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ILC2-derived IL-13 promotes skin cDC2 diversity

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Main text

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Dendritic cells (DCs) are critical players in discriminating between potentially dangerous pathogens, that require an immediate immune response, and the plethora of microbes and other stimuli that are harmless or even beneficial for the body. In response to both the diversity of the challenges that they may encounter and the distinct tissue environments in which they reside, DCs have evolved into multiple subsets and activation states, although the signals that drive this transcriptional adaptation is poorly understood for most tissue sites. A recent study by Mayer et al. in *Nature Immunology* [1] has provided insight into this issue in healthy skin by revealing that the cytokine IL-13, commonly associated with T helper (Th)2 immunity, provides a homeostatic signal that helps maintain a distinct population of type 2 conventional DCs (cDC2s) and inhibits inappropriate inflammatory responses.

cDCs can be divided into two major lineages, cDC1 and cDC2. While cDC1s depend on the transcription factor IRF8 for their development and are specialized into cross presenting cell associated antigens to $CD8^+$ T cells, cDC2s are IRF8-independent and more preferentially are required for $CD4^+$ T cell priming and Th responses [2, 3]. Mayer *et al.* [1] examined the cDC populations in the mouse skin and found that cDC2s can be separated into $CD11b^{hi}$ and $CD11b^{lo}$ fractions, a finding compatible with earlier studies [4, 5]. $CD11b^{lo}$ cDC2s were absent from other tissues examined, including the small intestine and lung, suggestive of the existence of a particular milieu imprinting cDC2 diversity in the skin. DNA motif analysis of the promoters of $CD11b^{lo}$ cDC2s specific genes revealed enrichment for several transcription factor binding sites including those bound by STAT6. In keeping with this, a major finding of Mayer *et al.* was that STAT6 knockout mice lacked skin $CD11b^{lo}$ cDC2s, whilst $CD11b^{hi}$ cDC2s development was unperturbed [1]. STAT6 is activated downstream of the cytokines IL-4 and IL-13. Analysis of knockout mice revealed that IL-4 was dispensable for $CD11b^{lo}$ cDC2 development, whereas IL-13 and components of the shared IL-4/13 receptor was essential for the formation of these cells (Figure 1). This finding suggests a role for IL-13 in regulating skin cDC diversity under steady-state conditions. Germfree mice, or those lacking the ability to respond to alarmins such as TSLP or IL-33, had normal numbers of $CD11b^{lo}$ cDC2s, further supporting a homeostatic function for IL-13 in maintaining cDC2 diversity.

To search for the cellular source of IL-13 in the skin, Mayer *et al.* used an IL-13-DsRed reporter strain and found that approximately half of the constitutive IL-13 expressing cells were type 2 innate lymphoid cells (ILC2s) [1]. This agrees with an earlier report identifying ILCs as the main source of IL-13 in unchallenged murine skin [6]. IL-13 was able to directly

promote the formation of CD11b^{lo} cDC2s in FLT3L cultures. The ability of IL-13 to promote the formation of CD11b^{lo} cDC2s absolutely depended on the transcription factor KLF4, a known regulator of cDC2 diversification [4]. Together these findings clearly show that an IL-13-STAT6 axis is required for the normal frequency of CD11b^{lo} cDC2s in healthy mouse skin and suggest a critical role for ILC2s in mediating their differentiation. Although ILC2s were shown to be the main source of IL-13 in the skin and their depletion resulted in a substantial reduction of CD11b^{lo} cDC2s, development of the latter in *Rag2*$^{-/-}\gamma C^{-/-}$ where all lymphoid cells, including ILCs, are ablated was not addressed. Additional experiments such as adoptive transfer of ILC2 progenitors into *Rag2*$^{-/-}\gamma C^{-/-}$ mice would further strengthen the authors conclusions implicating ILC2-derived IL-13 in promoting skin cDC2 diversity.

Mayer *et al.* [1] then investigated if this signalling axis also plays a role in the immune response in the skin. To exclude roles for this pathway in non-cDCs, such as T cells, these studies were conducted in chimeric mice where all cDC2s where either wildtype or STAT6-deficient and the wildtype T cells could be identified based on congenic markers. The chimeric mice were then vaccinated intradermally with inactivated microorganisms that typically induce Th1 (*Mycobacterium smegmatis*), Th2 (*Nippostrongylus brasiliensis*) or Th17 (*Candida albicans*) biased responses. As predicted, the Th1 response was intact without CD11b^{lo} cDC2s, whereas Th2 immunity, measured by the expression of IL-4, IL-13 or GATA3 in the T cells was clearly impaired, suggesting that CD11b^{lo} cDC2s are required for effective Th2 immunity (Figure 1). Although this observation corroborated an earlier finding [4], it is difficult to reconcile with the key role proposed for dermal cDC2s expressing CD301b/MGL2, in mediating Th2 immunity [7], as a later study showed that CD301b expression is restricted to the same skin migratory CD11b^{hi} cDC2s [4] that Mayer *et al.* demonstrate are IL-13 independent [1]. The nature of this discrepancy warrants further investigation.

In contrast to the induction of Th2 cell differentiation by CD11b^{lo} cDC2s, Th17 biased responses were increased in the absence of CD11b^{lo} cDC2s [8], an observation that resulted from the proportional increase in *Candida* responsive CD11b^{hi} cDC2s in the STAT6-deficient chimeras. Although these experiments used non-viable microorganisms and thus the longer-term impact of the loss of CD11b^{lo} cDC2s on protective immunity was not examined, these findings are in keeping with previous studies on mice lacking KLF4 in cDCs, where KLF4 loss selectively impaired the ability of the mice to survival skin infection with the parasite,

Schistosoma mansoni [4]. A major difference between these studies was that the impact of STAT6 loss was restricted to skin immunity, whereas KLF4 loss also alleviated the impact of the allergen house dust mite extract in the lung after intranasal exposure. The intact cDC2 compartment in the gut and lungs in STAT6 deficient mice, organs where IL-13 and IL-4 are known to promote Th2 responses in the appropriate settings, further supports for the authors contention that the IL-13 produced by ILC2s functions homeostatically in healthy mouse skin, whereas other factors such as IL-33 are additionally involved in the inflammatory responses in other organs.

Although the bulk of this study was performed on immune cells derived from mice, Mayer *et al.* did re-examine the data from several prior studies that have mapped the transcriptome of immune cells in human skin [9]. Single cell transcriptomic data confirmed that human skin contained *STAT6*, *KLF4* and IL-4/13 receptor subunit positive cDC2s. However, the case for *IL13* expression was less clear, with only a minority of human skin ILCs expressing the mRNA. In contrast, the expression of *IL13* in the T cell compartment was as pronounced as that observed for ILCs. Whether these differences are due to the limited sensitivity of scRNAseq approaches to detect *IL13* mRNA or a true difference between human and mouse skin remains to be determined. Additional analysis of human healthy and inflamed human skin should better resolve this issue.

Given the pre-eminence of CD11b^{lo} cDC2s in promoting Th2 immunity in the skin, it will be now important to address the role of ILC2-derived IL-13 and CD11b^{lo} cDC2s in the context of skin allergic diseases. Of particular interest, will be addressing the relevance of this axis in the development of atopic dermatitis a paradigmatic Th2-driven disease. A genome-wide association study performed on children with early onset atopic dermatitis revealed a strong association with a missense variant in the *IL13* gene[10], and in murine model, IL-13 production by ILC2s and mast cells increased following acute allergic skin inflammation[6]. Taken together, these studies highlight the need to address the role of CD11b^{lo} cDC2s in mediating immunity against allergic environments as they may pave the way for the design of therapeutic approaches for Th2-mediated immune disorders.

Collectively the study of Mayer *et al.* [1] adds to a growing body of evidence of tissue cDC2 diversity, identifying a unique subset of cDC2s in the skin that impacts on the capacity of the immune system to promote Th2 immunity. Although the IL-13-STAT6 homeostatic axis may

be restricted to the skin, cDC2 diversity is apparent in other tissues, as for example has been shown in the lung after allergen challenge [11]. Much remains to be discovered about the mechanisms underpinning the adaptation of immune cells like cDC2s to the many distinct anatomical locations they reside in throughout the body.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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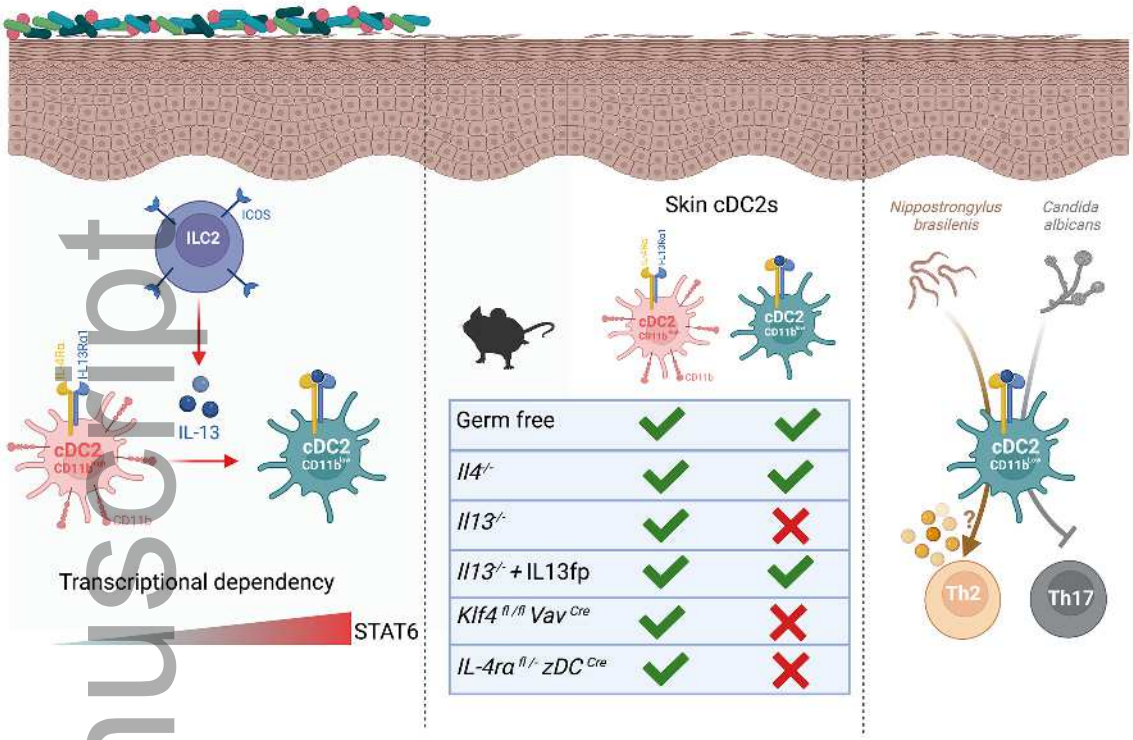
Figure Caption

Figure 1. IL-13 directs cDC2 diversity in the skin. Under homeostatic conditions, ILC2s constitutively secrete IL-13 that drives the STAT6-dependent development of CD11b^{lo} cDC2s (left). Production of IL-13 by ILC2s is independent of commensal bacteria or alarmin signals. IL-13 signalling in cDC2s promotes the differentiation of CD11b^{lo} cDC2s in a STAT6/KLF4 dependent manner (centre). Upon immune challenge of the skin CD11b^{lo} cDC2s prime Th2 immunity and directly or indirectly inhibit Th17 cell polarisation (right). This figure was created with BioRender.com.



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