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Human group 1 innate lymphocytes (ILC1) are negative for surface CD3ε but express CD5

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Abstract

Simoni and colleagues recently published a mass cytometry (CyTOF)-based analysis of innate lymphoid cell (ILC) subsets across different human tissues (Simoni et al., 2017). Helper-type ILC1 cells were undetectable in these analyses, and the authors suggest that ILC1s reported in previous studies were likely attributable to T cell contamination. Although much of the heterogeneity and complexity within the ILC1 population remains to be fully delineated, we believe there is ample evidence that these cells constitute a unique population(s) clearly distinct from conventional T cells.

In the t-SNE analysis of ILC1s performed by Simoni *et al.*, the authors show that most of the cells within their ILC1 gate clustered with T cells, with small fractions clustering with dendritic cells (DC) and hematopoietic stem cells (HSC). Cells expressing FcεR1α, CD123, CD14, and CD19 were excluded from the t-SNE analysis through bi-axial gating, but HSCs ($CD34^+$), DCs ($CD11c^+$), and T cells ($CD3^+$ or TCR^+) were not. Those cellular subsets not included in the lineage gating could therefore have shown up in analyses as ILC1s, which are $CD127^+$ lymphocytes that are defined largely by the lack of expression of surface markers for other lineages. We have found that $CD4^+$ T cells (Lin^+ $CD4^+$ cells in our analyses) are 100–1000× higher in frequency than total ILCs (Lin^- $CD127^+$ cells) in the peripheral blood and in certain tissues (data not shown). Thus, even if CD3 depletion by MACS has a 99% efficiency of depletion, the frequency of T cells would likely be higher than that of total ILCs in many tissues. Because CD3 or T cell receptor (TCR) surface expression was not evaluated in the analyses by Simoni *et al.*, T cells within their ILC1 gate may have obscured the ability to detect these rare populations.

We would also caution against the use of CD5 as a marker for T lymphocytes without concurrent evaluation of CD3 or TCR expression. Although CD5 is considered primarily to be a regulator of TCR and B cell receptor (BCR) signaling (and therefore complexed to TCR or BCR), a recent study demonstrated that IL-6 can signal via CD5 (Zhang et al., 2016). The identification of functional signaling through CD5 that appears independent of its antigen

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receptor regulatory functions suggests that it could be expressed and function independently of the antigen receptor complex. Furthermore, while Vely and colleagues have shown that a vast majority of ILC1s in their analyses expressed CD5, they also have shown that patients with RAG1 deficiency, who should therefore lack T cells, have circulating ILC1s at frequencies comparable to that of ILC2s and ILC3s (Vely et al., 2016). Thus, CD5 may be inadequate when used as the sole marker for T cells.

Recently, intra-epithelial ILC1s (ieILC1s) in mouse and human intestinal tissue have also been shown to exhibit T cell-like traits not found in other innate lymphocytes despite not expressing surface T cell receptor (TCR) or CD3 (Ettersperger et al., 2016). Our data and others suggest that ILC1s may also progress farther along a T cell developmental pathway than helper-like ILC2s or ILC3s, but still maintain an innate phenotype. We previously performed a comprehensive flow cytometric analysis of human ILC subsets in adult peripheral blood (Roan et al., 2016). The ILC1 cells in our study were distinct from NK cells since they did not express the NK markers CD16, CD56, CD94, perforin, and granzyme B. A large percentage of ILC1s did express T-bet, which was not seen in putative ILC1s in the analyses by Simoni *et al.* The frequency of T-bet expression in the ILC1 population in our studies varied from around 40% to 80%; this is consistent with the study by Bernink and colleagues, which also showed a substantial percentage of ILC1s (and conventional NK cells) lacked T-bet expression by flow cytometric analysis (Bernink et al. 2013). Whether these T-bet$^-$ ILCs are phenotypically and functionally distinct from Th1-like “helper ILCs” requires further study and highlights some of the ongoing challenges of defining the ILC1 population. A high percentage of ILC1s expressed CD4 or CD8 and had TCR rearrangements (Bjorklund et al., 2016; Roan et al., 2016; data not shown). However, in contrast to conventional T cells, ILC1s expressed intracellular CD3ε but not surface TCRαβ or CD3ε (Roan et al 2016). In addition, a CD127$^+$ CD4$^+$CD3$^-$ innate lymphocyte population described by Bekiaris and colleagues was not activated *in vitro* by αCD3/αCD28 (Bekiaris et al., 2013). Although CD4$^+$, CD8$^+$ and double-negative (DN) ILC1s did not express surface TCRαβ or CD3ε, most expressed CD5 (Supplemental Figure). In the study by Simoni and colleagues, the use of CD5 with CD4 or CD8 to identify CD4$^+$ and CD8$^+$ T cells without confirming surface CD3 or TCR expression may have led to inclusion of these innate populations within their T cell gate.

While there is substantial heterogeneity within the ILC1 population which clearly complicates the phenotyping of these cells, considering these populations as simply “contamination” from other classically described subsets overlooks unique populations that may be important in human disease and homeostasis. The degree to which increased frequencies of ILC1s in diseases such as inflammatory bowel disease (IBD) represent “ex-ILC3s” or other ILC1 subsets remains to be determined (Bernink et al. 2013). It is interesting to note that chemokine receptors and activation markers expressed by ILC1s are overlapping yet distinct from ILC2s and ILC3s (and conventional T cells) (Roan et al 2016), which suggests both shared and unique regulatory mechanisms in these different cellular subsets. Understanding how these populations differ from T cells in regulation and function may yield significant insights into the mechanisms and efficacy of T cell-targeted therapy in autoimmune disease and cancer. Whether these cells are innate-like T lymphocytes or T cell-like innate lymphocytes, they are distinct from conventional T cells, MAIT cells, and CD1-

restricted T cells such as NKT cells. Within this growing spectrum of lymphocytes, important questions remain regarding the plasticity, lineage relationships, regulation, and function of these cell populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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