-SIINFEKL-specific T-cells accumulation was found in the spleen. This suggests that self-reactive T cells primed in the skin can recirculate to the blood and finally to the spleen to constitute an OVA-specific memory T-cell pool.

Importantly, we showed that expansion of SIINFEKL-specific OT-I cells only occur upon induction of psoriasis-like inflammation and requires the presence of OVA at cutaneous level, as it does not occur in WT mice. This suggests that the phenomenon may require inflammation induced cross-presentation of cutaneous self-antigens.

Therefore, we consider that OT-I adoptively transferred imiquimod-treated K5-mOVA.tg mice can represent a suitable model of autoimmune psoriasis.

T-cell migration into peripheral tissues depends on their chemokine receptor profiles. Cells expressing Th1/Tc1 cytokines-type express high levels of CXCR3 and CCR5, and are recruited by chemokine CXCL10 and CCL4, respectively. CD8$^+$ T cells in their most represented $T_{EM}$ phenotype express CXCR3 and CCR5, whereas they express at much lower level CCR4 [28–31]. On the other hand, the recruitment of T cells to the skin is regulated by the expression of the specific skin-homing molecules: the chemokine receptor CCR4, and the cutaneous lymphocyte-associated antigen CLA. CCR4 has been defined as a skin-homing receptor that is upregulated by memory T cells primed in skin-draining lymph nodes and we have previously shown that, in circulating CD8$^+$ T cells, CCR4 is mainly expressed by $T_{CM}$ cells that also co-express cutaneous lymphocyte-associated antigen [32, 33]. A role for CCR4 in DC–T cell clusters formation and disease development has recently been shown in a mouse model of psoriasis [34]. In this study, the authors underline the production of IL-17 by CCR4$^+$ T cells. We previously observed in the human disease a marked production of IL-2 by CCR4$^+$ T cells together with intermediate level of IL-17 and low level of IFN-γ. This result is in line with both the evidence that CCR4$^+$ subset is a skin-primed IL-17 producing cell subset,as reported by Matsuo et al., and that it has a $T_{CM}$ phenotype [28, 34, 35].

Here, we envisaged a role for CCR4$^+$ $T_{CM}$ cells in patrolling healthy tissue and a high potential of T cell homing to the inflamed skin through the interaction with its ligand CCL17. In the light of these results, CCR4$^+$ $T_{CM}$ cells could represent a subset with high plasticity that upon antigen encounter can shift the phenotype to a more differentiated CXCR3$^+$ $T_{EM}$ phenotype.

Indeed, sorted CCR4-enriched CXCR3-depleted CD8$^+$ $T_{CM}$ cells upon in vitro stimulation clearly showed a shift toward a double positive CCR4$^+$ CXCR3$^+$ phenotype, thus supporting a phenotypic transition of antigen-stimulated CCR4 cells toward CXCR3. CXCR3 has recently been shown to be required for the recruitment of memory CD8$^+$ T cells at the site of infection [36]. It is also required for the T cell–DCs clustering and for T-cell bystander activation occurring early before the antigen-specific T-cell activation.

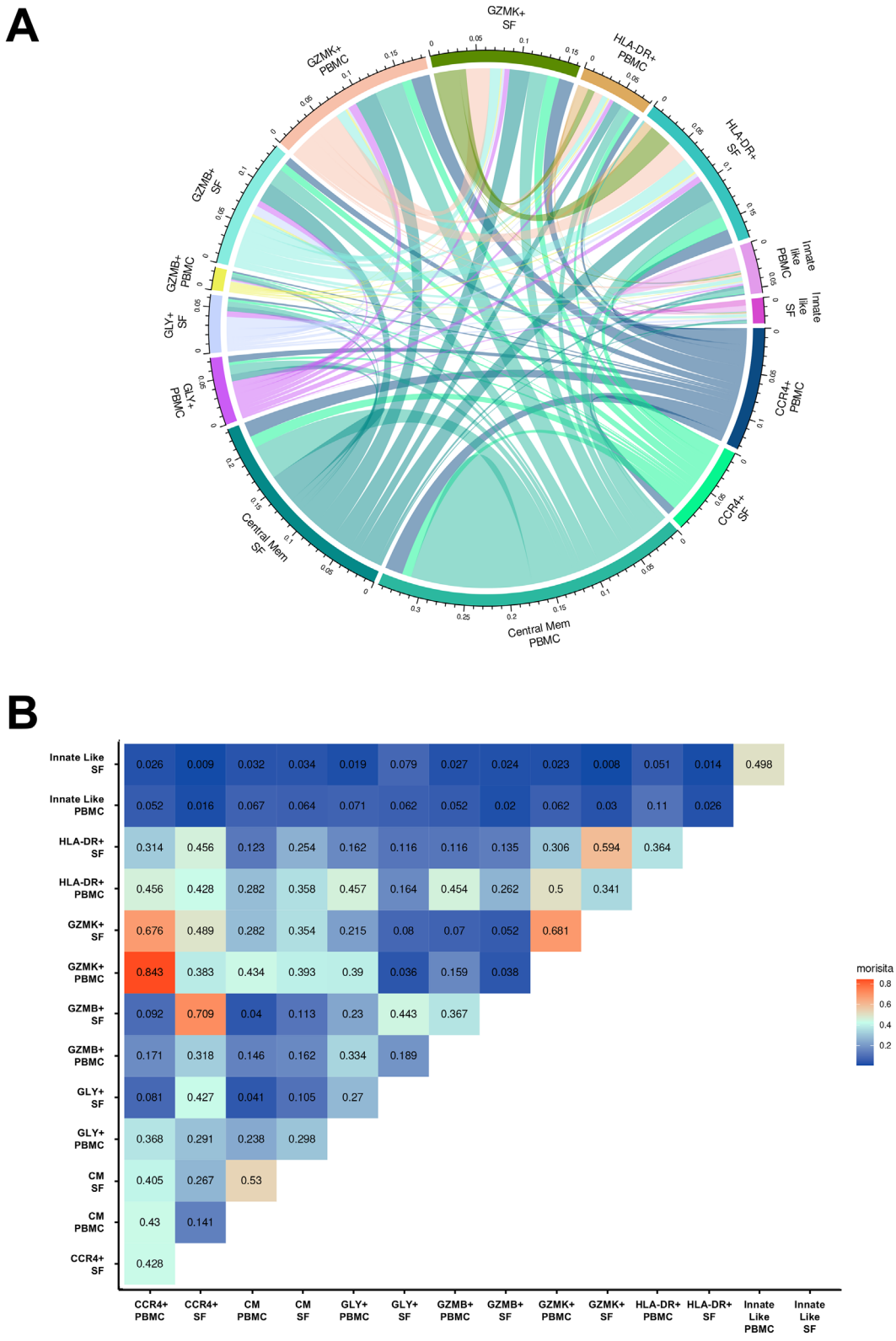


**Figure 5.** Clonotype sharing between peripheral blood and synovial fluid CD8$^+$ T-cell subsets in patients with PsA. (A) Circos plot showing TCR sharing among the CD8$^+$ T-cell subsets in peripheral blood (PBMC) and synovial fluid (SFMC). Arcs represent the number of clonotypes shared between subsets based on identically rearranged TCR genes. (B) The Morisita index is reported for each pair of CD8$^+$ T-cell subsets. Morisita index of 0 would indicate no TCR sequences shared between two subsets, while an index of 1 indicate identical repertoires, comprising the same clonotypes in the same proportions in both subsets. Abbreviations: CM, central memory, SF: synovial fluid.

We and other observed an accumulation of CXCR3$^+$ effector memory T cells in synovial fluid and in the skin of patients with psoriasis/PsA, indicating that effector T cells are largely recruited by a mechanism that involves the CXCL10/CXCR3 axis [18, 20, 37, 38].

In regard to the role of CD8$^+$ T cell in the development of psoriatic arthritis, Curran et al. reported the TCR β-chain nucleotide sequence analysis in peripheral blood and synovial tissues/fluid, showing that the majority of the clones in inflamed tissues were polyclonal and unexpanded, and only 26% were clonally expanded [39]. Together this evidence indicates that effector T-cell recruitment through CXCL10/CXCR3 could lead to both activation of antigen-specific clones and bystander activation of other clones, both contributing to the pathology.

Here, we confirmed the expansion of CCR4$^+$ $T_{CM}$ subset in circulating CD8$^+$ T cells in patients with either cutaneous or with psoriatic arthritis. Importantly, scRNA-seq analysis paired with TCR sequencing in peripheral blood and synovial fluid of patients with PsA showed that CCR4$^+$ CD8$^+$ $T_{CM}$ cells had an increased level of clonal expansion compared with the negative fraction of $T_{CM}$. Strikingly, CCR4$^+$ $T_{CM}$ cells further expanded in the synovial fluid and shared clonality with synovial fluid *CCR4*$^+$ $T_{CM}$ cells, as well as synovial effectors expressing *GZMK, GZMB*, and *HLADR*. This evidence strongly supports that skin-primed CCR4$^+$ $T_{CM}$ recirculating to the blood, and finally to synovial tissues, could shift toward effector phenotypes and play a role in the development of psoriatic arthritis.

Altogether, our data open the possibility that clonally expanded CD8$^+$ T cells primed in the skin toward a self-antigen in a context of prolonged inflammation could recirculate from the skin and establish a pool of memory T cells that can expand upon antigen re-encounter [40, 41]. This mechanism could play an important role in the generation and spreading of autoreactive T cells to distant organs and could represent a potential target for novel therapeutic interventions in patients with psoriasis to reduce the risk of development of psoriatic arthritis.

## Materials and methods

### Experimental design

We first set up a model in which TLR7/8-mediated activation of IL-23/IL-17 axis is paralleled by induction of T-cell-mediated adaptive responses against self-antigens expressed in the skin. For this purpose, we have used K5-mOVA.tg C57/BL6 mice, expressing chicken ovalbumin on the membrane of keratinocytes under the control of keratin 5 promoter [22]. Mice were adoptively transferred with naïve OVA-specific Vα2Vβ5 OT-I transgenic CD8$^+$ T cells at a low number ($1 \times 10^6$ cells) to avoid spontaneous skin reactions and were treated with imiquimod for a period of 7 days to induce psoriasis-like inflammation. We hypothesized that, during this time, prolonged inflammation could induce cutaneous self-antigen cross-presentation and cross-priming of OVA-reactive OT-I cells. Therefore, we analyzed OVA-specific T-cell responses at different time points. To evaluate skin-to-blood trafficking of activated OT-I CD8$^+$ T cells, we adoptively transferred OT-I T cells from CD45.1/CD45.2 congenic mice in recipient K5-mOVA.tg CD45.2 mice. We analyzed TCR.tg T cells in the skin and spleen of imiquimod-treated and -untreated recipient K5-mOVA.tg mice to determine their presence at different time points after the development of psoriasis-like inflammation. We also traced OVA-specific TCR.tg CD8$^+$ T cells and analyzed their memory phenotype and chemokine receptor expression in skin, blood, and spleen.

As an indicator of the OVA-specific OT-I cell activation and expansion, we also analyzed SIINFEKL-OVA tetramer positive cells in CD8$^+$ T cells or Vβ5$^+$CD8$^+$ T cells from the spleen and skin in the different groups of mice.

Finally, analysis of scRNA-seq data paired with TCR alpha/beta chain gene sequencing from patients with psoriatic arthritis was performed to evaluate the clonal expansion and clonotype sharing in the relevant T-cell subpopulations.

### Mice

All procedures involving mice and their care were performed in conformity with the ethical principles and EU Directive 2010/63/EU for animal experiments guidelines. The project was approved by the Italian Ministry of Health (Aut. No. 364/2019-pr) and all experiments were performed in accordance with the project and with the local ethical committee.

Mice were housed in pathogen-free conditions with food and water available ad libitum. Note that 6–8 weeks K5-mOVA.tg C57/BL6 female mice were used for the experiments. Female OT-I.tg mice (both CD45.2 and congenic CD45.1/CD45.2) were used in the same age range.

OT-I CD8$^+$ T cells for adoptive transfer experiments were purified from splenocytes by MACS positive selection using CD8 microbeads (MACS microbeads, Miltenyi Biotech, Auburn, CA). Purity was analyzed by flow cytometric (FACS) analysis and was routinely >90%.

### Imiquimod-induced psoriasis-like inflammation in K5-mOVA.tg mice and OT-I CD8$^+$ T-cell adoptive transfer

For the induction of psoriasis-like inflammation, each mouse was treated with imiquimod (ALDARA 50 mg/days) for 7 consecutive days according to the treatment schedule.

At day +1 after the first imiquimod application, Vα2Vβ5 OT-I cells that recognize ovalbumin peptide residues 257–264 (SIINFEKL) in the context of H2Kb MHC class I allele were isolated from the spleen of donor OT-I.tg mice and transferred by tail vein injection in K5-mOVA.tg mice ($1 \times 10^6$ OT-I cells/mouse).

## Phenotype analysis of skin-infiltrating T cells

For the analysis of the T-cell infiltrate in inflamed skin tissue, ears were split into dorsal and ventral halves and digested for 150 min at 37°C with rotation in RPMI 1640 containing penicillin-streptomycin, L-glutamine, 200 μg/mL Liberase (Roche Diagnostics Corporation, Indianapolis, IN, USA), 200 μg/mL DNAse I (Sigma Aldrich, St. Louis, MO, USA), and 5% FCS. Ear digests were disaggregated by passage through a 70-μm cell strainer (BD Bioscience, San Josè, CA, USA), and digestion enzymes were quenched by the addition of 2 mM EDTA in RPMI1640 medium with 10% FCS.

Single cell suspensions were stained by using the following panels of fluorochrome-conjugated antibodies: anti-CD45.1, anti-CD45.2, anti-CD3, anti-CD4, anti-CD8a, anti-CD44, anti-CD62L, anti-CCR4, and anti-CXCR3.

### Phenotype analysis of splenocytes

The spleen of each mouse was removed, weighted, and sized at time of sacrifice. Single-cell suspension prepared from the spleen was stained with the panel of antibodies listed above. The samples were incubated in the dark for 30 min at 4°C in PBS and 2% FBS. Cells were acquired using a Gallios Flow Cytometer Beckman Coulter Inc., Brea, CA, USA). Data were analyzed with the FlowJo software (Tree Star, Ashland, OR, USA).

### Analysis of adoptively transferred OT-I.tg T cells

To distinguish adoptively transferred cells from recipient cells, we used CD45.1/CD45.2 heterozygous donors and CD45.2 recipients. Migration and localization of OT-I OVA-specific T cells in the recipient K5-mOVA.tg mouse was followed by staining with anti-CD45.1 and anti-CD45.2 fluorochrome-conjugated antibodies in the skin, blood, and spleen of recipient mice.

At day 15, we also evaluated the presence of SIINFEKL/OVA-specific $CD8^+$ T cells in the spleen by tetramer staining using SIINFEKL-linked fluorochrome-conjugated Kb tetramer (kindly provided by the NIH Tetramer Core Facility) together with fluorochrome-conjugated anti-CD45.2, anti-CD3 anti-CD4, anti-CD8, and anti-Vβ5 antibodies for flow cytometric analysis of spleen and skin T cells.

### scRNA-seq and TCR sequencing data analysis

We analyzed a publicly available dataset of scRNA-seq and single-cell TCR sequencing of memory-enriched ($CD45RA^-$) $CD3^+CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ cells isolated from peripheral blood of three patients with psoriatic arthritis [20]. Processed scRNA-seq data were downloaded from ArrayExpress (E-MTAB-9492), while the filtered contig annotation files for TCR analysis were kindly provided by the authors of the study.

Data processing and analysis of scRNA-seq data was performed using Seurat [42] (version 4.0.1) in R (version 4.0.3). First, gene-expression matrices were filtered to remove cells with >10% mitochondrial genes, <500 or >3500 genes, and >25,000 UMI. Counts were then normalized and log-transformed using sctransform [43], while regressing out UMI counts and the percentage of mitochondrial counts. Principal component analysis was performed to reduce dimensionality. Principal components were fed to Harmony [44] for the integration of datasets from the three patients. Clusters were identified using the shared nearest neighbor modularity optimization-based clustering algorithm, followed by Louvain community detection.

Clusters were then annotated as $CD4^+$ or $CD8^+$ T cells using a two-method approach: (1) we manually checked the expression of CD8A, CD8B, and CD4 markers across the clusters and (2) we used the SingleR package [45] for the automatic annotation of clusters based on the correlations of the single-cell expression values with transcriptional profiles from pure cell populations in the Blueprint/ENCODE dataset as reference [46, 47]. We then retained only the clusters annotated as $CD8^+$ T cells: a total of 13 $CD8^+$ T-cell clusters were identified and annotated based on the expression of marker genes.

Single-cell TCR sequencing data were analyzed using the R package scRepertoire (version 1.1.4) [48] following the official vignette. Filtered contig annotation files from the output of cell ranger vdj were used as input. Clonotypes were removed when any cell barcode had more than two immune receptor chains and merged with scRNA-seq data based on the cell barcodes. Expansion was categorized in five classes spanning the dynamic range from single clones up to hyperexpanded clones. Overlap between the samples was visualized using clonalOverlap using Morisita index, which accounts for both the TCR species presence and abundance.

### Statistical analysis

Data presented as means were compared by either unpaired or paired parametric *t*-tests or nonparametric test depending on the normality of the distribution, evaluated by Shapiro–Wilk test. Data were expressed and plotted as mean ± SEM values. Sample sizes for each experimental condition were provided in the figure legends. All *p* values were calculated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA) and tests were considered significant when the *p* values were lower than 0.05. Correction for multiple comparisons was obtained using Benjamini and Hochberg adjustment.

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**Author contributions:** G.M. F.M., F.C., and L.G. performed the experiments in the mouse models. G.P. performed bioinformatics analysis of scRNA-seq data and wrote the results. E.M. and R.G. synthesized peptides and discussed the results. G.B. and P.S. discussed the results and critically read the manuscript. S.V. supervised the bioinformatics part of the study, F.G. participated to the supervision of the study and edited the manuscript, and E.R. coordinated and supervised the study and wrote the final version of the manuscript.

**Ethics statement:** All procedures involving mice and their care were performed in conformity with the ethical principles and EU Directive 2010/63/EU for animal experiments guidelines. The project was approved by the Italian Ministry of Health (Aut. No. 364/2019-pr) and all experiments were performed in accordance with the project and with the local ethical committee.

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