SEB Stimulation Induces Functional Pathogenic Features in Th17 Cells from Psoriasis Patients


TO THE EDITOR

Psoriasis is a chronic inflammatory cutaneous disease in which CD4+ T-cell lymphocytes expressing IL-17 have been identified (Lowes et al., 2014), and IL-17 blockers have shown outstanding results in psoriasis treatment (Leonardi et al., 2012). Chronic inflammatory models have shown plasticity of T helper type (Th) 17 cells to acquire Th1 features. Conventional Th17 cells express RORyt and IL-17 but not IFN-γ, whereas after plasticity, Th17 cells express RORyt, T-bet, Runx1, and IFN-γ and lose IL-17 expression (Lee et al., 2012). Thus, the balance of these master transcription factors and the cytokine environment including IL-1β and IL-23 (Annunziato et al., 2014) is critical in the regulation of the Th17 phenotype. After plasticity, Th17 cells appear to have increased proliferation (Maggi et al., 2011; Jorge et al., 2016), and they are defined as pathogenic because they can exacerbate disease in experimental models (Hirota et al., 2011).

In human pathologies including psoriasis, high numbers of IL-17/IFN-γ double-positive CD4+ T cells have been identified (Lowes et al., 2008; Kagami et al., 2010). Recently, the lesional skin microbiome in psoriasis patients exhibited an increased presence of Staphylococcus aureus (Alekseyenko et al., 2013; Tett et al., 2017). In addition, Staphylococcus aureus enterotoxin-B (SEB) superantigen has been associated with psoriasis severity (Balci et al., 2009). Therefore, we evaluated the Th17 cell features in 52 lesional (LS) and 30 nonlesional skin (NLS) biopsy samples of psoriasis vulgaris patients after SEB stimulation (see Supplementary Table S1 online). Medical ethics...

Abbreviations: LS, lesional skin; NLS, nonlesional skin; PB, peripheral blood; SEB, Staphylococcus aureus enterotoxin-B; Th, T helper lymphocyte

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committees from the Centro Dermatológico Dr. Ladislao de la Pascua and Instituto Mexicano del Seguro Social approved this study, and written informed consent was obtained from all patients.

In LS, we found several CD4$^+$ and CD4$^+$ROR$\gamma^+$ T cells and some CD4$^+$ROR$\gamma^+$ cells compared with NLS from the same patient (Figure 1a). The number of Th17 cells determined through the expression of transcription factor

Figure 1. Increased pathogenic phenotype in Th17 cells after SEB stimulation in LS of psoriasis patients. (a) NLS and LS immunofluorescence with anti-CD4 (green), anti-ROR$\gamma^+$ (red), and nuclei (blue). Arrows indicate CD4$^+$/ROR$\gamma^+$ (yellow) and CD4$^+$ROR$\gamma^+$ cells (red). (b) Percentage and absolute numbers of Th17 cells in NLS and LS. (c) Median fluorescence intensities (MFIs) of ROR$\gamma^+$, T-bet, and Runx1 before and after SEB treatment. (d) Representative plots and percentages of conventional and pathogenic Th17 cells in NLS and LS. (e) Pathogenic/conventional index, according to the cell percentage in each condition. (f) Percentage of conventional and pathogenic Th17 cells in patients with small and large plaques after SEB stimulation. NLS, n = 23; LS, n = 38. *P < 0.05, ***P < 0.001; Mann-Whitney U test, Wilcoxon signed-rank test. LS, lesional skin; NLS, nonlesional skin; SEB, Staphylococcus aureus enterotoxin-B; Th, T helper lymphocyte.
Figure 2. SEB and CD3/CD28 induce functional plasticity features in the Th17 lymphocytes of psoriasis patients. (a) NLS and LS immunofluorescence for CD4 (green), IL-17 (red), IFN-γ (cyan), and nuclei (blue). Zoom region (white square). Arrows indicate CD4⁺/IL-17⁺ (yellow), CD4⁺/IFN-γ⁺ (cyan), and CD4⁺/IL-17⁺/IFN-γ⁺ (white) cells. Representative of three patients. (b) Plots and percentage of the Th17 cells IL-17⁺ or IFN-γ⁺ after SEB or phorbol 12-myristate 13-acetate/ionomycin activation (P/I) (n = 10). (c) CD4⁺ T-cell proliferation and expression of RORγt and T-bet after SEB treatment. (d) IL-17⁺ or IFN-γ⁺ Th17 cells after SEB or anti-CD3/CD28 stimulation. (LS orange plots, PB red plots). (e) Expression of RORγt, T-bet, IL-17, IFN-γ and (f) proliferation in purified PB Th17 cells after CD3/CD28 or CD3/CD28 plus IL-1β/IL-23 stimulation. *P < 0.05, **P < 0.01, ***P < 0.001. Wilcoxon signed-rank test. CTV, CellTrace Violet; LS, lesional skin; NLS, nonlesional skin; PB, peripheral blood; SEB, Staphylococcus aureus enterotoxin-B; Th, T helper lymphocyte.
in NLS and LS, we sought to determine the functional and pathogenic Th17 lymphocytes of the disease. Suggesting an association with the large plaque form of the disease, we indicate that SEB stimulation increases the percentage of Th17 cells with a pathogenic phenotype increased only in patients with large plaques (Figure 1f). These results suggest that SEB stimulation increases the pathogenic phenotype of Th17 cells, suggesting an association with the large plaque form of the disease.

Considering the mixture of conventional and pathogenic Th17 lymphocytes in NLS and LS, we sought to determine the in situ expression of IL-17 and IFN-γ in CD4+ T cells. NLS contains few CD4+ T cells, and we could not detect the expression of IL-17 and IFN-γ. In contrast, LS images of the same patient showed high CD4+ T-cell infiltration that expressed either IL-17 or IFN-γ and, strikingly, IL-17/IFN-γ double-positive CD4+ T cells (Figure 2a, white arrows).

Next, we evaluated whether SEB stimulation affected the function of Th17 cells. In LS, most Th17 cells lack IL-17 but highly express IFN-γ, whereas a small population expresses both IL-17 and IFN-γ after SEB stimulation. In contrast, there were IL-17+ and IL-17+/IFN-γ+ and fewer IFN-γ+ Th17 cells after phorbol 12-myristate 13-acetate/ionomycin activation (Figure 2b). In NLS, healthy skin, or skin with another pathology such as atopic dermatitis, Th17 cells lack or have a lower expression of IL-17 and IFN-γ after activation (see Supplementary Figure S2a online). In addition, in patients with a high Psoriasis Area Severity Index score, SEB stimulation induces a pathogenic microenvironment as evidenced by significant quantities of IFN-γ and tumor necrosis factor-α and low levels of IL-17 and IL-9 (see Supplementary Figures S3a-d online). These results strongly suggest the acquisition of functional plasticity features in Th17 cells from LS after SEB stimulation; this could be explained by the increase in Runx1 only in LS, which could dimerize with T-bet and in turn inhibit IL-17 expression (Lazarevic et al., 2010).

To elucidate whether the acquisition of plasticity features in Th17 cells was restricted to skin-resident Th17 cells, we compared the proliferation and cytokine expression of Th17 cells from LS and peripheral blood (PB) from the same patient. LS cells without stimulation showed proliferation, which increased after SEB stimulation, whereas PB cells showed lower proliferative capabilities (Figure 2c). The expansion of CD4+ T cells from LS occurred with increased expression of the transcription factors RORγt and T-bet (Figure 2c). In addition, the plasticity features were evaluated with another TCR stimulus such as anti-CD3/CD28. After SEB stimulation, the Th17 cells from LS only expressed IFN-γ, whereas the anti-CD3/CD28-stimulated cells were IL-17+ and IFN-γ+. In contrast, after SEB or anti-CD3/CD28 activation of the PB Th17 cells, we found IL-17+, IFN-γ+/IL-17+, and IFN-γ− cells (Figure 2d). These results suggest that the acquisition of plasticity features in Th17 cells occurs in LS and PB after SEB or anti-CD3/CD28 stimulation; however, fully functional plasticity features were observed only in LS. Finally, using CD161 as a Th17 cell marker (Cosmi et al., 2008), we sorted the Th17 cells from the PB of psoriasis patients (see Supplementary Figure S4a online) and investigated whether plasticity features are acquired in isolated cells with TCR stimulation only or TCR stimulation with IL-1β and IL-23, which are increased in LS (Pietrzak et al., 2008). After activation with anti-CD3/CD28 or anti-CD3/CD28 plus cytokines, we found an increased expression of RORγt and T-bet. The Th17 cells activated with anti-CD3/CD28 expressed both IL-17 and IFN-γ; however, anti-CD3/CD28 plus cytokine stimulation showed a decrease in IL-17+ and increase in IFN-γ− cells (Figure 2e). We also found the proliferation of Th17 cells after anti-CD3/CD28 activation, whereas the cytokines induced a more robust proliferation in the last cycle (Figure 2f). These results suggest that proliferation and plasticity features are acquired in isolated Th17 cells after TCR stimulation and can be enhanced by the presence of inflammatory cytokines. This research unveils a role for SEB in the physiopathology of psoriasis through the induction of plasticity features of Th17 cells toward a pathogenic profile that could contribute to inflammation. The acquisition of Th17 plasticity features might have implications in disease severity and in the biological treatment of psoriasis patients.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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An Integrated Data Resource for Genomic Analysis of Cutaneous T-Cell Lymphoma


TO THE EDITOR


Analyzed cutaneous T-cell lymphoma (CTCL) genomes to identify disease driving mutations and therapeutic targets (Choi et al., 2015; da Silva Almeida et al., 2015; McGrattan et al., 2016; Prasad et al., 2016; Silva Almeida et al., 2015; McGirt et al., 2015; Ungewickell et al., 2015; Wang et al., 2012). The low incidence rate of CTCL (Korgovar et al., 2013) makes it difficult to conduct such investigations; therefore, combining multiple small cohorts may increase the statistical power for identifying rarer mutations and key disease pathways (Park et al., 2017) and may uncover genomic types of CTCL, which may guide future therapies via selective targeting of mutation-specific patient populations. For many cancers, integrated genomic datasets are accessible via centralized portals such as eBioPortal, but a similar resource for CTCL is not available. Here we have collected, selected, and re-analyzed individual datasets whenever raw data were available to generate high-quality, homogeneous data, and we present the compilation and analysis of this integrated dataset in CTCL, including matching genomic mutations and gene copy number profiling for all cases. Most importantly, our database can be expanded as more CTCL studies are published.

We compiled genomic data of 139 CTCL cases from seven sequencing studies of mycosis fungoides and Sézary syndrome (SS) (Supplementary Table S1 online). We identified 11,520 single nucleotide mutations and 1,248 insertions/deletions in 121 SS cases (Supplementary Table S2 online), and 1,774 single nucleotide mutations and 37 insertions/deletions in 18 mycosis fungoides cases (Supplementary Table S3 online). In parallel, gene copy number analysis resulted in 682,298 and 60,067 genomic regions in SS and mycosis fungoides cases (Supplementary Tables S4 and S5 online), providing additional information and confirming consistency with previous studies (Lin et al., 2012) (Supplementary Figure S1 online). To identify significantly mutated genes in the entire database, we took a Poisson statistics-based approach (Crescenzo et al., 2015), taking into account the effective length of a gene (Nei and Gojobori, 1986). Briefly, Poisson probabilities of observing a given number of mutations or more were calculated and corrected for multiple hypothesis testing by the Benjamini-Hochberg method. We found 125 significantly mutated genes with a corrected P-value <0.05 (Supplementary Table S6 online). Twenty of these genes were known oncoproteins in the COSMIC cancer gene census. Most frequently mutated oncoproteins were TP53 (19%), PLCG1 (10%), CARD11 (7%), DNMT3A (6%), FAS (6%), POT1 (6%), RHOA (3%), KIT (5%), and tumor necrosis factor receptor 2 (TNFRSF1B) (4%). The complete genomic metadata including the identified frequently mutated genes are available for download from the Dryad Digital Repository (https://www.datadryad.org).

One of the important problems we were able to address, which was not

Abbreviations: CTCL, cutaneous T-cell lymphoma; SS, Sézary syndrome

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