cDC1s: New Orchestrators of Tissue Innate Immunity

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ABSTRACT

Type-I conventional dendritic cells (cDC1s) are key in the induction of adaptive immunity. Using single-cell sequencing, Ginhoux and colleagues find that a subset of activated cDC1s in bacteria-infected skin is critical for neutrophil recruitment via the production of VEGF-α. These results reveal a crucial function for cDC1s beyond antigen presentation.

SPOTLIGHT

The skin represents the first line of defense against the environment, creating both a mechanical and immunological barrier [1]. Dendritic cells (DCs) are professional antigen presenting cells that maintain an equilibrium between tolerance and immunity. DCs sense and integrate a wide range of signals from internal and external environments in order to initiate and shape adapted immune responses. In the skin, three main DC subsets ($CD11c^+$ $MHCII^+$) can be found: $XCR1^+$ conventional type I (cDC1s), $SIRP\alpha^+$ conventional type II (cDC2s) and $EpCAM^{bright}$ $Langerin^+$ Langerhans cells (LCs) [2]. Understanding the specific roles of each of these populations and their crosstalk is needed to develop therapies targeting skin pathologies, including infections, allergy, autoimmunity or cancer.

One of these pathologies is acne vulgaris, characterized by infection of pilosebaceous follicles by *Propionibacterium acnes* (*Cutibacterium acnes*) [3]. High numbers of neutrophils are recruited to infected follicles, triggering further inflammation resulting in skin damage, pronounced thickness, formation of dermal granulomas and in severe cases, scarring [4]. Notably, systemic infection with *P. acnes* results in the accumulation of $CD11c^+$ DCs that produce interleukin (IL)-15 and mediate an inflammatory response, contributing to the formation of hepatic granulomas [5]. However, the function of skin DCs in response to dermal *P. acnes* infection had remained elusive.

To decipher the role of different DC subsets during *P. acnes* infection, Janela *et al*. [6] performed deep immunophenotyping following ear skin intradermal infection, combining mass cytometry by time of flight (CyTOF) and conventional cytometry. This analysis showed massive hematopoietic infiltration

into the infected ear parallel to notorious thickening of the ear associated with the formation of a granuloma. cDC1s were required for the infiltration of neutrophils into the infected site, along with monocytes and macrophages -- a conclusion that was supported by different experimental approaches. First, the authors took advantage of the differential repopulation kinetics of LCs versus cDC1s after conditional Langerin-diphtheria toxin receptor (DTR)-based depletion of both cell types in the mice; the depletion was long-lasting for LCs, remaining fully depleted 30 days post-diphtheria toxin (DT) treatment, at which time point, cDC1s had fully repopulated the dermis. Furthermore, infection with *P. acnes* in the absence of both LCs and cDC1s (24 hours after depletion) prevented sustained immune infiltration and ear swelling, reverted by adoptive transfer of bone marrow-derived cDC1s at the time of the infection. In addition, infection at 30 days post-depletion, at which time cDC1s were present and LCs absent, led to massive immune cell infiltration and increased ear thickness relative to infection 24 hours after depletion. Moreover, neutrophil recruitment and inflammation following *P. acnes* infection was prevented in cDC1-deficient *Batf3*$^{-/-}$ mice relative to wild type (WT) mice, further supporting the notion that cDC1s mediate inflammation in this setting. Taking advantage of LC radioresistance, specific depletion of cDC1 in Langerin-DTR bone marrow chimeras further confirmed the observation that cDC1s were essential for inflammation and cell infiltration following *P. acnes* ear infection [6].

In the study by Ginhoux and co-workers [6], *P. acnes* infection 24 hours after depletion of DCs led to reduced expression in skin neutrophils of genes related to infiltration, migration and survival compared with infected skin in non-depleted mice. Subsequently, neutrophils in langerin-DTR mice treated with DT

and infected with *P. acnes* exhibited reduced motility along with enhanced apoptosis relative to neutrophils from infected non-depleted mice.

To assess the potential mechanisms of cDC1-mediated neutrophil recruitment in these mice, , the authors performed single-cell mRNA sequencing on skin cDC1s shortly after *P. acnes* infection (16h post-infection) – also because of the low number of cDC1s normally found in ear skin. This analysis revealed the presence of a minor cDC1 subpopulation defined by the expression of genes encoding CD59, Ly6D and EpCAM, with an apparent activated phenotype. Gene and protein expression of Vascular endothelial growth factor alpha (VEGF-α) -- which recruits neutrophils into inflamed tissues [7]-- was also induced in this activated cDC1 subset compared with other cell subsets in the skin (Figure 1) [6]. Infection of *Xcr1Cre*$^{+/-}$ *Vegf*$^{fl/fl}$ mice (a mouse model allowing the excision of the *Vegf* gene only in cDC1s) prevented inflammation, showing a relevant contribution of cDC1-derived VEGF-α in the recruitment of neutrophils and ear swelling, relative to WT. However, additional sources of neutrophil chemoattractant generated by cDC1s may have been relevant (e.g. CXCL2, see below [8]), given that full cDC1depletion resulted in greater inhibition of neutrophil infiltration than by only depleting VEGF-α in the cDC1-specific subset . Concomitantly, VEGFR1 expression on skin neutrophils was reduced in cDC1-depleted mice, indicating that the binding of cDC1-derived VEGF-α to neutrophil VEGFR1 is important in mediating granuloma formation upon *P. acnes* infection in the mouse ears [6].

Moreover, the relevance of the CD59^{+} Ly6D^{+} EpCAM^{+} activated cDC1 subset in skin neutrophil infiltration was also shown for other bacterial infections, including Gram negative (*Escherichia coli*), Gram positive (*Staphylococcus*

aureus) and mycobacteria (BCG). In addition, human cytometry-sorted cDC1s from UV-killed *E. coli*-induced skin blisters also demonstrated enhanced expression of *VEGF* relative to blood cDC1s. This correlated with higher VEGFR1 expression on neutrophils recovered from these blisters compared to blood neutrophils. Altogether, these data suggest a similar crosstalk between cDC1s and neutrophils in humans upon bacterial skin infections [6]

This study highlights the relevance of cDC1s as regulators of innate immunity and particularly, of neutrophil infiltration. This notion is also supported by recent data showing that cDC1s regulate the infiltration of neutrophils into damaged kidneys after systemic candidiasis in mice, where the proposed mechanism depended on the expression of CXCL2 in $XCR1^+$ cDC1s [8]. In contrast, Janela *et al*. did not detect CXCL2 in their single-cell analysis, although, as discussed by the authors, the limited detection rate of the single-cell sequencing technique could not identify this transcript [6]. Alternatively, the bulk analysis of the cDC1 population examined by our group could have masked specific qualitative differences in the transcription profiles of minor subpopulations [8].

In addition, cDC1s could be orchestrating inflammation through different mechanisms depending on the innate stimuli that activate them. How cDC1s modulate neutrophil infiltration upon sensing different challenges (either infectious or sterile injury/inflammation) warrants further investigation. Moreover, as the VEGF-α-dependent cDC1 and neutrophil infiltration responses were associated with bacterial skin infections, while the CXCL2-mediated responses were found upon fungal infection in damaged mouse kidneys, we cannot rule out tissue- or possibly pathogen-specific reactions developed by cDC1s. In support

of this hypothesis, despite unbiased definition of lineage-imprinted surface markers to determine DC populations, organ-specific environmental cues dictate DC heterogeneity [9].

Previous reports have indicated that there is a differential priming capacity of DC subsets in the skin which leads to specialized adaptive immunity [10]. The work by Ginhoux and colleagues [6] provides new knowledge regarding the differential phenotypic and functional roles performed by distinct DC subsets upon infection, and even by discrete subpopulations within these subsets, as defined at the single-cell level. However, the more striking feature uncovered by these studies [6,8] is the capacity of cDC1s to regulate innate immune responses, including neutrophil infiltration; these characteristics go beyond the classical role of cDC1s in antigen presentation and priming of T cell responses. Therefore, these such concepts open up novel research avenues, designating cDC1s as intriguing orchestrators of both adaptive and innate immunity.

FIGURE LEGEND

Figure 1. A Subset of Conventional Type I Dendritic Cells (cDC1s) Promotes Neutrophil Infiltration upon Bacterial Skin Infection in Mice and Humans. cDC1s, characterized by the specific expression of XCR1, are one of the DC populations present in the dermal layer of the skin. After bacterial infection of the dermis (e.g. *Propionibacterium acnes*), a minor subset of cDC1s expressing CD59, EpCAM and Ly6D differentially produces vascular endothelial growth factor alpha (VEGF-α). VEGF-α promotes massive neutrophil recruitment (e.g. to the infected ear skin) that eventually contributes to the generation of a skin granuloma. These results support a newly-recognized role for cDC1s beyond antigen presentation, placing this rare population as a relevant regulator subset of tissue innate immunity [6].

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