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抄読会　Jun, 23, 2023

Single-cell transcriptome analysis of human skin identifies novel fibroblast subpopulation and enrichment of immune subsets in atopic dermatitis.

He H, Suryawanshi H, Morozov P, et al. J Allergy Clin Immunol. 2020 Jun;145(6):1615-1628.

アトピー性皮膚炎（AD）は免疫細胞と表皮異常からなる複雑な病態を有する皮膚炎症性疾患であり病態解明にむけて多くの研究が行われているが，まだよく解明されてはいない．筆者らはADと健常者の皮膚検体を用いてsingle-cell解析を行った．対象は5人のAD（病変部4，非病変部5）と7人の健常者．合計39042個の皮膚細胞のプロファイルが作製された．（Fig. 1）

1)　AD病変部のfibroblastに*COL6A5*$^{+}$*COL18A1*$^{+}$の発現が新規に認められ，それらは*CCL2*と*CCL19*サイトカインを発現していた．（Fig.3）

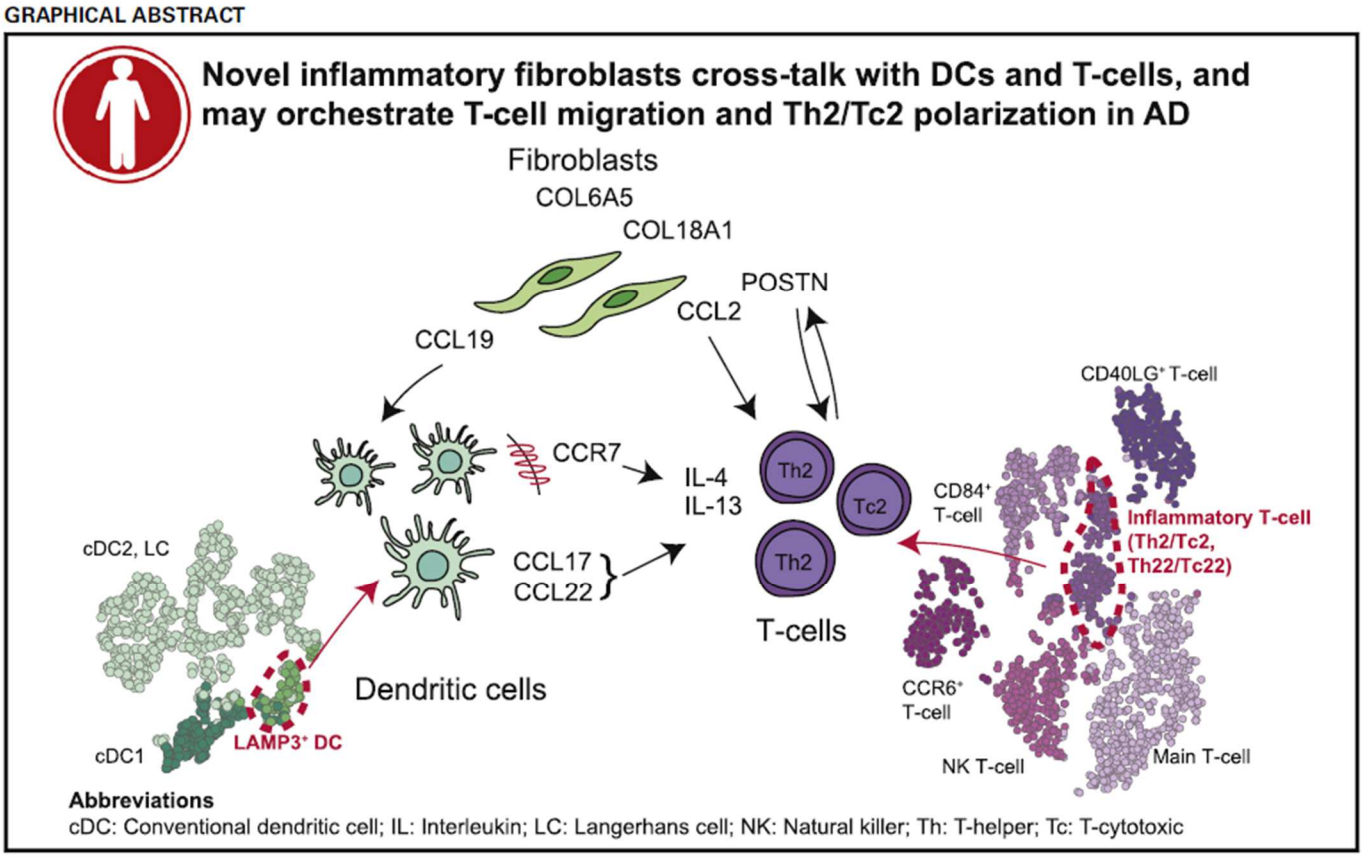
*COL6A5*のADにおける病因的な役割としては不安定なヘテロダイマーを形成し，fibroblastの異常な結合，コラーゲンの生成と代謝，バリア機能破綻に関与．また，他のアレルギー疾患（皮膚，肺，腸管など）においてもみられ，LC細胞やT細胞を制御することによりアレルギー感受性，炎症をpromoteしている可能性が考えられている．

*COL18A1*は血管新生を阻害，他の細胞外マトリックスに結合し，ADにおける細胞外マトリックスの乱れ，不安定性を関与しているのかもしれない．

2)　*CCL19*のレセプター*CCR7*の発現に対応している*LAMP3*$^{+}$ dendritic cellはAD病変部で多く認められた．（Fig.5）

3)　Type2(IL13+)/type 22(IL22+)のT細胞の割合はAD病変部で最も多く，AD非病変部，健常者の順で減少していった．（Fig.6）

まとめ（Visual abstractを参照）：ADはtype2/type22 T細胞と炎症性のDC，さらに今回確認された新規の炎症性fibroblastに特徴づけられており，他の免疫細胞と相互関係しながらADの微小環境をつくっていると考えられた.



Take Home Message

・ADの微小環境においてfibroblastが重要な役割を果たしている可能性が示唆された.

・ADの微小環境においてfibroblastが何を発現しているか，調べる価値があると考えられた.

（査読者　柳川直樹）

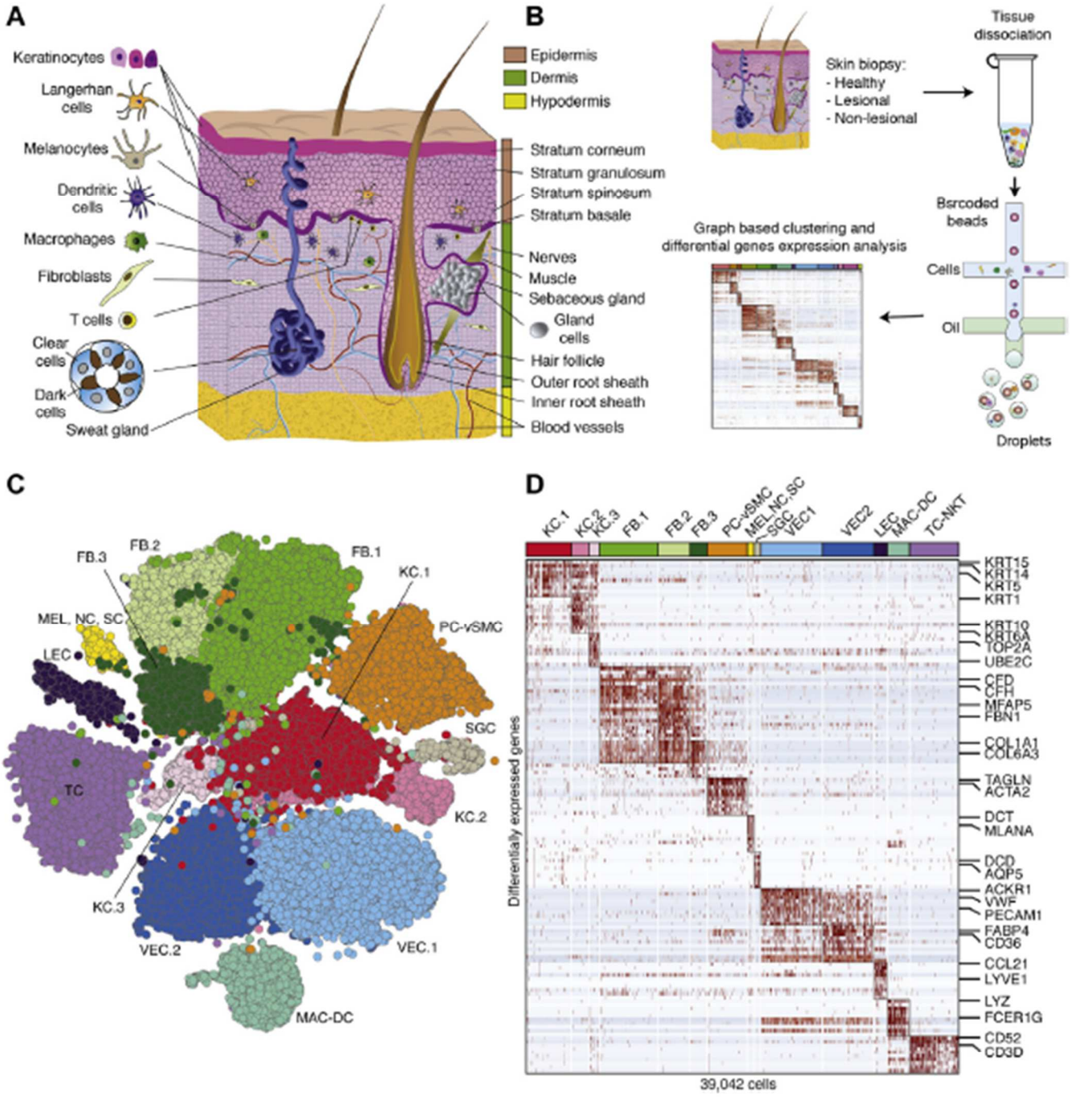


FIG 1. scRNA-seq atlas of cell populations in AD and healthy skin. **A,** Cross-sectional schematic illustrating diverse cell populations in skin. **B,** Workflow for single-cell profiling of skin, including biopsy specimen dissociation, droplet-based scRNA-seq, and graph-based clustering. **C,** t-Distributed stochastic neighbor embedding plot for 39,042 skin cells, derived from 5 patients with AD (4 lesional and 5 nonlesional samples) and 7 healthy subjects. **D,** Distinct gene signatures (top 10 differentially expressed genes; Wilcoxon rank sum test) of skin cell populations. *MAC,* Macrophage; *MEL,* melanocyte; *NC,* neuronal cell; *NKT,* natural killer T cell.

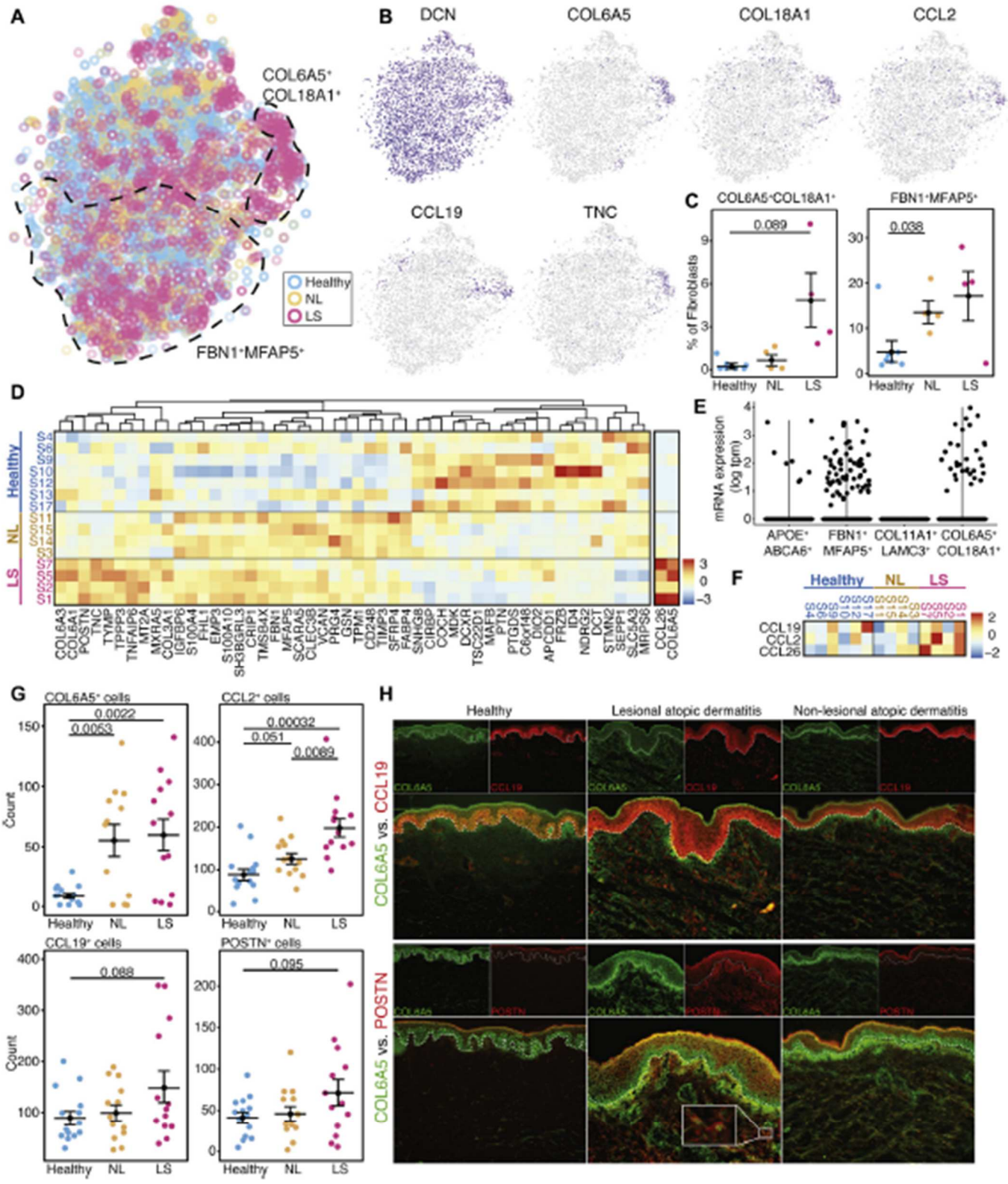


FIG 3. Novel fibroblast subpopulation in lesional AD. **A,** t-Distributed stochastic neighbor embedding plot of combined fibroblast cells, color-coded by tissue type/condition (healthy, nonlesional [NL] AD, and [LS] AD), with the *COL6A5*$^+$*COL18A1*$^+$ and *FBN1*$^+$*MFAP5*$^+$ fibroblast clusters delineated by a dotted black line. **B,** Feature plots displaying expression of specific markers among the set of all fibroblasts: *DCN*, *COL6A5*, *COL18A1*, *CCL2*, *CCL19*, and *TNC*. **C,** Frequencies of *COL6A5*$^+$*COL18A1*$^+$ and *MFAP5*$^+$*FBN1*$^+$ fibroblasts, as a proportion of all fibroblasts, with pairwise comparisons (with accompanying *P* values with *P* less than .1) among disease conditions. **D,** Unsupervised clustering heatmap showing relative expression (*z* score) levels of the top 50 most abundantly and differentially expressed genes in fibroblasts,

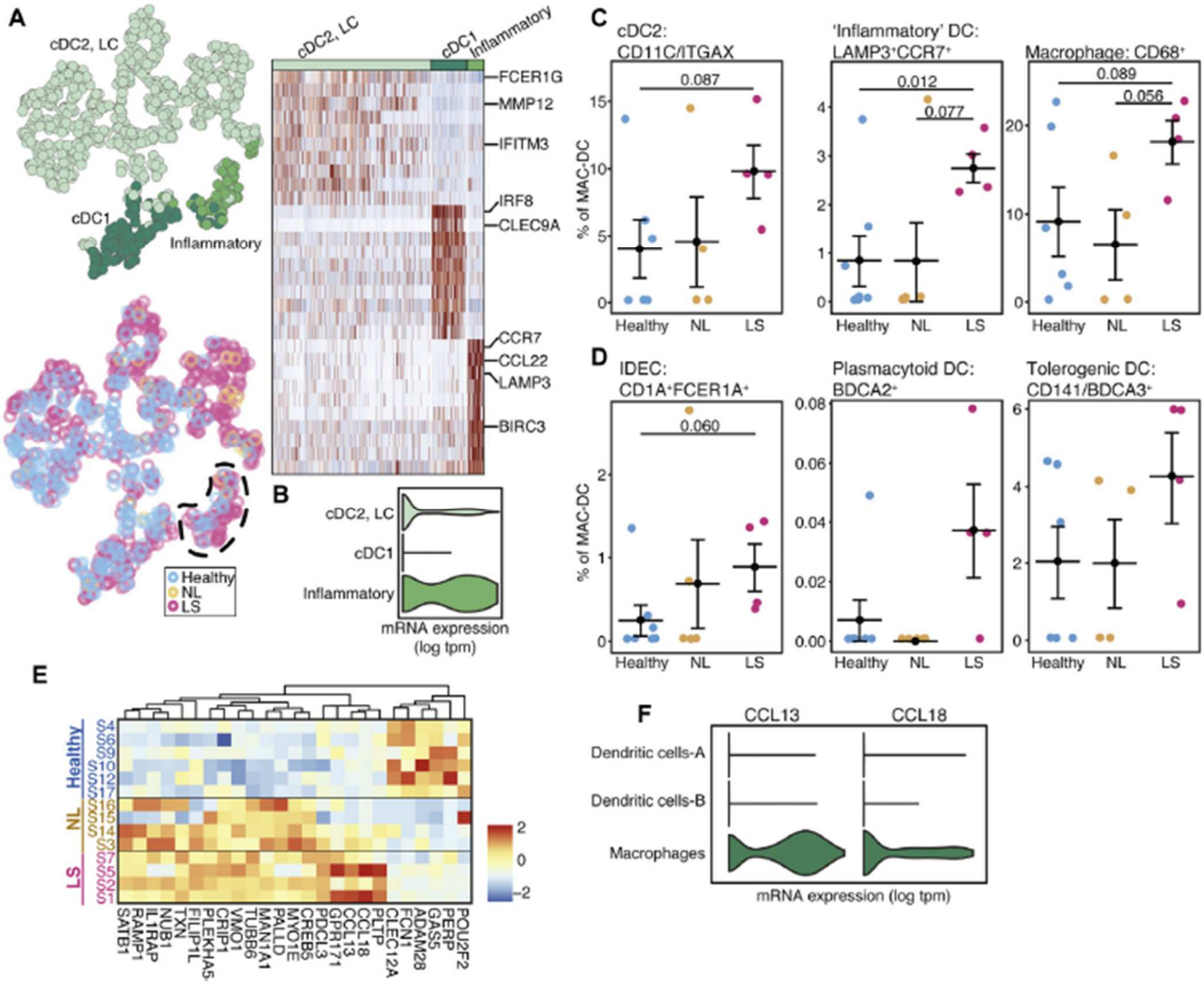
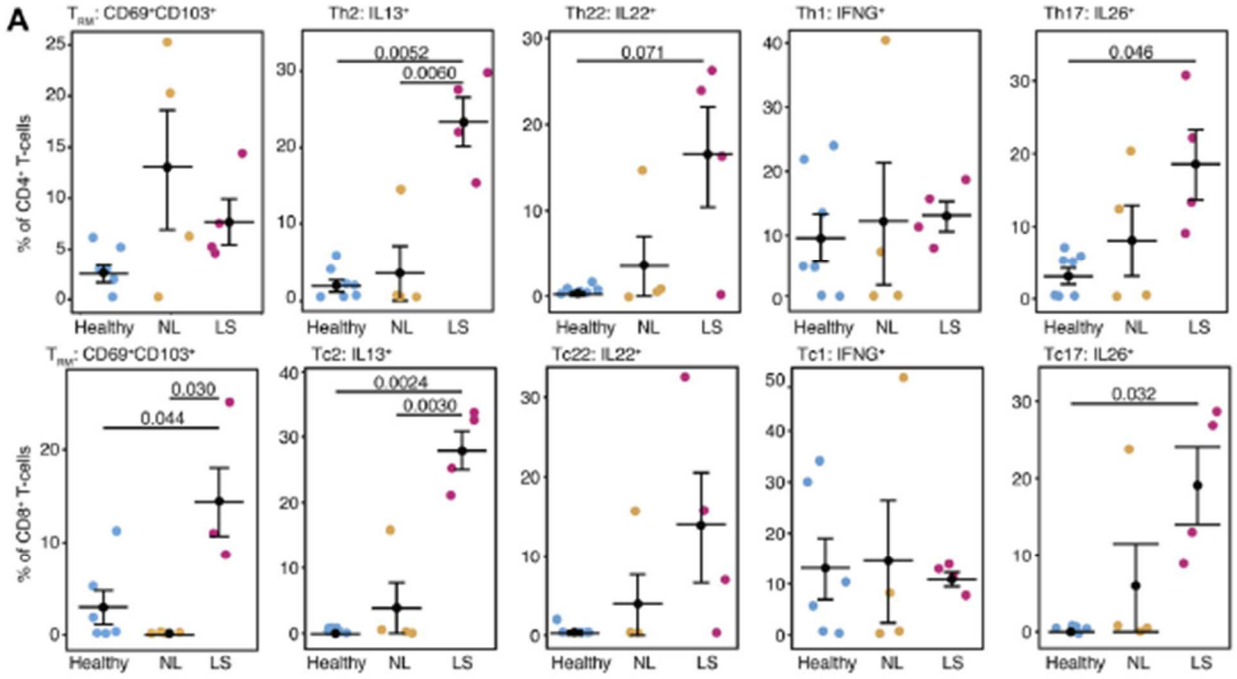
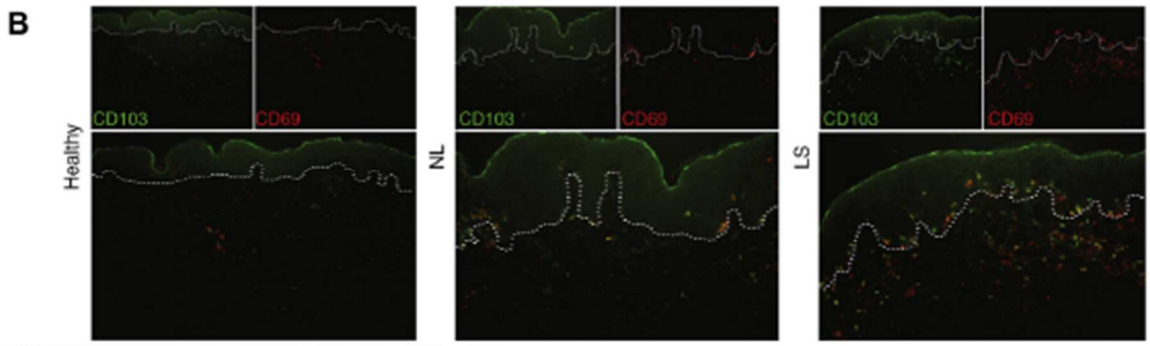


FIG 5. Compositional and gene expression changes among macrophages and DCs in AD. **A,** t-Distributed stochastic neighbor embedding plot of DCs, color-coded by cell subpopulation and by disease condition, with an accompanying heatmap of distinct gene signatures (top 10 differentially expressed genes, Wilcoxon rank sum test). **B,** Violin plot showing *CCL17* mRNA expression, written as log transcripts per kilobase million (tpm) value, within DC subpopulations. **C** and **D,** Frequencies of DC and macrophage subsets as a percentage of all macrophages and DCs in the sample, with pairwise comparisons among disease conditions (healthy, nonlesional [NL] AD, and lesional [LS] AD), as obtained from subclustering **(C)** and from previously defined lineage markers **(D)**. **E,** Unsupervised clustering heatmap showing relative expression (*z* score) levels of differentially expressed genes in macrophages and DCs, as determined by using criteria log fold change greater than 1 and FDR less than 0.05, calculated on samples with more than 10 macrophages and DCs. **F,** Violin plot showing *CCL13* and *CCL18* mRNA expression, written as log tpm within macrophage/DC subpopulations. *IDEC,* Inflammatory dendritic epidermal cell; *MAC,* macrophage.





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All	Healthy	NL	LS
IL13+	2.66	11.94	22.91
IL22+	0.95	12.69	20.08
IL26+	4.38	11.94	17.63
IFNG+	5.07	5.97	5.92
IL13+/IFNG+	0.53	2.00	3.87
IL22+/IFNG+	0.19	2.13	3.39
IL26+/IFNG+	0.86	2.00	2.98

CD4+	Healthy	NL	LS
Th2	3.42	12.73	19.10
Th22	0.99	12.73	16.58
Th17	4.38	11.82	16.40
Th1	2.76	2.73	3.42
Th2/Th1	1.24	4.67	5.58
Th22/Th1	0.36	4.67	4.84
Th17/Th1	1.88	4.33	4.79

CD8+	Healthy	NL	LS
Tc2	0.00	11.76	25.41
Tc22	1.18	11.76	17.21
Tc17	0.00	17.65	15.57
Tc1	9.41	11.76	9.02
Tc2/Tc1	0.00	1.00	2.82
Tc22/Tc1	0.13	1.00	1.91
Tc17/Tc1	0.00	1.50	1.73