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The role of innate lymphoid cells in healthy and inflamed skin

Charlotte M. Bonefeld*, Carsten Geisler

Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

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ABSTRACT

The skin constitutes the interface between the organism and the environment, and it protects the body from harmful substances in the environment via physical, chemical and immunological barriers. The immunological barrier of the skin comprises both cells from the innate and the adaptive immune system. During the last years, it has become clear that innate lymphoid cells play a role in homeostasis and inflammation of the skin in humans and mice. In this review, we will discuss the role of innate lymphoid cells in healthy and inflamed skin with special focus on their role in atopic dermatitis.

1. ILC in healthy skin

The skin can be divided into two major compartments, namely the epidermis and the dermis, separated by the basement membrane. The keratinocytes constitute 90–95% of the cells in the epidermis, and the remaining cells include Langerhans cells, skin-resident memory $CD8^+$ T cells, melanocytes and Merkel cells [1,2]. In addition, mouse epidermis contains a highly specialized γd T cell subset called dendritic epidermal T cells (DETC) [3]. A much more heterogeneous population of immunological cells reside in dermis, including dermal subsets of dendritic cells, mast cells, $CD4^+$ and $CD8^+$ T cells, γδ T cells, B cells, macrophages and natural killer (NK) cells [1,2]. Recently, it has become clear that NK cells are part of a family of innate cells termed innate lymphoid cells (ILC). The ILC lack expression of antigen-specific receptors and are activated by specific cytokines [4,5]. The ILC family can be subdivided into three groups based on their requirement for activating cytokines, expression of transcriptions factors and production of effector cytokines [4,5]. The group 1 ILC contains NK cells and ILC1 that both are activated by IL-12, dependent on T-bet and produce IFNγ. The group 2 ILC contains ILC2 that are activated by IL-25, IL-33 and TSLP, express GATA-3 and produce IL-4, IL-5 and IL-13. The group 3 ILC contains LTi and ILC3. ILC3 can be further subdivided based on their expression of natural cytotoxicity receptors (NCR). They are activated by IL-1β and IL-23, are dependent on RoRγt and produce IL-17A and/or IL-22 [4,5]. Interestingly, all of the activating cytokines can be produced by keratinocytes, Langerhans cells and/or dendritic cells in the skin [6–11]. Besides NK cells, ILC2 were the first subset of ILC to be identified in healthy skin of both human and mice [12]. Roediger et al. found that ILC are present in both the epidermis and dermis, but that the majority of ILC were found in the dermis, where ILC2 constitute 5–10% of all $CD45^+$ cells [13]. Subsequently, we and others have shown that all three subsets of ILC are present in healthy human skin (Fig. 1A) [14–16]. ILC2 seem to play an important role in immune surveillance, immune regulation and in wound healing of the skin [13,17]. Roediger et al. showed that dermal ILC2 constitutively produce IL-13 in an IL-7-dependent way [13]. By the use of intravital multiphoton microscopy they found that ILC2 can interact with mast cell, and that ILC2 through this interaction can suppress IgE-dependent cytokine release from mast cells via IL-13 (Fig. 1A). However, following IL-2 stimulation *in vivo*, dermal ILC2 rapidly start to proliferate and express higher levels of *IL13* and *IL5* turning them into inflammatory cells [13]. The role of ILC1 and ILC3 in skin homeostasis is yet to be defined, but results from the gut suggest that ILC3 might play an important role in tolerance towards commensal bacteria [18,19]. $RoR\gamma t^+$ ILC3 purified from intestinal and lymphoid tissue of healthy mice and humans express MHCII [18]. In addition, $MHCII^+RoR\gamma t^+$ ILC3 were shown to lack expression of the classical co-stimulatory molecules CD40, CD80 and CD86 indicating that these cells induce T cell anergy instead of T cell activation [18]. A role for $MHCII^+RoR\gamma t^+$ ILC3 in limiting the immune response of $CD4^+$ T cells to intestinal commensal

* Corresponding author at: Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark. Fax: +45 3532 7881.
E-mail address: cmenne@sund.ku.dk (C.M. Bonefeld).

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bacteria was further confirmed, as mice lacking MHCII-expressing ILC developed intestinal inflammation that could be reduced by treating the mice with antibiotics [18]. Interestingly, the expression of MHCII by ILC3 was found to be significantly reduced on intestinal ILC3 from pediatric patients with Crohn's disease compared to ILC3 from patients without inflammatory bowel disease [19]. We find it likely that similarly mechanisms exist within the skin, however this needs clarification (Fig. 1A).

2. ILC and wound healing

Cutaneous wound healing is a complex immunological process involving cells from both the innate and the adaptive immune response [3,20]. ILC2 isolated from healthy human skin have been shown to express higher levels of the amphiregulin gene *AREG* than ILC2 purified from blood [21]. As amphiregulin it a known regulator of wound healing, this indicates that ILC2 might play a role in cutaneous wound healing [22]. This notion was recently supported by a study which found that ILC2 probably play an important role in the healing of acute wounds [17]. In this study, an increased expression level of *IL33* was found in wounded compared to non-wounded skin of C57BL/6 mice, and IL-33KO mice had delayed wound closure underlining the important role of IL-33 in the wound healing process [17]. Furthermore, an increase number of IL-5 and IL-13-producing ILC2 were found in wounded compared to intact skin [17]. Depletion of ILC by treating *Rag1*$^{-/-}$ mice with anti-CD90.2 mAbs resulted in a delayed wound closure compared to control mice [17]. The depletion of ILC seems to delay the wound closure more than found in the IL-33KO mice indicating that other subtypes of ILC might also be involved in wound healing [17]. IL-17A has been detected in human wounds [23]. Delayed wound healing was found in *Il17a*$^{-/-}$ mice, and even though DETC were shown to be important producers of IL-17A during wound healing, the delayed wound healing seemed more delayed in *Il17a*$^{-/-}$ mice than in *Tcrd*$^{-/-}$ mice, suggesting that other IL-17A-producing cells might be involved in wound healing [24]. This could indicate that ILC3 also play an important role during wound healing. However, the role of ILC in cutaneous wound healing needs further investigations.

3. The role of ILC in atopic dermatitis

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease that results in itchy, red, swollen, and cracked skin. AD is a common disease with a lifetime prevalence of 10–20% in developed countries [25,26]. The etiopathogenesis of AD is unclear, but defects in the skin barrier and an imbalanced immune response seem to be the key drives of AD [26]. Several recent papers indicate that ILC2 might play a central role in driving the immune response during AD (Fig. 1B) [12,21,27–30]. Kim et al. provided the first evidence that ILC2 are involved in AD in both humans and mice [12]. They found an enrichment of ILC2, characterized by being Lin-CD25^{+}IL-33R^{+}, in lesional AD skin compared to healthy skin [12]. Interestingly, they found that whereas ILC2 isolated from healthy skin did not express CRTH2 and CD161, ILC2 isolated from AD skin did [12]. In contrast, although other studies have confirmed the enrichment of ILC2 in human AD lesions, they found that ILC2 isolated from both healthy and AD lesional skin expressed CRTH2 and CD161 [21,28]. However, ILC2 derived from AD skin expressed increased level of the IL-25 and IL-33 receptor subunits IL-17RB and ST2 and of the TSLP receptor [21]. Furthermore, it was found that stimulation with prostagladin D_2 (PGD_2) induced an up-regulation of IL-17RA and ST2 on ILC2 [28]. Thus, taken together these observations indicate that skin inflammation induces an altered phenotype of ILC2 making these cells more responsive to cytokines in the environment.

Salimi et al. found that stimulation of ILC2 isolated from healthy human skin with IL-33 induced production of IL-5, IL-6 and IL-13 and that this cytokine production was further increased by addition of IL-25 and TSLP. In contrast, IL-25 and TSLP by them-selves only had a minor effect on IL-5, IL-6 and IL-13 production by ILC2 [21]. In addition to cytokine activation, both PGD_2 and stimulation via NKp30 seem to be involved in the activation of ILC2 in AD skin [27,28]. Binding of PGD_2 to CRTH2 expressed on ILC2 isolated from healthy skin induced the migration of ILC2 and induced the production of IL-4, IL-5 and IL-13 [28]. Xue et al. further investigated the physiological role of PGD_2-induced ILC2 activation by stimulating these cells with supernatants from anti-IgE-activated mast cells that are major producers of PGD_2 [28]. Stimulation of skin ILC2 with supernatants from activated mast cells induced IL-4, IL-5 and IL-13 production by the ILC2 [28]. The cytokine production could be blocked by addition of a CRTH2 antagonist showing that the mast cell-induced ILC2 activation is mediated by a PGD_2-CRTH2-dependent pathway [28]. Several studies have shown that subsets of human group 1 and group 3 ILC express different members of NCR, but the role of NCR on ILC2 are poorly defined [4,5]. Interestingly, a recent study showed that NKp30 is expressed on ILC2 and that stimulation via NKp30 induces the production of IL-5 and IL-13 in ILC2 [27]. The expression level of B7-H6, which is the ligand for NKp30, is up-regulated in lesional AD skin and furthermore, the study found that the expression of B7-H6 was up-regulated on keratinocytes following stimulation with IL-4 or IL-13 [27]. Taken together, these results indicate that the effector function of ILC2 is likely to be enhanced during the inflammatory response *via* stimulation by other skin resident cells.

The role of ILC2 in AD has been further investigated by different AD mouse models [12,21,29,30]. Kim et al. found an enrichment

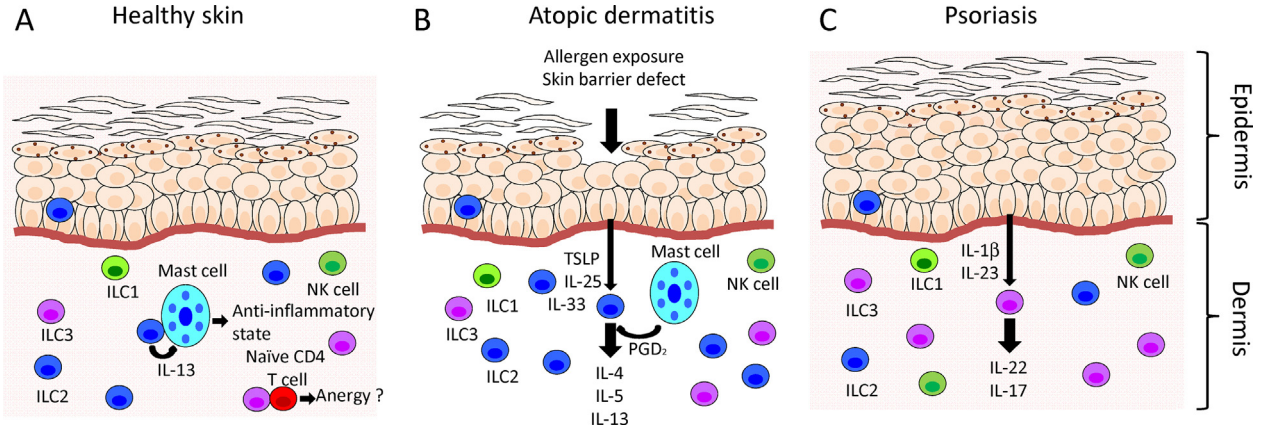

Fig. 1. Schematic presentation of the role of ILC in (A) healthy skin, (B) atopic dermatitis and (C) psoriasis.