# A Subset of Type I Conventional Dendritic Cells Controls Cutaneous Bacterial Infections through VEGFa-Mediated Recruitment of Neutrophils

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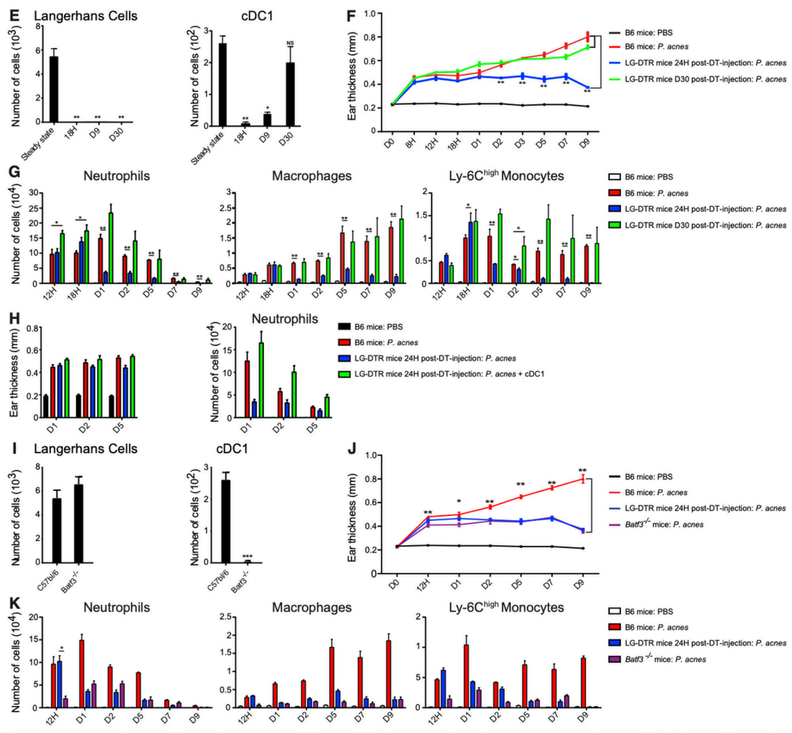
## Introduction

Dendritic cells (DCs) are professional pathogen-sensing and antigen-presenting cells (APCs) that are central to the initiation and regulation of immune responses (Schlitzer et al., 2015). In the skin, two subsets of ontogenetically distinct and functionally specialized conventional DCs (cDCs) exist: cDC1s, identified in mice as expressing CD103 (integrin αE) and XCR1; and cDC2s, expressing CD11b (integrin αM) and SIRPα (Schlitzer et al., 2015). Together, they maintain the balance of immunity to pathogens and tolerance to self and microbiota. P. acnes is a commensal of the human skin, mouth, and upper respiratory tract, but it is also an opportunistic pathogen (Fitz-Gibbon et al., 2013). The inflammatory response to this bacterium drives the development of acne lesions. The injection of P. acnes into the skin results in recruitment of polymorphonuclear cells, macrophages, and T lymphocytes (De Young et al., 1984). **Our hypothesis suggests that DCs control the immune response to P. acnes, especially in the formation of granulomas.** CD11c+ DCs are critical for hepatic granuloma formation in mice injected with heat-killed P. acnes (Ohteki et al., 2006), but little is known of the role of skin DCs in the pathophysiology of dermal P. acnes infection.

## FIGURE 1

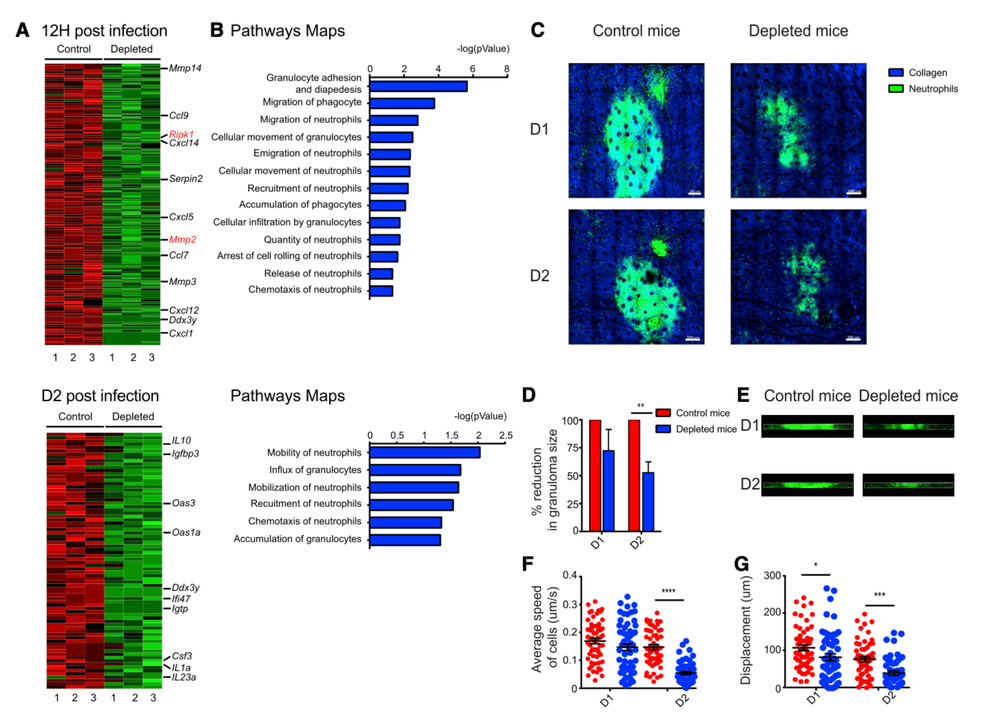



(E) Flow-cytometry quantification of the absolute numbers of LCs and cDC1s in total ear skin cell suspensions at steady state, 18 h, 9 days, or 30 days after DT injection of LG-DTR mice.
(F) Ear-thickness measurements during the 9 days following intradermal *P. acnes* injection of WT B6 mice (red line), LG-DTR mice 24 h after DT injection (blue line), LG-DTR mice 30 days after DT (green line), and WT B6 mice injected with PBS as control (black line).
(G) Flow-cytometry quantification of absolute numbers of immune cell populations after *P. acnes* intradermal injection, with mouse groups as in (F).
(H) Ear-thickness measurements and absolute neutrophil numbers in ear skin during the 5 days following intradermal *P. acnes* injection of WT B6 mice (red bars), LG-DTR mice 24 h after DT injection (blue bars), and LG-DTR mice 24 h after DT injection and adoptive transfer with 5,000 cDC1s derived from the bone marrow of WT B6 mice (green bars); control WT B6 mice were injected with PBS only (black bars).
(I) Flow-cytometry quantification of the absolute numbers of LCs and cDC1s in total ear skin cell suspensions from WT B6 and *Batf3*$^{-/-}$ mice at steady state.
(J) Ear-thickness measurements during the 9 days following intradermal *P. acnes* injection of WT B6 mice (red line), LG-DTR mice 24 h after DT injection (blue line), and *Batf3*$^{-/-}$ mice (purple line); control WT B6 mice were injected with PBS (black line).
(K) Flow-cytometry quantification of absolute numbers of immune cell populations after *P. acnes* intradermal injection and mouse groups as in (J).
Data are representative of two independent experiments with three replicates per condition and per time point. Shown is the mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.001) (see also Figures S1 and S2).

Fig 1. cDC1s Are Required for Sustained Immune Cell Recruitment Following Intradermal Injection of P. acnes

- In Langerin-DTR mice (LG-DTR mice), specific depletion of dermal cDC1s via exposure to diphtheria toxin (DT) causes the mice to exhibit significantly less ear swelling when exposed to P. acnes compared to controls and after repopulation of cDC1s.

## FIGURE 2

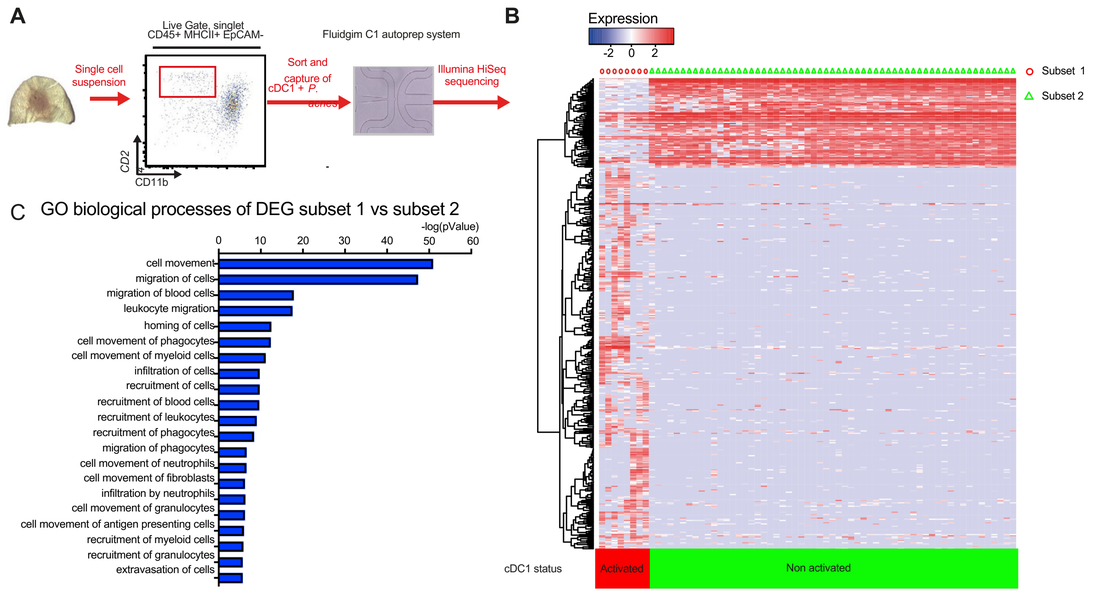



(A) Heatmap showing the expression of the DEGs (obtained by bulk RNA sequencing) downregulated in neutrophils infiltrating the skin in the absence of cDC1s 12 h and 2 days after infection (see also Table S2).
(B) Visualization of the gene-associated GO biological processes related to the neutrophil immune response (see also Figures S3A–S3C).
(C) Intravital microscopy images of neutrophils around the *P. acnes* ($10^7$ CFU) injection site within the ears of control and cDC1-depleted lysozyme M-GFP/Langerin-DTR mice at day 1 and day 2 after injection (see also Videos S1, S2, S3, and S4).
(D) Volume of the granuloma formed at the *P. acnes* injection site in control and cDC1-depleted mice at day 1 and day 2 after injection.
(E) Cross-sectional view of ear skin granulomas at *P. acnes* injection site in control and cDC1-depleted mice at day 1 and day 2 after injection (see also Figures S3D and S3E).
(F) Mean speed of neutrophils infiltrating the skin after *P. acnes* injection in control and cDC1-depleted lysozyme M-GFP/Langerin-DTR mice over 30 min (symbols represent individual cells).
(G) Displacement of neutrophils in (F).

Fig 2. Dermal cDC1 Depletion Alters Neutrophil Gene Expression and Activity

- Dermal cDC1s are required for sustained inflammatory immune response to P. acnes.
- LG-DTR mice 24 h after DT injection (depleted mice) had downregulated differentially expressed genes (DEGs) in neutrophils.
- The result was decreased motility of neutrophils, less neutrophil recruitment to the P. acnes injection site, reduced granuloma formation and inflammatory response to P. acnes.

## FIGURE 3

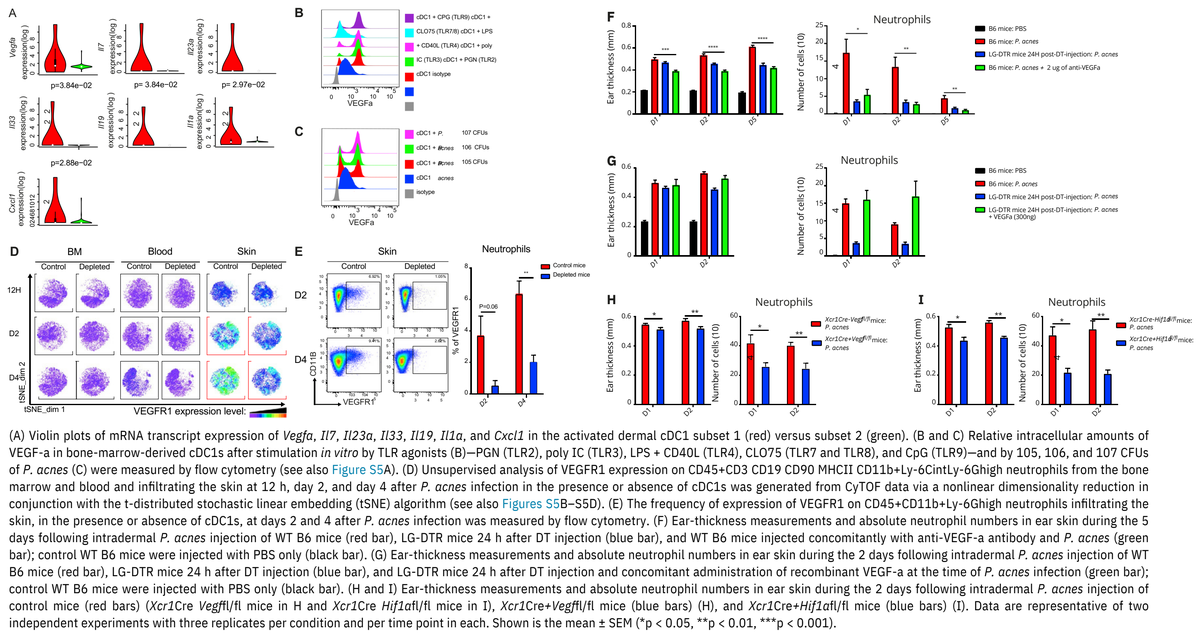



(A) Experimental workflow for isolation of individual cDC1s from WT B6 mouse ears 16 h after *P. acnes* injection via the Fluidigm C1 Auto Prep System. (B) Supervised clustering via the Fluidigm SINGuLAR method identified two clusters of cDC1s in *P. acnes*-injected ears (see also Figures S4D–S4F). (C) Visualization of the GO biological processes associated with DEGs more abundantly expressed in cDC1 subset 1 than in subset 2. (D–J) Violin plots show mRNA transcript levels of cell-surface-protein-encoding genes selected by ANOVA and highlight the activated status of subset 1 (red) versus subset 2 (green), which are associated with cell migration pathways (D), maturation and activation during immune responses (E), antigen priming and presentation by MHCI (F), and downregulation of molecules associated with MHCII presentation (G). The frequency and intensity of expression of the surface proteins EpCAM, Ly-6D, and CD59 (encoded by genes identified by the violin plot in H) across the cDC1 (I) and cDC2 (J) populations from PBS- and *P. acnes*- injected ear skin were measured by flow cytometry (see also Figure S4 and Table S3).

*Fig 3. Single-Cell Gene Expression Analysis Reveals an Activated Subset of cDC1s in P. acnes-Injected Skin*

- Single-cell mRNA sequencing identified two subsets of dermal cDC1s (minor subset 1 and major subset 2) after P. acnes injection. (Fig 3B.)
- Minor subset 1 showed enriched expression of genes related to antigen capture, activation, migration, and immune cell recruitment, but reduced MHC-II presentation (Fig 3C.)
- Surface markers EpCAM, CD59, and Ly-6D distinguished the minor subset and were enriched in granulomatous areas.
- The minor subset was specifically linked to cDC1s and not cDC2s, highlighting its unique role.
- This minor subset represents an activated cDC1 population involved in the immune response to P. acnes.

## FIGURE 4




(A) Violin plots of mRNA transcript expression of *Vegfa*, *Il7*, *Il23a*, *Il33*, *Il19*, *Il1a*, and *Cxcl1* in the activated dermal cDC1 subset 1 (red) versus subset 2 (green). (B and C) Relative intracellular amounts of VEGF-a in bone-marrow-derived cDC1s after stimulation *in vitro* by TLR agonists (B)—PGN (TLR2), poly IC (TLR3), LPS + CD40L (TLR4), CLO75 (TLR7 and TLR8), and CpG (TLR9)—and by 105, 106, and 107 CFUs of *P. acnes* (C) were measured by flow cytometry (see also Figure S5A). (D) Unsupervised analysis of VEGFR1 expression on CD45+CD3 CD19 CD90 MHCII CD11b+Ly-6CintLy-6Ghigh neutrophils from the bone marrow and blood and infiltrating the skin at 12 h, day 2, and day 4 after *P. acnes* infection in the presence or absence of cDC1s was generated from CyTOF data via a nonlinear dimensionality reduction in conjunction with the t-distributed stochastic linear embedding (tSNE) algorithm (see also Figures S5B–S5D). (E) The frequency of expression of VEGFR1 on CD45+CD11b+Ly-6Ghigh neutrophils infiltrating the skin, in the presence or absence of cDC1s, at days 2 and 4 after *P. acnes* infection was measured by flow cytometry. (F) Ear-thickness measurements and absolute neutrophil numbers in ear skin during the 5 days following intradermal *P. acnes* injection of WT B6 mice (red bar), LG-DTR mice 24 h after DT injection (blue bar), and WT B6 mice injected concomitantly with anti-VEGF-a antibody and *P. acnes* (green bar); control WT B6 mice were injected with PBS only (black bar). (G) Ear-thickness measurements and absolute neutrophil numbers in ear skin during the 2 days following intradermal *P. acnes* injection of WT B6 mice (red bar), LG-DTR mice 24 h after DT injection (blue bar), and LG-DTR mice 24 h after DT injection and concomitant administration of recombinant VEGF-a at the time of *P. acnes* infection (green bar); control WT B6 mice were injected with PBS only (black bar). (H and I) Ear-thickness measurements and absolute neutrophil numbers in ear skin during the 2 days following intradermal *P. acnes* injection of control mice (red bars) (*Xcr1*Cre *Vegf*fl/fl mice in H and *Xcr1*Cre *Hif1a*fl/fl mice in I), *Xcr1*Cre+*Vegf*fl/fl mice (blue bars) (H), and *Xcr1*Cre+*Hif1a*fl/fl mice (blue bars) (I). Data are representative of two independent experiments with three replicates per condition and per time point in each. Shown is the mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.001).

*Fig 4.Skin cDC1s Regulate Neutrophil Influx during P. acnes Infection through VEGF-a Secretion*

- VEGF-a Expression by cDC1s: Activated dermal cDC1s express VEGF-a, a critical neutrophil chemoattractant, in response to P. acnes and other TLR agonists. (Fig 4B. and 4C.)
- Role of VEGF-a in Neutrophil Recruitment: VEGF-a regulates neutrophil infiltration into tissues by upregulating VEGFR1 expression, which is necessary for neutrophil migration and inflammation at the infection site. (Fig 4F.)
- Neutralization and Rescue Experiments: Blocking VEGF-a reduced neutrophil infiltration and ear inflammation, while recombinant VEGF-a restored the inflammatory response in cDC1-depleted mice. (Fig 4G.)
- cDC1-Specific VEGF-a Deletion: Mice with cDC1-specific VEGF-a knockout showed decreased neutrophil recruitment and inflammation after P. acnes injection, confirming the role of cDC1-derived VEGF-a in immune response. (Fig 4H.)
- HIF-a Dependency: HIF-a was identified as a critical transcription factor for VEGF-a expression in cDC1s, essential for neutrophil recruitment and inflammation in P. acnes-infected skin. (Fig 4I.)

## DISCUSSION

Hence in this study, we uncovered an essential role for cDC1s in regulating neutrophil biology through the VEGFa-VEGFR1 pathway in the context of bacterial infection of murine skin and highlighted a specific role of a CD59+EpCAM+Ly-6D+ minor cDC1 subset in the regulation of tissue innate immunity that goes beyond antigen presentation and T cell priming. Further work should investigate the similarities in term of cell composition, pathways activated, and cDC1 transcriptomic profiles during the immune response to skin bacterial infection in humans.

## REFERENCES

Janela et al., A Subset of Type I Conventional Dendritic Cells Controls Cutaneous Bacterial Infections through VEGFa-Mediated Recruitment of Neutrophils, Immunity (2019), https://doi.org/10.1016/j.immuni.2019.03.001