Clinically resolved psoriatic lesions contain psoriasis-specific IL-17–producing αβ T cell clones

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In psoriasis, an IL-17–mediated inflammatory skin disease, skin lesions resolve with therapy, but often recur in the same locations when therapy is discontinued. We propose that residual T cell populations in resolved psoriatic lesions represent the pathogenic T cells of origin in this disease. Utilizing high-throughput screening (HTS) of the T cell receptor (TCR) and immunostaining, we found that clinically resolved psoriatic lesions contained oligoclonal populations of T cells that produced IL-17A in both resolved and active psoriatic lesions. Putative pathogenic clones preferentially utilized particular Vβ and Vα subfamilies. We identified 15 TCRβ and 4 TCRα antigen receptor sequences shared between psoriasis patients and not observed in healthy controls or other inflammatory skin conditions. To address the relative roles of αβ versus γδ T cells in psoriasis, we carried out TCR/δ HTS. These studies demonstrated that the majority of T cells in psoriasis and healthy skin are αβ T cells. γδ T cells made up 1% of T cells in active psoriasis, less than 1% in resolved psoriatic lesions, and less than 2% in healthy skin. All of the 70 most frequent putative pathogenic T cell clones were αβ T cells. In summary, IL-17–producing αβ T cell clones with psoriasis-specific antigen receptors exist in clinically resolved psoriatic skin lesions. These cells likely represent the disease-initiating pathogenic T cells in psoriasis, suggesting that lasting control of this disease will require suppression of these resident T cell populations.

Introduction

Psoriasis is a T cell– and IL-17–mediated inflammatory skin disease, characterized by inflamed skin lesions and systemic complications as well as arthritis in some patients (1, 2). Psoriatic skin lesions appear to resolve with therapy, but often recur in the same locations after therapy is discontinued. Identification of the phenotype and antigen specificity of pathogenic T cells in psoriasis is needed to better understand the disease and to develop novel therapies for long-term disease suppression. In particular, identifying and sequencing the antigen receptors of the pathogenic T cells is a critical step in identifying new antigens and validating existing antigen candidates. Transcriptional and histologic studies of resolved psoriatic skin lesions show that these areas of skin contain residual populations of $CD8^+$ and $CD4^+$ T cells making IL-17 and IL-22, 2 key pathogenic cytokines in psoriasis (3, 4). Pathogenic T cells capable of initiating psoriatic lesions are also present in the nonlesional skin of patients with psoriasis, but not in healthy controls (5). We postulate that the T cells resident in healed psoriatic lesions may represent the pathogenic T cells of origin in this disease. If this is the case, healed psoriatic lesions should contain expanded clonal or oligoclonal populations of T cells, these clonal T cells should produce IL-17, a key pathogenic cytokine in psoriasis, and these clones should be present at a lower, but detectable, frequency in nonlesional skin from the same individual. In this manuscript, we utilized high-throughput screening (HTS) of the CDR3 region of the T cell receptor (TCR) and immunostaining to evaluate the clonality and cytokine production of T cells in active and psoriatic lesions. Below, we present our findings that the residual T cell populations in clinically resolved psoriatic lesions contain oligoclonal expanded αβ T cell populations, that these expanded populations produce IL-17, and that unique TCR sequences are observed in these lesions that are not found in healthy controls. By taking steps toward identifying the TCR sequences of putative pathogenic T cells in psoriasis, we hope to contribute to a better understanding of what antigens are recognized in this disease and how pathogenic T cell clones may be stably suppressed.

Results

Oligoclonal populations of T cells are present in clinically resolved psoriatic skin lesions. We carried out HTS analyses of the CDR3 region of the TCRα, -β, -δ, and -γ genes in patients with psoriasis and in healthy controls. In psoriatic patients, the same skin lesion was biopsied before and after complete clinical resolution of skin inflammation with etanercept therapy or ultraviolet B (UVB) therapy (Figure 1A), and in some patients, nonlesional skin before therapy was also sampled. HTS is a powerful technique that measures the total number of T cells and the diversity of T cells and identifies the sequences of the TCR CDR3 regions — the antigen recognition domains — for all T cells in a sample. Not surprisingly,

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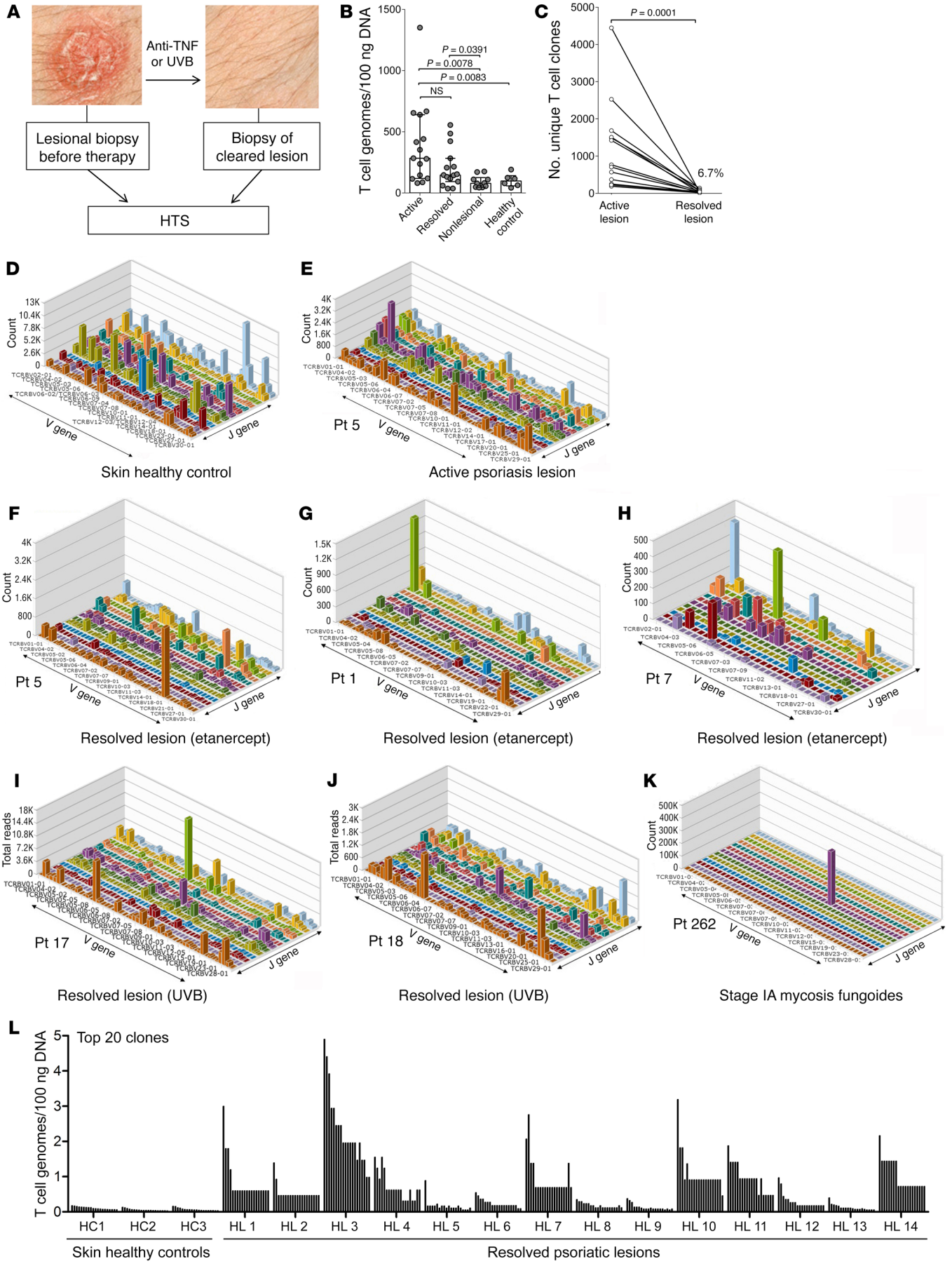
A
Anti-TNF or UVB
Lesional biopsy before therapy
Biopsy of cleared lesion
HTS
B
T cell genomes/100 ng DNA
P = 0.0391
P = 0.0078
P = 0.0083
NS
Active
Resolved
Nonlesional
Healthy control
C
No. unique T cell clones
P = 0.0001
6.7%
Active lesion
Resolved lesion
D
Count
V gene
J gene
Skin healthy control
E
Pt 5
Active psoriasis lesion
F
Pt 5
Resolved lesion (etanercept)
G
Pt 1
Resolved lesion (etanercept)
H
Pt 7
Resolved lesion (etanercept)
I
Total reads
Pt 17
Resolved lesion (UVB)
J
Pt 18
Resolved lesion (UVB)
K
Pt 262
Stage IA mycosis fungoides
L
Top 20 clones
T cell genomes/100 ng DNA
HC1
HC2
HC3
HL 1
HL 2
HL 3
HL 4
HL 5
HL 6
HL 7
HL 8
HL 9
HL 10
HL 11
HL 12
HL 13
HL 14
Skin healthy controls
Resolved psoriatic lesions

Figure 1. Oligoclonal populations of T cells are present in clinically resolved psoriatic skin lesions. (**A**) Experimental design: single psoriatic lesions were biopsied before and after clearance on etanercept (anti-TNF) or UVB therapy, and T cells were evaluated using HTS of the TCR. (**B**) The total T cells per unit skin (100 ng of skin DNA) are shown for active psoriatic lesions (active, *n* = 15), clinically resolved lesions following etanercept therapy (resolved, *n* = 15), nonlesional skin (*n* = 10) (samples compared by Wilcoxon matched-paired signed rank test), and the skin of healthy individuals (healthy control, *n* = 6) (samples compared by Mann-Whitney *U* tests). (**C**) The number of unique T cell clones, as measured by the total number of unique CDR3 sequences, are shown for 14 patients before (active lesion) and after (resolved lesion) clinical resolution of psoriasis on etanercept therapy (Wilcoxon matched-paired signed rank test). The total numbers of unique T cell clones decreased by a mean of 93.3% following clinical clearance. (**D**–**K**) The skin T cell repertoires of a healthy control (**D**), an active psoriatic lesion (**E**), resolved psoriatic lesions after clearance on etanercept (**F**–**H**) or UVB therapy (**I** and **J**), and the clonal T cell lymphoproliferative disease mycosis fungoides (**K**) are shown. Oligoclonal populations of T cells were evident in resolved psoriatic lesions. Pt, patient. (**L**) The absolute number of individual T cell clones per unit skin (100 ng total skin DNA) of the top 20 clones are shown for 3 healthy controls and resolved psoriatic lesions from 14 etanercept-treated patients.

the total number of T cells per unit skin was greater in active psoriatic lesions than in nonlesional skin or healthy controls (Figure 1B). However, T cell density in clinically resolved psoriatic lesions was also significantly greater than in nonlesional skin and healthy controls and was not significantly different from that in active lesions before therapy. The T cell diversity in a sample can be characterized by describing the number of unique T cell clones that are present, as measured by the unique CDR3 sequences identified by HTS. T cell populations in active psoriatic skin lesions were very diverse, as previous studies have shown (6). After clinical resolution of skin inflammation with etanercept, only 6.7% of these T cell clones remained in skin (Figure 1C), demonstrating that over 93% of the T cell clones present in active psoriatic skin lesions disappear from the skin when the tide of inflammation recedes. We observed that oligoclonal T cell populations were present at increased frequencies in resolved psoriatic lesions (Figure 1, D–J). Unlike in mycosis fungoides, a lymphoproliferative disorder of clonal malignant T cells (Figure 1K), the skin of resolved psoriatic lesions had multiple expanded T cell clones. When the absolute numbers of these expanded T cell clones were calculated using HTS, it was clear that these oligoclonal populations were expanded in both frequency and absolute number when compared with those in the skin of healthy controls (Figure 1L).

Expanded T cell clones in clinically resolved and active psoriatic lesions produce IL-17A. Neutralization of IL-17A induces complete disease remission in a subset of patients, demonstrating that IL-17 is an important cytokine in psoriasis; IL-22 is also known to contribute to pathology (1). To identify the cytokines produced by residual T cells in clinically resolved psoriatic lesions, we isolated T cells from clinically resolved psoriasis and analyzed their cytokine production by flow cytometry (Figure 2A). We observed expanded populations of T cells producing IL-17A and IL-22. To confirm that IL-17A was produced by the T cell clones identified by our sequencing studies, we immunostained expanded T cell clones to determine whether they produced IL-17A. To do this, we identified the Vβ subunit utilized by top oligoclones and stained resolved psoriatic lesions using antibodies against these TCR Vβ subunits (Figure 2, B–E). These studies are challenging because only 65%–75% of TCR Vβ subunits are recognizable by commercially available antibodies by flow cytometry and, in our hands, only 4 TCR Vβ antibodies functioned in immunostaining of frozen sections (Vβ2, -5.1, -8, -9; clones included in Methods) and none successfully stained T cells in formalin-fixed paraffin-embedded (FFPE) samples. We were fortunate that 3 of our patients had expanded oligoclonal T cell populations using the VB03 and VB05 genes that correspond to the use of the TCR Vβ9 and Vβ5.1 protein subunits by T cells, respectively (using the distinct protein/antibody Vβ nomenclature). We immunostained clinically resolved psoriatic lesions from these patients and found that these expanded Vβ5.1 and Vβ9 T cell populations were producing IL-17A, even in the absence of clinically appreciable psoriatic inflammation (Figure 2, B–E). A mean 78% of $Vβ^+$ T cells produced IL-17A (SEM 4.7, *n* = 3 donors), and these $Vβ^+$ T cells contributed a mean of 60% of the total IL-17A produced (SEM 4.8, *n* = 3 donors; Figure 2, C and D). Prior studies have shown that residual T cell populations in clinically resolved psoriatic lesions expressed mRNA for IL-17A (4); our studies demonstrate that IL-17A protein is also actively expressed by residual T cell clones in quiescent psoriatic lesions. We next immunostained active psoriatic lesions from the same patients to estimate what proportion of the IL-17A produced in active lesions may be contributed by these oligoclonal T cell populations. In patient 15, over 90% of the Vβ9-expressing T cell populations produced IL-17A, and approximately 40% of the total T cell–derived IL-17A was contributed by Vβ9-expressing T cells (Figure 3, A and B). However, it should be noted that Vβ9 immunostaining would be expected to identify both the expanded oligoclones in resolved lesions and any T cell clones recruited into the skin that also use the TCR Vβ9 subunit. In the clinically resolved psoriatic lesion from patient 15, a single T cell clone made up 57% of the total TCR Vβ9–expressing T cell population (Figure 3C), whereas in the active lesion from the same patient, 10 T cell clones expressed TCR Vβ9 and the top single T cell clone made up only 21% of the total TCR Vβ9 population. Thus, although we can be confident that almost all TCR Vβ9–expressing T cells, including the oligoclonal populations in resolved lesions, express IL-17A in active lesions, our calculations of how much IL-17A in active lesions is contributed by the residual Vβ9 oligoclones are only estimates.

Putative pathogenic T cell clones are most frequent in resolved lesions, but can also be found in nonlesional skin. Elegant studies using transplantation of nonlesional skin from psoriasis patients to immunodeficient mice demonstrated that T cells capable of generating a full-blown active psoriatic lesion were present in the nonlesional skin of patients with psoriasis, but were not present in healthy controls (5). Our HTS studies of nonlesional skin demonstrated that oligoclonal populations of expanded T cells were also present in the nonlesional skin from patients with psoriasis, although at a lower level than in clinically resolved lesions (Figure 4A). However, this does not prove that the same T cell clones were expanded in both healed and nonlesional samples. To investigate this question, we first developed a working definition of putative pathogenic T cell clones, defining them as clones that were most frequent in resolved lesions and were also present in lesional skin. Clone-tracking analyses demonstrated that many of these puta-

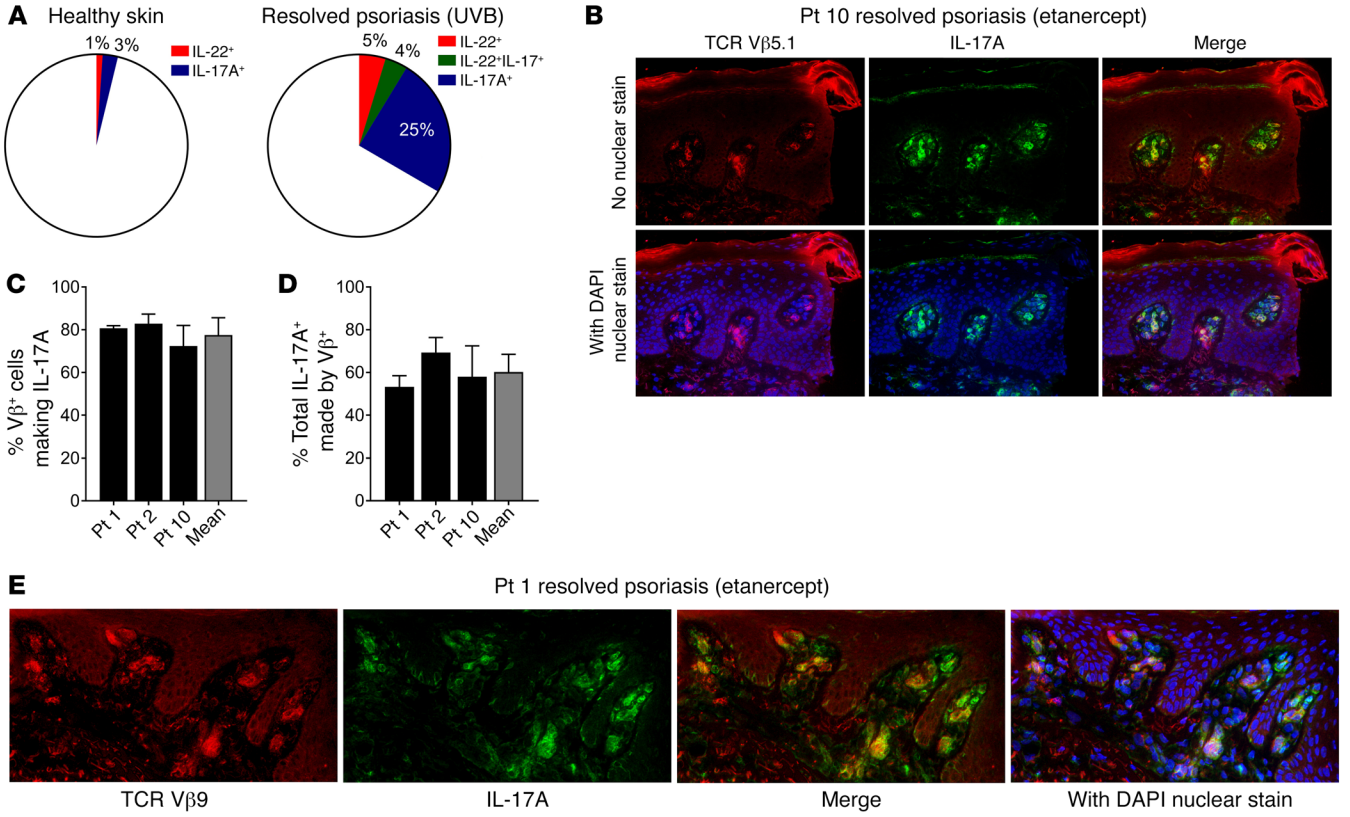

Figure 2. Residual T cell populations and expanded T cell clones in clinically resolved psoriatic lesions produce key pathogenic cytokines in psoriasis. (**A**) IL-17A and IL-22 are produced by residual T cell populations in clinically resolved psoriatic lesions. T cells were isolated from biopsies of clinically resolved psoriasis after UVB phototherapy, and their cytokine production was studied by intracellular cytokine staining and flow cytometry. The mean results of healthy skin (n = 4) and resolved psoriasis lesions (n = 3) are shown. (**B**–**E**) Expanded T cell clones identified by HTS produce IL-17A in healed lesions. HTS was used to identify the TCR Vβ subunit used by T cell clones expanded in resolved lesions. Three patients in the cohort had oligoclones in resolved lesions that used the VB03 or VB05 genes that correspond to the use of the TCR Vβ9 and Vβ5.1 protein subunits by T cells, respectively. Costaining for (**B**) Vβ5.1-expressing and (**E**) Vβ9-expressing T cells and IL-17A in these patients is shown. Individual values and the mean from 3 patients of the (**C**) percentage of Vβ+ T cells producing IL-17A and (**D**) the proportion of total IL-17A produced by Vβ+ T cells are shown.

tive pathogenic T cell clones were also present in nonlesional skin from the same patients (Figure 4, B and C). Putative pathogenic T cell clones were more frequent in resolved psoriatic lesions than in active lesions, where they may have been diluted out by other T cells recruited into the skin by the inflammatory process. Likewise, putative pathogenic T cell clones were more frequent and numerous in resolved lesions than in nonlesional skin, perhaps because these T cell clones had not been driven to locally expand and were sharing space with diverse healthy skin–resident and recirculating T cells. When individual T cell clones were tracked across active lesions, resolved lesions, and nonlesional skin of the same patients, T cell clones shared among all these tissues were among the most frequent in both active and resolved psoriatic lesions, lending indirect support to the concept that they may participate in the pathogenic process (Figure 4D).

Putative pathogenic T cell clones utilize a skewed TCR Vβ repertoire and have unique psoriasis-specific TCR sequences. Utilizing our definition of putative pathogenic T cell clones as those sequences highest in resolved lesions that were also shared in active lesions, we measured the TCR Vβ gene usage of our putative pathogenic T cell clones and compared them with 6,918 TCR Vβ sequences obtained from sequencing the skin of 6 healthy donors (Figure 5A). Putative pathogenic clones had significantly increased usage of TCR Vβ2, Vβ6, and Vβ13. These results are in agreement with 2 prior studies demonstrating preferential use of Vβ13 by T cells in psoriatic lesions and by a melanocyte antigen–specific autoreactive T cell clone from a patient with psoriasis (7, 8).

If the residual T cell clones that we have identified are truly involved in the pathologic process of psoriasis, we would expect to observe in patients TCRs from psoriasis that are not present in healthy controls or in patients with other inflammatory skin diseases. We compared the CDR3 antigen receptor sequences of 162 putative pathogenic T cell clones (Supplemental Table 1; supplemental material available online with this article; https://doi.org/10.1172/JCI93396DS1) from psoriatic skin and compared them with sequences isolated from the skin of healthy controls (6,918 sequences), 11 patients with biopsy-proven eczematous dermatitis (398,927 sequences), 11 patients with allergic contact dermatitis (96,783 sequences), and 7 patients with pityriasis lichenoides (365,100 sequences). None of the 162 putative pathogenic T cell clones were present in these patients, providing strong evidence that these sequences are psoriasis specific.

We also observed some similarities and overlap of putative pathogenic clones between psoriasis patients. In 8 psoriasis

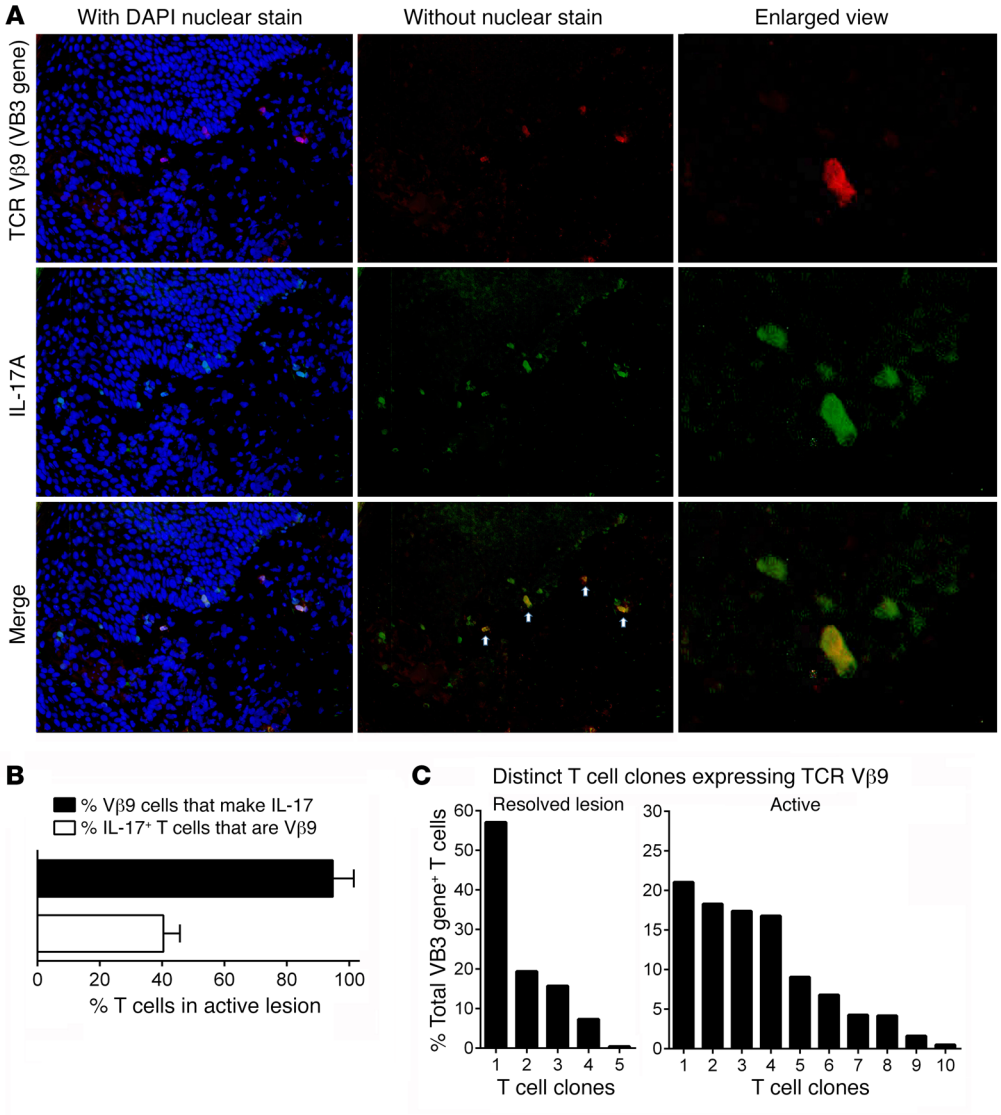

Figure 3. T cell oligoclones identified in resolved lesions also produce IL-17A in active lesions from the same patient. Studies of the active psoriatic lesion from patient 15 are shown. This patient had expanded VB03 gene/Vβ9 protein–expressing T cells in resolved lesions. (**A**) Costaining for Vβ9-expressing T cells and IL-17A are shown. (**B**) Over 90% of Vβ9 T cells produced IL-17A in active lesions, and Vβ9-expressing T cells contributed approximately 40% of the T cell–derived IL-17A in the active lesion. (**C**) However, this population of Vβ9 T cells contained 5 T cell clones in the resolved lesion (one of which made up 57% of the total Vβ9 population), but the active lesion from the same patient had 10 distinct Vβ9 T cell clones contributing to this population. Total Vβ9 T cell–derived IL-17A therefore contains contributions from Vβ9 T cell clones recruited into skin in the active lesion and is not an exact measurement of the contribution of putative pathogenic V9 T cell clones to total IL-17A production. Results from patient 15 are shown; comparable results were obtained in 2 additional patients.

patients, 2 different donors had T cells with identical CDR3 nucleotide sequences that gave rise to identical amino acid sequences (Figure 5B and Table 1). In 11 additional pairs of psoriasis donors, there were T cells with distinct nucleotide sequences that produced identical CDR3 amino acid sequences in both donors (Figure 5C and Table 1). Finally, in 7 psoriatic donors, there were multiple distinct T cell clones within the psoriatic lesions of a particular individual that gave rise to the same amino acid sequence (Figure 5D). None of these sequences were present in our samples of healthy skin, eczematous dermatitis, allergic contact dermatitis, or pityriasis lichenoides.

αβ T cells predominate in psoriatic and healthy human skin. γδ T cells have been found in low (<5%) but detectable frequencies in human psoriatic skin lesions and are the main source of IL-17 in the mouse model of imiquimod-induced psoriasiform dermatitis (9, 10). To evaluate the relative importance of αβ T cells versus γδ T cells in psoriatic and healthy skin, we sequenced the TCRα/δ loci using HTS. The vast majority of T cells in both psoriatic and healthy human skin were αβ T cells (Figure 6, A–C, and Table 2). δ T cells made up only 1% of the T cells in active psoriatic lesions and less than 1% in resolved psoriatic lesions. In healthy human skin, γδ T cells made up less than 2% of the total T cell population. Of the 70 most frequent putative pathogenic T cell clones identified in 14 psoriatic patients, 100% were confirmed α T cells by HTS. These results suggest that human psoriatic inflammation is a process driven by αβ T cells.

Putative pathogenic T cell clones utilize a skewed TCR Vα repertoire and have unique TCR sequences not shared with healthy controls. We next utilized TCRα/δ HTS to examine the frequencies of Vα gene usage in putative pathogenic T cell clones versus T cells from the skin of healthy controls. Putative pathogenic T cell clones were again defined as the most frequent T cell clones in resolved skin lesions that were also present in lesional skin. TCR Vα 20, 30, and 41 genes were preferentially utilized by putative pathogenic T cells (Figure 6D). There were also 4 TCRα CDR3 sequences that were unique to psoriasis and shared between donors (Figure 6, E and F, and Table 1). For one of these common TCRα sequences, the same amino acid sequence was produced by a convergence of 2 clearly distinct nucleotide sequences. For the remaining 3 common TCRα sequences, the nucleotide sequences differed by only 1 or 2 bases. In this situation, it is difficult to tell whether the nucleotide sequences are truly distinct or whether these differences represent sequencing errors.

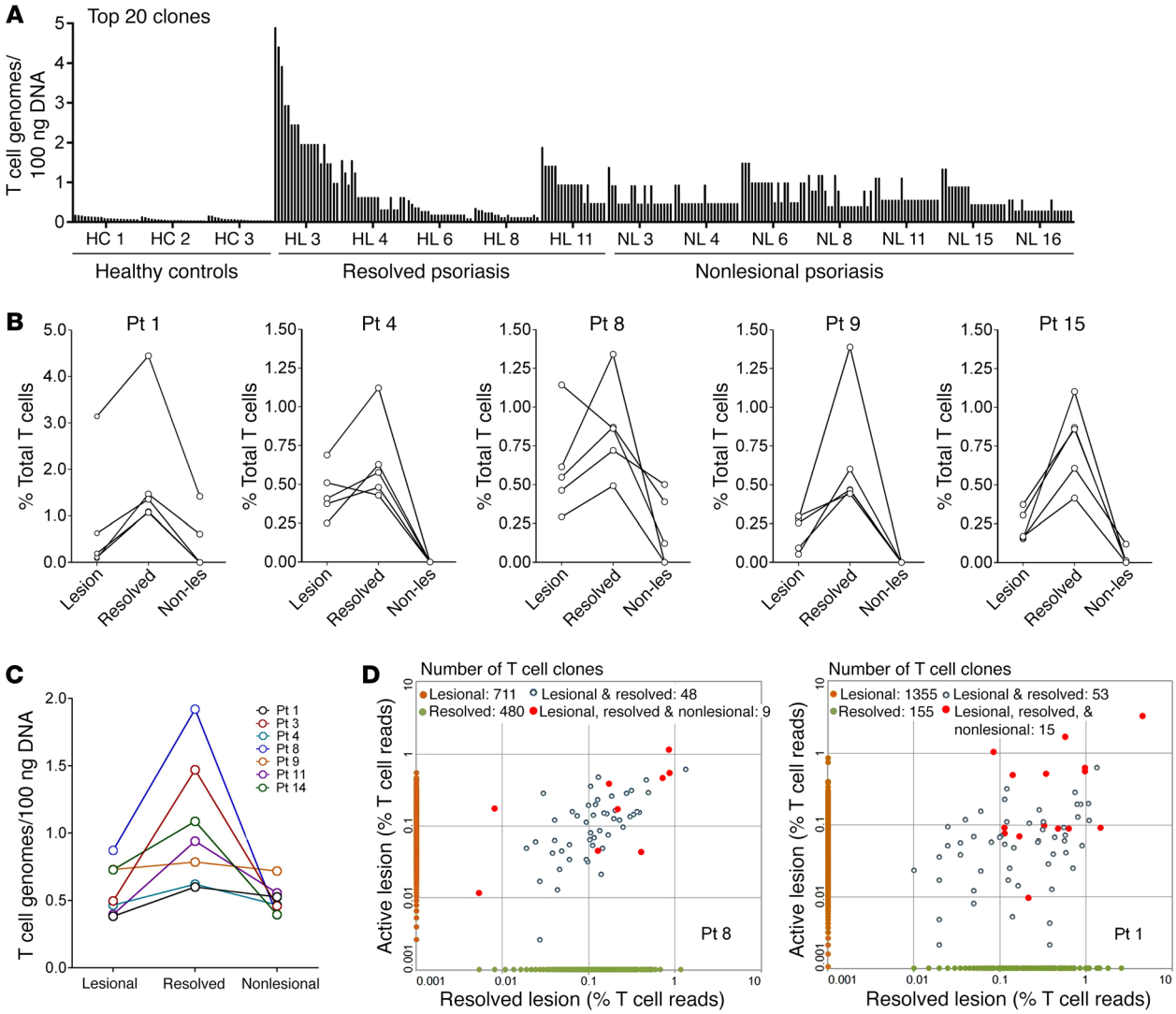

Figure 4. Putative pathogenic T cell clones are also present in lower numbers in the nonlesional skin from psoriasis patients. (**A**) Expanded oligoclonal populations of T cells are present in nonlesional skin. The total T cells per unit skin (100 ng of skin DNA) of the top 20 most frequent T cell clones are shown for nonlesional skin from 7 psoriatic patients (nonlesional psoriasis [NL], far right). Three skin samples from healthy individuals (healthy controls [HC]) and 4 clinically resolved psoriatic lesions (resolved psoriasis, healed lesion [HL]) are included for comparison. (**B**) Putative pathogenic T cells are detected in the nonlesional skin of patients with psoriasis. Putative pathogenic clones were defined as the most frequent T cell clones in resolved lesions that were also present in active lesions. Clone-tracking analyses were used to measure the frequency of the top 5 most frequent of these T cell clones in active lesions (Lesion), resolved lesions (Resolved), and nonlesional skin from the same patient (Non-les). The frequency of these T cell clones was highest in resolved lesions, followed by active lesions, and was lowest in the nonlesional skin. Data from 5 patients are shown; 5 additional patients showed a similar pattern. (**C**) In addition to being more frequent, putative pathogenic clones were present in highest absolute numbers in resolved lesions. The absolute number of T cells per 100 ng of total DNA is shown for the most frequent putative pathogenic T cell clone in 7 patients. (**D**) Overlap analyses demonstrate that shared T cell clones among lesional, resolved, and nonlesional skin (red circles) are among the most frequent T cell clones in both active and resolved psoriatic lesions. Two representative analyses out of a total of 7 are shown.

Discussion

Residual populations of T cells containing RNA for IL-17 and IL-22 are present in clinically resolved psoriatic skin lesions (4). We suggest that these are critical pathogenic T cells that, when activated by autologous or commensal-derived antigens, can reinitiate the inflammatory cascade and give rise to new or recurrent psoriatic skin lesions. If this hypothesis is correct, these residual T cells should be clonal or oligoclonal, should produce IL-17A, should be present to some extent in nonlesional skin, and should have unique TCR sequences that distinguish them from the T cells found in healthy controls. In this study, we used HTS and immunostaining to study residual T cells and found that all of these suppositions are true. Clinically resolved psoriatic skin lesions contained oligoclonal populations of T cells that were present to a lower extent in nonlesional skin from the same patients. Three patients in our cohort had expanded oligoclones that were recognizable by Vβ antibodies, and immunostaining studies demonstrated that IL-17A was produced by these T cells both in clinically resolved and active psoriatic skin lesions. Additionally, we identified 15 TCRβ and 4 TCRα CDR3 sequences

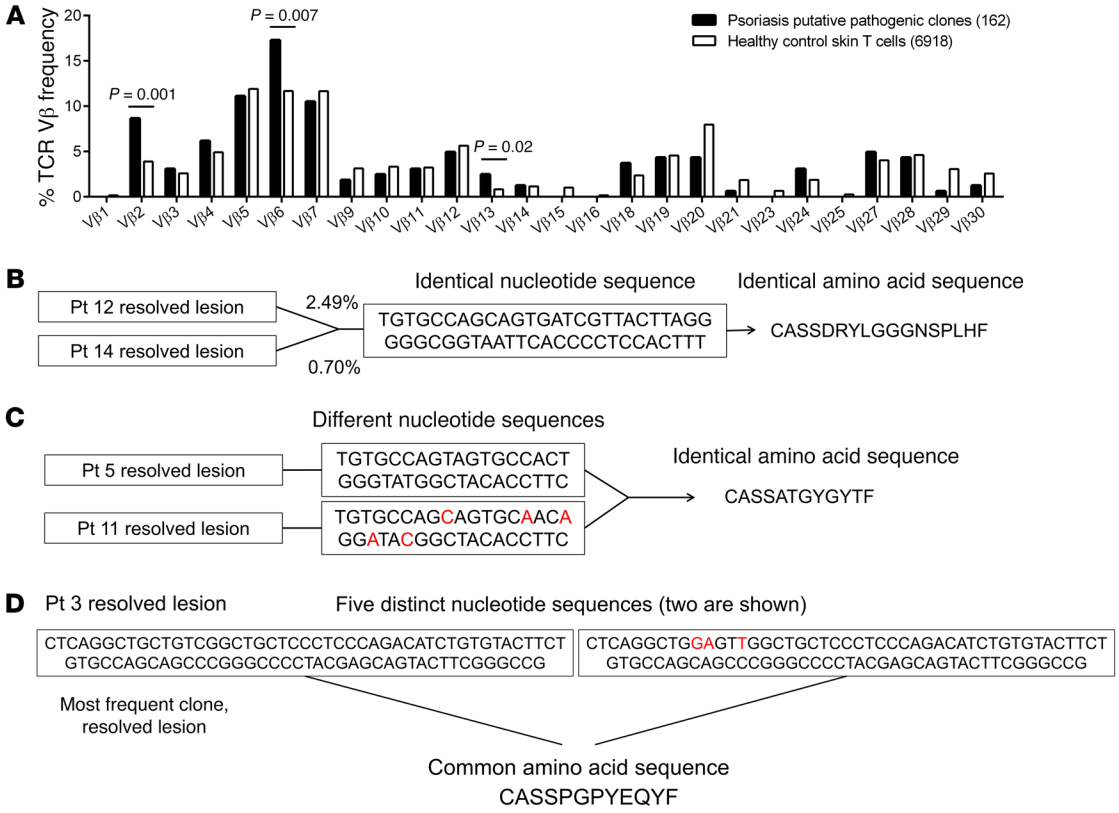

Figure 5. Putative pathogenic T cell clones utilized a skewed TCR Vβ repertoire and had unique TCRs not shared with healthy controls. (**A**) The TCR Vβ gene usage of putative pathogenic T cell clones from 14 psoriasis patients was compared with that in a pool of 6,918 T cell CDR3 sequences from the skin of 6 healthy controls. Use of TCR Vβ2, V6, and Vβ13 was significantly more frequent among putative pathogenic T cell clones (χ^2 test). (**B**–**D**) T cells from different psoriatic patients had identical TCRβ CDR3 amino acid sequences that were not observed in healthy controls. (**B**) In 8 psoriasis patients, T cells with identical CDR3 nucleotide sequences giving rise to identical amino acid sequences were found in 2 different patients; these sequences were not observed in healthy controls. The sequences of all shared receptors are also shown in Table 1. (**C**) In 11 cases, different CDR3 nucleotide sequences in 2 different patients converged to produce the same amino acid sequence; these sequences were not observed in healthy controls. (**D**) Within individual psoriasis patients, multiple distinct nucleotide sequences were found in 7 patients who produced the same amino acid sequence; these sequences were also not observed in healthy controls.

that were unique to psoriasis, shared among multiple psoriasis patients, and not observed in healthy controls.

Identification of the phenotype and antigen specificity of pathogenic T cells in psoriasis is critical to furthering our understanding of this disease. The persistence of T cells in peripheral tissues after resolution of inflammation is a hallmark of resident memory T cells (T_{RM} cells) (11). T_{RM} cells are generated by immune challenges in peripheral tissues, provide rapid responses to rechallenge, and persist in the absence of antigen (12). Studies using mouse infection models have demonstrated that immune challenges in skin, whether they are infectious or hapten driven, lead to colonization of the entire skin surface with antigen-specific T_{RM} (13, 14). These cells are present in the highest numbers at the site of primary infection and at sites of subsequent rechallenge, are long lived, and are capable of rapid reactivation when specific antigen is reencountered. Our results in combination with prior studies suggest that a similar distribution of pathogenic T cells is observed in psoriasis. Xenograft studies in mice showed that the entire skin surface, e.g., the nonlesional skin, of psoriatic patients is colonized by T cells capable of initiating a psoriatic skin lesion in the absence of T cell recruitment from blood (5). Our work shows that T cell clones enriched in active and clinically resolved psoriatic skin lesions, some of which had TCR antigen receptors unique to psoriasis, were also found in nonlesional skin from the same patients, although at lower frequencies. T_{RM} cells are by nature long lived and difficult to kill. Psoriasis and mycosis fungoides, a lymphoma arising from skin T_{RM} cells (15), are both characterized by inflammatory skin lesions that appear to completely resolve with therapy, but then often recur in the same anatomic locations once therapy is discontinued. This behavior suggests that most conventional therapies probably do not eradicate pathogenic T cells, but instead suppress the activity of these cells. Withdrawal of active therapy allows these T cells to reactivate and reinitiate inflammatory lesions. We found that putative pathogenic T cell clones in clinically resolved lesions were still actively producing IL-17A, even in the absence of clinical inflammation. We believe that these cells represent a smoldering nidus of inflammation that can lead to reactivation of overt inflammation and recurrence of psoriatic skin lesions. T_{RM} cells have a unique biology; a better understanding of the signaling pathways that allow long-term survival of T_{RM} cells in skin could lead to novel therapies capable of eradicating these cells and thereby inducing long-term remissions in psoriasis.

Table 1. Shared CDR3 amino acid sequences in patients with psoriasis

	Shared in patients	Nucleotide sequences identical?	Present in healthy controls?
Shared TCRβ CDR3			
CASSQDLAGGPDTQYF	3, 13	Yes	No
CSARGGNTIYF	1,9	Yes	No
CASSISATGDTEAFF	3, 5	Yes	No
CASSDRYLGGGNSPLHF	12, 14	Yes	No
CASSPQETQYF	3, 4	No	No
CASSLVGQAYEQYF	4, 13	No	No
CASSESNQPQHF	11, 13	No	No
CASSFYNEQFF	3, 13	No	No
CASSLGEKLFF	9, 13	No	No
CASSLGAGQPQHF	8, 13	No	No
CASSEGYEQYF	6, 8	No	No
CASSPGPSYEQYF	10, 12	No	No
CASSPLAGSYNEQFF	3, 10	No	No
CASSATGYGYTF	5, 11	No	No
CASSLGTGELFF	3, 11	No	No
Shared TCRα CDR3			
CALSKAAGNKLTF	1, 3	Similar[A]	No
CALRNTGGFKTIF	1, 3	Similar[A]	No
CIVRVYGGATNKLIF	7, 3	Similar[A]	No
CAVDSGGSNYKLTF	9, 10	No	No

[A]Similar: nucleotide sequences differed by 1 or 2 nucleotides; it is not clear whether this represents sequencing errors or true differences in the sequences.

Several putative antigens have been identified in psoriasis. Two-thirds of patients with psoriasis have T cells reactive to LL37 (16), and neolipid antigens were recognized by a subset of CD1a-restricted T cells in psoriatic skin lesions (17, 18). Reactivity to the melanocyte antigen ADAMTSL5 was observed in $CD8^+$ T cells from psoriasis patients, but not healthy controls (7), although patients with psoriasis do not generally develop vitiligo, a sequelae of autoimmune targeting of melanocytes. Identification and sequencing of the TCRs of pathogenic T cells in psoriasis will be an important step forward in validating current antigen candidates and identifying new ones. Although psoriasis is considered to be an autoimmune disease, pathogenic T cells in this disorder also could be responding to antigens derived from commensal organisms on the skin surface. Our studies demonstrated that there were TCRα and -β antigen receptor sequences that were shared among psoriatic patients and not found in healthy controls or patients with eczematous dermatitis, allergic contact dermatitis, and pityriasis lichenoides. We found that multiple distinct nucleotide sequences converged to produce the same antigen receptor amino acid sequences that were shared between patients and not present in healthy controls. Moreover, multiple T cell clones with distinct nucleotide sequences giving rise to the same antigen receptor existed among putative pathogenic T cells from individual patients. These findings suggest that a common and potentially discoverable antigen is driving T cell responses in these patients. One shortcoming of our studies is the fact that separate HTS of the TCRα and -β chains did not allow pairing of putative pathogenic α and β subunits to provide a complete TCR sequence. Current approaches that allow paired sequencing of the TCRα and -β chains, such as pairSEQ (19) and single-cell RNA sequencing, require viable single cell suspensions and cannot be performed on biopsy specimens. However, techniques are rapidly evolving, and we would suggest a focus on residual T cells in healed psoriatic lesions as a viable approach to identifying the full TCR of pathogenic T cells. We did not have access to 2 or more resolved lesions from the same patient, but sequencing multiple resolved lesions in a single patient would provide supportive evidence that the residual T cell clones, if common across 2 lesions, participate in the pathogenic process.

The relative importance of αβ versus γδ T cells in human psoriatic inflammation has remained an unanswered question. γδ T cells produce the majority of IL-17 in the mouse model of imiquimod-induced psoriasiform dermatitis, and γδ T cells have been found in low but detectable levels in human psoriatic skin lesions (9, 10). We quantified the αβ and γδ T cells in active psoriatic lesions, resolved psoriatic lesions, and healthy skin using HTS of the TCRα/δ loci. We found that the vast majority of T cells in both psoriatic and healthy human skin were αβ T cells. Only 1% of T cells in active psoriatic lesions and less than 1% of T cells in clinically resolved lesions were γδ T cells. γδ T cells made up less than 2% of T cells in healthy human skin. All 70 of the most frequent putative pathogenic T cell clones identified in our 14-patient cohort were confirmed αβ T cells. Although γδ T cells clearly play a role in the psoriasiform dermatitis induced in topical imiquimod and intradermal IL-23 injection mouse models, our findings suggest that human psoriasis is a process primarily driven by αβ T cells.

Taken together, our studies support the concept that psoriasis is a disease mediated by oligoclonal populations of αβ T cells, that these pathogenic T cell clones remain resident in previously lesional skin, and that they may recognize common antigens and are likely responsible for lesional recurrence after cessation of therapy. A better understanding of pathogenic T_{RM} cells may lead to novel therapies that can eradicate these cells from skin and induce long-term remission in this disease.

Methods

Skin and blood samples. Samples of pretreatment active psoriasis lesions, nonlesional skin, and resolved lesions (the same lesion after clearance on etanercept therapy [16 patients] or UVB therapy [6 patients]) were obtained from patients seen at Rockefeller University, and 3 additional skin samples from resolved psoriatic lesions after UVB therapy were obtained from the Department of Dermatology at the Academic Medical Center, University of Amsterdam. Nonlesional samples were taken from an area without psoriasis 5 to 10 cm away from the biopsied psoriatic plaque. Nonlesional samples were always from the same body region as the plaque biopsy, e.g., arm or thigh. Skin from healthy controls was obtained from patients undergoing cosmetic surgery procedures. Lesional skin from patients with mycosis fungoides was obtained from patients seen at the Cutaneous Lymphoma Program at the Dana-Farber/Brigham and Women's Cancer Center. Deidentified samples of biopsy-proven eczematous der-

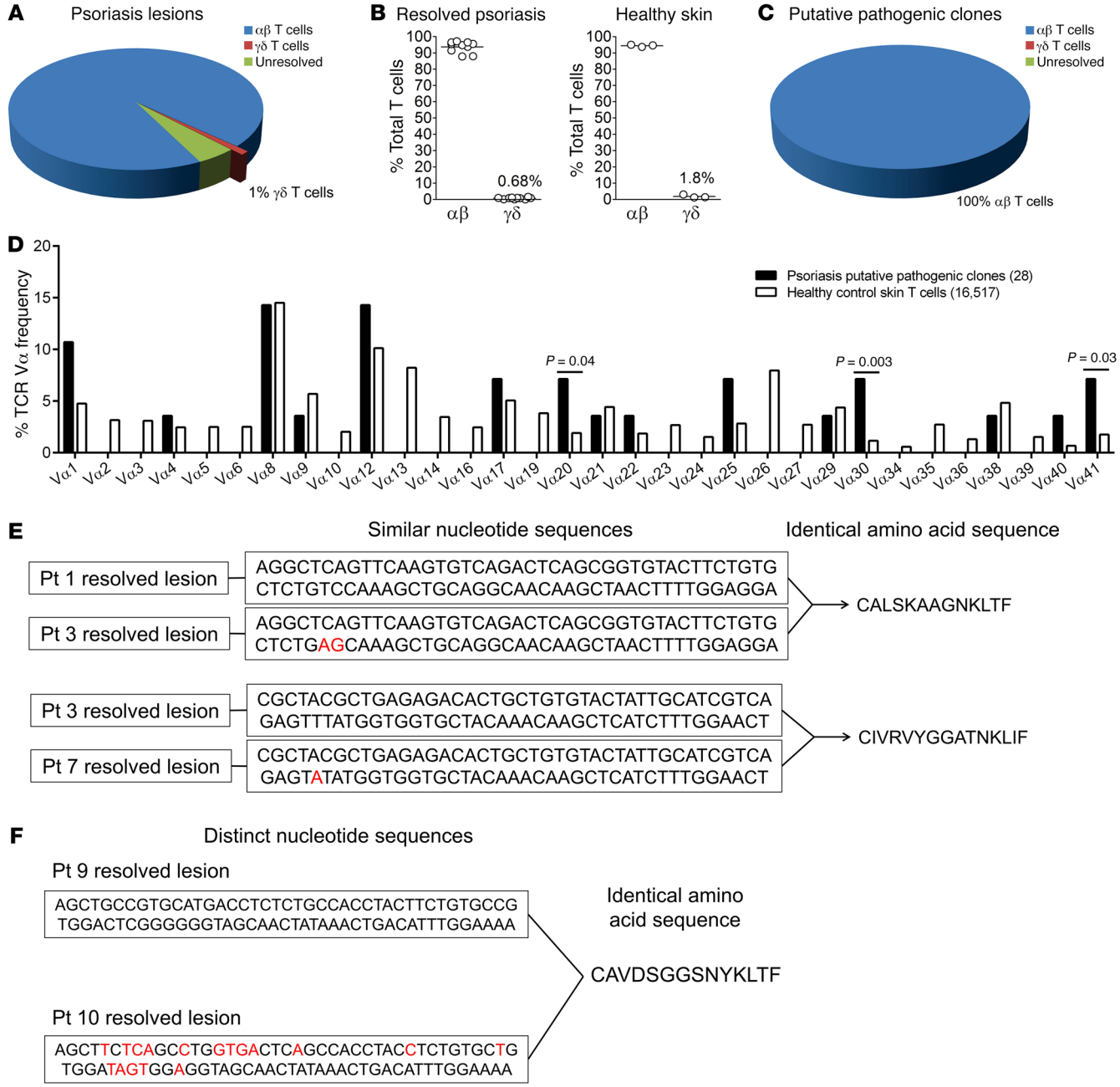

Figure 6. αβ T cells predominate in psoriatic and healthy human skin, and putative pathogenic clones in psoriasis were αβ T cells that utilized a skewed Vα repertoire and contained common TCRα CDR3 sequences not observed in healthy controls. (**A**–**C**) Sequencing of the TCRα/δ locus revealed that αβ T cells predominate in psoriatic lesions and healthy human skin and putative pathogenic T cells were universally α T cells. The percentages of αβ versus γδ T cells in (**A**) active psoriatic lesions (n = 10), (**B**) resolved psoriatic lesions (n = 10), and skin from healthy controls (n = 3), and among (**C**) putative pathogenic T cell clones in psoriasis are shown. Results are also summarized in Table 2. (**D**) Putative pathogenic T cell clones preferentially utilized Vα20, Vα30, and Vα41, as compared with 16,517 TCRα sequences from the skin of 6 healthy controls (χ^2 test). (**E** and **F**) T cells with highly similar TCR Vα CDR3 sequences (1 or 2 nucleotides different, 4 pairs of patients) or clearly distinct Vα CDR3 sequences (1 patient pair) giving rise to identical CDR3 amino acid sequences were found in common between patients with psoriasis. These shared CDR3 amino acid sequences were not observed in healthy controls. Shared receptors are listed in Table 1.

matitis, allergic contact dermatitis, and pityriasis lichenoides were obtained with IRB approval from the Pathology Specimen Locator Core at Brigham and Women's Hospital.

DNA isolation from skin. DNA was isolated from frozen, OCT-embedded skin samples. 30 Cryosections of 10-μm thickness were cut, and DNA extraction was carried out using the QIAamp DNA Mini Kit (QIAGEN) per the manufacturer's instructions, with overnight tissue digestion. This method generated 155–3730 ng DNA per sample.

HTS analyses: immunosequencing. For each sample, DNA was extracted from skin biopsies, then TCRβ CDR3, TCRγ CDR3, TCRα CDR3, and TCRδ CDR3 regions were amplified and sequenced using immunoSEQ (Adaptive Biotechnologies). Bias-controlled V and J gene

Table 2. αβ versus γδ T cells in psoriasis (active lesions and resolved) and healthy skin

Sample	Confirmed αβ T cells (%)	Confirmed γδ T cells (%)	Discordant (%)	Unresolved (%)
Active, Ps[3] Pt 1	92.9	1.4	0.2	5.5
Active, Ps Pt 2	94.8	1.0	1.2	3
Active, Ps Pt 3	94.2	1.4	1.1	3.2
Active, Ps Pt 5	94.7	0.6	0.2	4.5
Active, Ps Pt 7	96.9	0.3	1.0	1.7
Active, Ps Pt 9	95.4	0.7	0.7	3.2
Active, Ps Pt 10	95.8	0.2	1.5	2.5
Active, Ps Pt 12	92.8	2.5	0.2	4.5
Active, Ps Pt 14	93.6	1.8	0.3	4.3
Active, Ps Pt 16	93.6	0.4	1.5	5.7
Mean	**94.5**	**1.0**	**0.8**	**3.8**
Resolved, Ps Pt 1	94.7	0.8	0.3	4.2
Resolved, Ps Pt 2	91.4	0.7	1.8	6.1
Resolved, Ps Pt 3	87.9	1.6	1.1	9.5
Resolved, Ps Pt 5	95.4	0.9	0.1	3.6
Resolved, Ps Pt 7	96.4	1	1	1.5
Resolved, Ps Pt 8	96.4	0.8	0.8	2
Resolved, Ps Pt 9	95.4	0.9	0.7	3
Resolved, Ps Pt 10	93.7	0	1.5	4.8
Resolved, Ps Pt 14	97.1	0.1	0.8	2.0
Resolved, Ps Pt 15	87.8	0	0.7	11.5
Mean	**93.6**	**0.7**	**0.9**	**4.8**
Healthy skin, Pt 1	93.7	3.0	0.2	3.1
Healthy skin, Pt 2	94.6	1.3	0.2	3.9
Healthy skin, Pt 3	95.0	1.2	0.2	3.6
Mean	**94.4**	**1.8**	**0.2**	**3.5**

Discordant, TCR sequences that contain genes from both α and δ; unresolved, TCR sequences that lack sufficient resolution to discriminate α from sequences – most are αβ T cells; Ps, psoriasis; Pt, patient.

primers were used to amplify rearranged V(D)J segments for HTS at approximately 20× coverage. After correcting sequencing errors via a clustering algorithm, CDR3 segments were annotated according to the International ImMunoGeneTics collaboration, identifying which V, D, and J genes contributed to each rearrangement (20–22).

Identification of putative pathogenic T cell clones. Putative pathogenic T cell clones were identified as T cell sequences from clinically resolved psoriatic lesions that (a) were also present in active lesions from the same patients, (b) were increased in frequency in the resolved versus active lesions, and (c) were not present in the skin of healthy donors. T cell clones with an increase in frequency between resolved and active lesions were defined as those with frequency differences between clinically resolved and active lesions that were larger than the median of the aggregate difference in frequency for all clones.

Analysis of Vα and Vβ usage. The above criteria for putative pathogenic clones in Vβ analyses were met by 162 distinct T cell clones (Figure 5A), and the criteria for Vα analyses were met by 28 T cell clones (Figure 6D). The data are shown as percentage of pooled pathogenic T cell clones from all patients versus pooled sequences from 6 healthy skin donors. Significance was determined using χ^2 analyses.

Determination of αβ versus γδ T cell identity. HTS of the α/δ TCR loci were carried out, and confirmed assignment of T cells was possible when discrete α or δ genes were detected. The term discordant refers to TCR sequences that contain genes from both α and δ that should be discrete to one set or the other. This can occur when there is misidentification of genes or when genes are present in both loci that have not been characterized previously as shared. Unresolved sequences refers to TCR sequences that lack sufficient resolution to definitively identify the gene and therefore assign it as α or δ. This generally results when the sequence amount is less than that needed to capture all the genetic variation needed to annotate a sequence. This results from longer N1DN2 segment and key differences discriminating between genes lying outside the length sequenced. Most unresolved sequences have been found on multiple retests to be αβ T cells.

Cryosection immunohistochemistry. Psoriasis skin samples were embedded in OCT, frozen, and stored at −80°C until use. Cryosections of 5 μm were cut, air dried, fixed for 10 minutes in acetone, rehydrated in TBS with 0.1% saponin (wash buffer, Boston Bioproducts), and blocked with 20 μg/ml of human IgG (Jackson ImmunoResearch Laboratories, catalog 009-000-002) for 20 minutes at room temperature. Sections were incubated with rabbit anti-human IL-17A (Abcam, catalog ab79056, rabbit polyclonal) for 2 hours at room temperature, then rinsed in wash buffer for 10 minutes. Sections were incubated with secondary Alexa Fluor goat anti-rabbit IgG (Life Technologies, catalog A11054) and directly conjugated mouse anti-human TCR Vβ9 (Beckman Coulter, clone FIN9) or Vβ5.1 (Beckman Coulter, clone IMMU157) for 30 minutes at room temperature and rinsed in wash buffer for 10 minutes. Sections were mounted using ProLong Gold AntiFade with DAPI (Life Technologies) and examined within 2 to 4 hours by immunofluorescence microscopy. Sections were photographed using a microscope (Eclipse 6600; Nikon) equipped with a 40×/0.75 objective lens (Plan Fluor; Nikon). Images were captured with a camera (SPOT RT model 2.3.1; Diagnostic Instruments) and were acquired with SPOT 4.0.9 software (Diagnostic Instruments). Images were also acquired at 20× with the Mantra Quantitative Pathology Imaging System and analyzed using inForm software (all from PerkinElmer).

Flow cytometry analyses of residual T cell populations. Biopsies of clinically resolved psoriatic lesions following UVB phototherapy were collagenase digested as previously described (23). Cells were stimulated with 50 ng/ml PMA (Sigma-Aldrich) and 750 ng/ml ionomycin (Life Technologies) plus 10 μg/ml Brefeldin A (BD Biosciences) for 4.5 hours. Cells were then surface stained, fixed, permeabilized, stained with anti-cytokine antibodies, and examined by flow cytometry. Antibodies used included CD4 (BioLegend, clone OKT4), CD8 (BioLegend, clone SK1), CD69 (BioLegend, clone FN50), CD103 (BioLegend, clone Ber-ACT8), CD62L (EuroBiosciences, clone LT-TD180), and CCR7 (BioLegend, clone G043H7). Analysis of flow cytometry sam-

ples was performed on BD FACSCanto instruments, and data were analyzed using FlowJo software (10.2).

Statistics. Primary methods of data analysis included descriptive statistics (means, medians, and SD). Differences between nonlesional, lesional, and resolved samples were detected using the Wilcoxon matched-paired signed rank test. χ^2 testing was used to assess the difference in distribution of Vβ and Vα usage between psoriatic patients and healthy controls. Distribution of unpaired continuous data was assessed by means of Mann-Whitney *U* tests. A *P* value of 0.05 was deemed significant.

Study approval. The protocols of this study were performed in accordance with the Declaration of Helsinki and were approved by the IRBs of the Partners Human Research Committee (Partners Research Management, Boston, Massachusetts, USA) and Rockefeller University, and the Institutional Medical Ethical Committee at the Academic Medical Center, University of Amsterdam. All subjects provided informed consent prior to their participation in the study.

Author contributions

TRM carried out experiments, analyzed data, drafted figures, provided fresh biopsy specimens of resolved psoriasis after UVB, assisted in drafting the manuscript, and carried out statistical analyses. RAC supervised experiments, analyzed data, carried out statistical analyses, drafted figures, and wrote and revised the manuscript. JTOM carried out immunofluorescence studies. ELL assisted in carrying out experiments. DH, IRK, and HSR carried out HTS analyses and helped to analyze data. TSK provided advice, provided the sample of mycosis fungoides, and assisted in editing the manuscript. JGK provided the skin samples of psoriasis used in these studies as well as valuable advice.

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