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Immunity
Letter

Human ILC1: To Be or Not to Be

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http://dx.doi.org/10.1016/j.immuni.2017.05.001

In a recent publication, Simoni et al. (Simoni et al., 2017) extensively analyzed the phenotypic characteristics of human innate lymphoid cells (ILCs). It is now well established that there are three ILC subsets based on the cytokines they produce and transcription factors they depend on. Whereas ILC2s and ILC3s are well defined by a collection of markers, the definition of ILC1s is problematic because of the lack of a specific marker (Spits et al., 2016). Simoni et al. have used mass-cytometry (CyTOF) to simultaneously analyze 29 parameters in multiple primary healthy and pathological human samples. Visualizing cell populations by t-distributed stochastic neighbor embedding (t-SNE) analysis resulted in separate clusters of ILC2s and ILC3s, whereas ILC1s were found scattered throughout other cell populations. Moreover classical bi-axial gating of ILC1s did not clearly show enhanced levels of the type 1 transcription factor T-bet. The researchers could identify epithelium-residing ILC1s, but these cells fell within the same cluster as NK cells, raising the possibility that intra-epithelial (IE) ILC1s are a subset of NK cells. On the basis of these observations, the authors concluded that ILC1s not residing in the epithelial layers are in fact contaminating T cells, dendritic cells (DCs), hematopoietic stem cells (HSCs), ILC3s, and NK cells and that the previously described plasticity of ILC2s and ILC3s toward ILC1s should be interpreted as an artifact. These observations and the associated interpretation and conclusions triggered us to explore possible explanations for the discrepancies between this publication and previous studies on human ILC1s.

In general, ILCs are defined by the expression of the IL-7Rα (CD127) and CD161 but lack of surface markers that define T cells (CD3, TCRαβ, and TCRδ), B cells (CD19), NK cells (CD16 and CD94), myeloid cells (CD1a, CD14, and CD123), granulocytes (FcεR1α and CD123), hematopoietic stem cells (HSCs) (CD34), and plasmacytoid DCs (BDCA2 and CD123), collectively called "lineage." Within this lineage$^-$, CD127$^+$, CD161$^+$ pool of ILCs, ILC2s and ILC3s are subdivided by the surface expression of CRTH2 and c-Kit, respectively (Bernink et al., 2015). In contrast, there is currently no defining marker for ILC1s besides their expression of CD127 and CD161 and their lack of c-Kit and CRTH2.

For their analysis of ILCs by CyTOF, Simoni et al. first used magnetic bead sorting with the goal of removing T and B cells. However, this method is notoriously inadequate for achieving complete depletion. Indeed, after CyTOF acquisition the authors further enriched for ILCs by using a cocktail of antibodies to remove residual B cells and other lineage cell types, but this cocktail contained no antibodies, such as anti T cell receptor (TCR) or anti-CD34, that would remove T cells and HSCs. Hence CD127-expressing T cells and a portion of HSCs are likely to have contaminated the ILC1 population and obscured the results of the t-SNE analysis.

In the interpretation of their data, Simoni and colleagues have not taken into account that human ILC1s can express markers that are historically considered to be lineage specific. For example, two groups have reported that human peripheral-blood ILC1s are defined by the absence of cell-surface CD3, TCRαβ, and TCRγδ but express CD5 (Roan et al., 2016), a finding that has been confirmed by single-cell RNA-sequencing analysis of tonsillar ILC1s (Björklund et al., 2016) and in our lab by flow cytometry. Furthermore, ILC1s can be cloned under T-cell-promoting conditions without developing into T cells. However, Simoni et al. state that CD5$^+$ cells are T cells without performing validating functional analyses to support their statements. Similarly, the complement receptors CD11b and CD11c considered by Simoni et al. to only be expressed on DCs are in fact also expressed by ILCs (Björklund et al., 2016). Thus, given the largely unexplored protein expression profile of ILCs, it is important to realize that the grouping of cells in a certain lineage on the basis of a limited set of markers from historical classifications should be approached with caution. Similarly, manually grouping cell populations in t-SNE maps and visualizing cell populations in heat maps can largely obscure small differences between clusters that represent distinct populations.

We have demonstrated that ILC1s are particularly prominent under inflammatory conditions. ILC1s are present in tonsils, but much larger numbers have been found in inflamed intestinal tissues of patients suffering from Crohn's disease, for instance, a chronic inflammatory disease of the bowel. Some of those ILC1s are most likely derived from ILC3s under the influence of IL-12, and ILC3s possess the capacity to transdifferentiate toward IFN-γ-producing ILC1s in the presence of IL-12 and IL-18, as we have validated by proliferation assays and single-cell cloning experiments (Bernink et al., 2015; Bernink et al., 2013). Simoni et al. now consider those findings to be an artifact because, first, they fail to find ILC1s and, second, ILC3s exposed to IL-23 and IL-18 did not acquire an ILC1 phenotype. However, we also found that IL-23 and IL-18 were unable to induce transdifferentiation of ILC3s; IL-12 is here

essential. This cytokine was not used by Simoni et al. in their stimulation of ILC3s.

To address the issue of whether an alternative analysis strategy would result in a separate cluster of ILC1s, we re-analyzed Simoni's CyTOF dataset generated from tonsil and gut tissues (supplemental figure 1). In this analysis we first used bi-axial gating to identify cells that were Lin^- $CD94^-$ $CD34^-$ $CD127^+$ $CD161^+$. Although we could not exclude T cells on the basis of either CD3 or TCR$\alpha\beta$, TCR$\gamma\delta$ t-SNE mapping demonstrated a clear ILC1 cluster in addition to ILC2 and ILC3. These ILC1s expressed T-bet, albeit at lower levels than $CD94^+$ NK cells, as we have previously reported (Bernink et al., 2013). Because some of these cells co-express $CD5^+$ and T-bet, it is possible that these cells represent a distinct subset of ILC1s.

We expect that clustering of ILC1s will be more pronounced if intestinal specimens of patients with Crohn's disease were used. Because of the lack of specific markers for ILC1s, it is possible that these cells represent a heterogeneous population—rather than a homogeneous cluster—but which nevertheless contains a subset of IL-12-responsive IFN-γ-producing ILC1s that can be stably cloned and that do not differentiate to T cells or NK cells in culture, as demonstrated by multiple publications.

Furthermore, the conclusion that trans-differentiation of ILC3s into ILC1s and vice versa should be considered an artifact cannot be justified with the cytokine stimulations employed. In light of all of this, therefore, we believe that the conclusion drawn by Simoni et al. that $CD127^+$ $CD161^+$ ILC1s do not exist in humans is not sufficiently supported by their data. It is of note that Tbet is expressed on approximately 70% and 20% of gut and tonsil tissue, respectively. Because T-bet is expressed only on a fraction of human ILC1s, the identification of human ILC1s remains challenging.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2017.05.001.

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Immunity, Volume *46*

Supplemental Information

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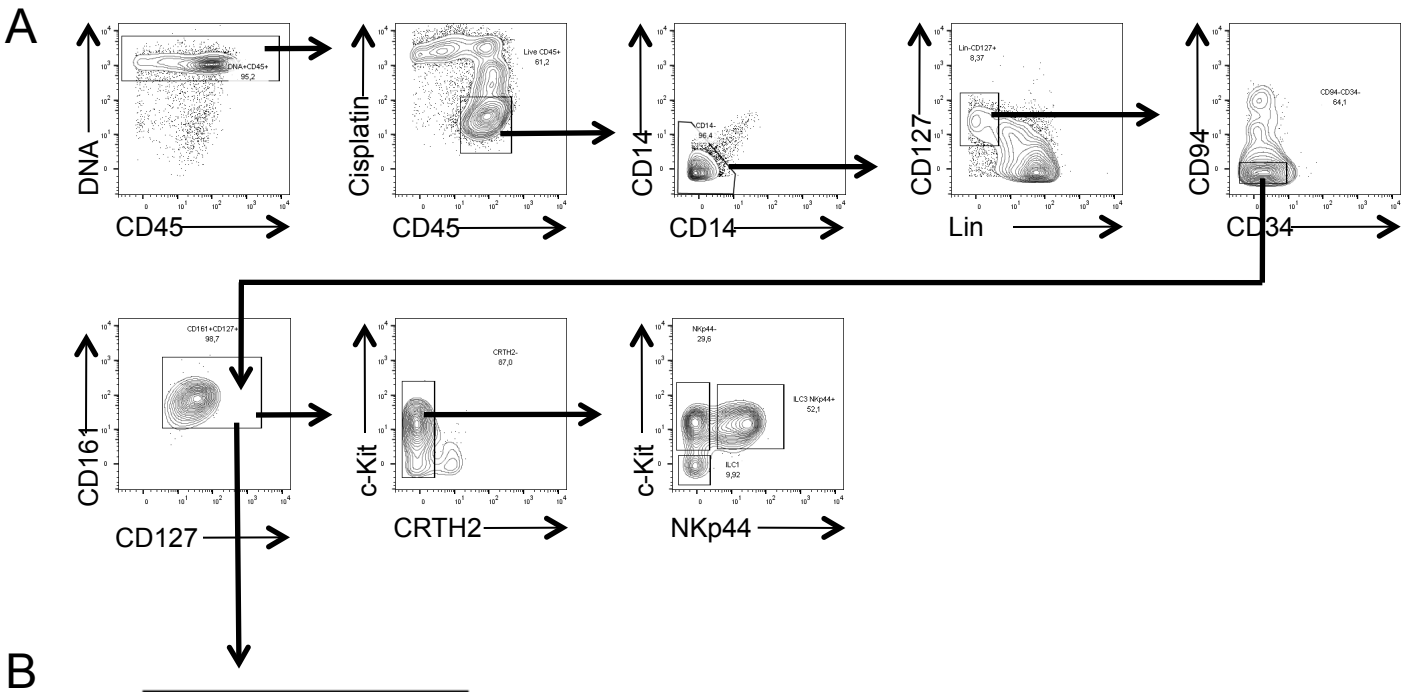
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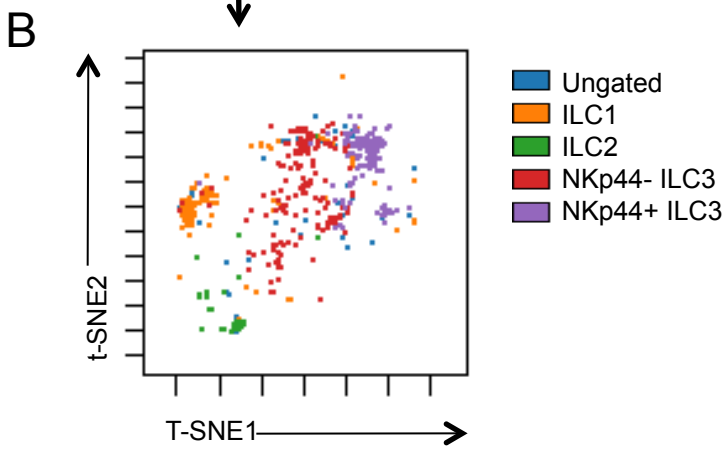
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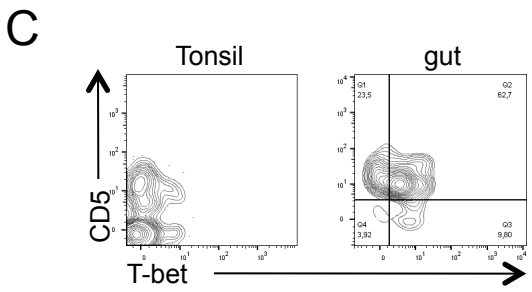
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