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Innate lymphoid cells (ILCs) are typically categorized into three subsets — ILC1, ILC2 and ILC3 — that are characterized by transcription factor and cytokine expression. Single-cell genomic analysis of ILCs in the mouse small intestinal lamina propria now shows not only that these cells are more heterogeneous than the current classification allows for but also that the commensal microbiota is required to maintain this diversity.

RNA sequencing (RNA-seq) of the three canonical subsets of ILCs (RORγt$^-$NKp46$^+$ ILC1s, RORγt$^-$KLRG1$^+$ ILC2s and RORγt$^+$ ILC3s) identified subset-specific transcriptomes involving 1,161 differentially expressed genes. Characterizing the chromatin landscape of the three ILC subsets showed that a large set of enhancers (marked by H3K4me2) are unique to each subset and probably determine these subset-specific patterns of gene expression. The presence of open chromatin regions within enhancers of the loci encoding the subset-specific transcription factors (*Tbx21*, *Gata3* and *Rorc*) correlated with the respective ILC subset (ILC1, ILC2 and ILC3, respectively).

However, this genomic analysis of bulk subsets predetermined based on marker expression is likely to miss intermediate states or diversity within subsets, so the authors extended their analysis to single-cell RNA-seq of the entire CD127$^+$ cell population from the mouse intestinal lamina propria. Clustering cells on the basis of expression level of 6,637 differentially expressed genes created 15 transcriptionally homogeneous ILC clusters. Comparing these clusters to the previously identified ILC1, ILC2 and ILC3 gene modules identified four ILC1 clusters, four ILC2 clusters and five ILC3 clusters. Two of the transcriptome-based clusters (referred to as ILCXa and ILCXb) did not clearly belong to any of the three canonical ILC subsets.

Further characterization showed a gradient of *Tbx21* expression within the ILC1 clusters with expression levels highest in the ILC1d cluster and lowest in the ILC1a cluster. The ILC1a cluster had detectable expression of *Gata3*, indicating that it might represent previously described plasticity between ILC1s and ILC2s. Similarly, there was a gradient of expression of *Gata3* decreasing from the ILC2d to ILC2a clusters. Within the ILC3 clusters, expression of the hallmark cytokine interleukin-22 was almost exclusively restricted to the ILC3c cluster, whereas MHC class II-associated genes were almost exclusively restricted to the ILC3e cluster. The ILCXa cluster had transcriptional features of both ILC1 and ILC3 subsets, which is consistent with earlier reports of ex-ILC3, ILC1-like cells. The ILCXb cluster did not have strong similarity to any of the ILC gene modules. These data illustrate a high level of compartmentalization within ILCs and provide evidence of ILC plasticity.

The authors went on to show — by administration of broad-spectrum antibiotics or in germ-free mice — that the commensal microbiota accounts for much of this ILC diversity and plasticity. Disrupting the microbiota rendered the transcriptional profile and enhancer landscape of ILC1s and ILC2s more similar to that of ILC3s in both bulk populations and at the single-cell level.

In summary, this first comprehensive description of the transcriptional and regulatory landscape of intestinal ILCs identifies high levels of diversity under conditions of homeostasis that are maintained by the commensal microbiota and that tend to an ILC3-like profile in the absence of microbial stimulation.

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ORIGINAL ARTICLE Gury-BenAri, M. *et al.* The spectrum and regulatory landscapes of intestinal innate lymphoid cells are shaped by the microbiome. *Cell* http://dx.doi.org/10.1016/j.cell.2016.07.043 (2016)