CD6 is expressed on human airway and blood innate lymphoid cells (ILCs) and is upregulated by epithelial alarmins IL-33 and TSLP

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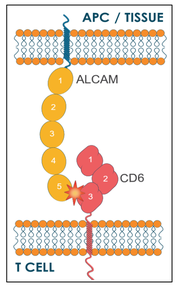
Introduction

Rationale:
Innate lymphoid cells (ILCs) are recently discovered counterparts of T helper subsets. ILC2s produce type 2 cytokines in response to epithelial alarmins IL-33 and TSLP, contribute to asthma features in animal models, and are elevated in human asthma. CD6 is a co-stimulatory receptor that contributes to pathogenic T cell responses in allergic and autoimmune inflammation. CD6 expression on ILCs has been briefly described, however no role has been elucidated.

Regulation of Adaptive Immunity by ILCs:
ILCs regulate T cells both directly through antigen presentation on MHC II, and indirectly through the regulation of DCs. The cross-talk between ILCs, DCs, and T cells establishes a complex regulatory network involving positive and negative feedbacks, the dynamics of which remain to be elucidated.[5] Furthermore, CD6 expression has not been fully characterized on all three ILC subsets.

CD6-ALCAM Pathway:

- CD6 is a co-stimulatory receptor expressed predominantly on T cells, that binds to activated leukocyte cell adhesion molecule (ALCAM), a ligand expressed on antigen presenting cells and various epithelial and endothelial tissues.
- The CD6-ALCAM pathway plays an integral role in modulating T cell activation and trafficking, making this an integral pathway in immuno-inflammation.
- The CD6-ALCAM pathway may contribute to disease pathogenesis, as increased levels of CD6 are associated with pathogenic T cell responses.



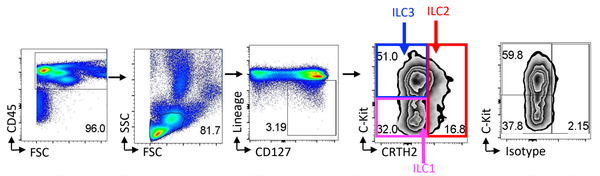
Objective:
Determine the expression of CD6 on all three human ILC subsets and identify novel aspects of CD6 regulating ILC function.

Methods

Human:
Flow cytometry for CD6 expression on human ILC subsets (ILC1, ILC2, and ILC3) was performed on human blood, lung, and nasal polyps. ILC subsets (CD45+lineage- lymphocytes) were identified as follows: ILC1s as CD127+CD117-CRTH2-; ILC2s as CD127+CRTH2+; and ILC3s as CD127+CD117+CRTH2-. RNA-seq was performed by GENEWIZ with sorted CD6+ and CD6- blood ILC subsets. Differential expression was calculated with limma package.[6] Fresh leukopaks were enriched for pan ILCs (Stemcell kit and protocol) prior to sorting. Human PBMCs were cultured with IL-33, TSLP, or both and ILC2 CD6 expression levels was assessed after 72 hours of stimulation.

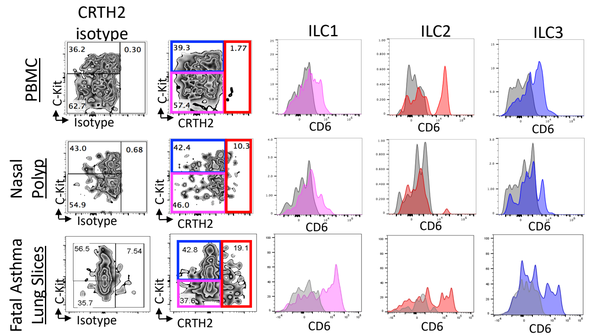
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Figure 1



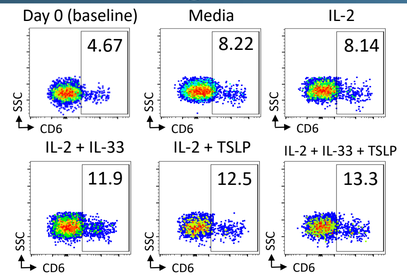
Representative Gating Scheme of all ILC three Subsets in human PBMCs. ILCs were identified as CD45+Lin-CD127+ lymphocytes. The lineage cocktail included CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD203c, and FceR1a. ILC1s were gated as c-kit-CRTH2-; ILC2s were gated as CRTH2+; ILC3s were gated as c-kit+CRTH2-.

Figure 2



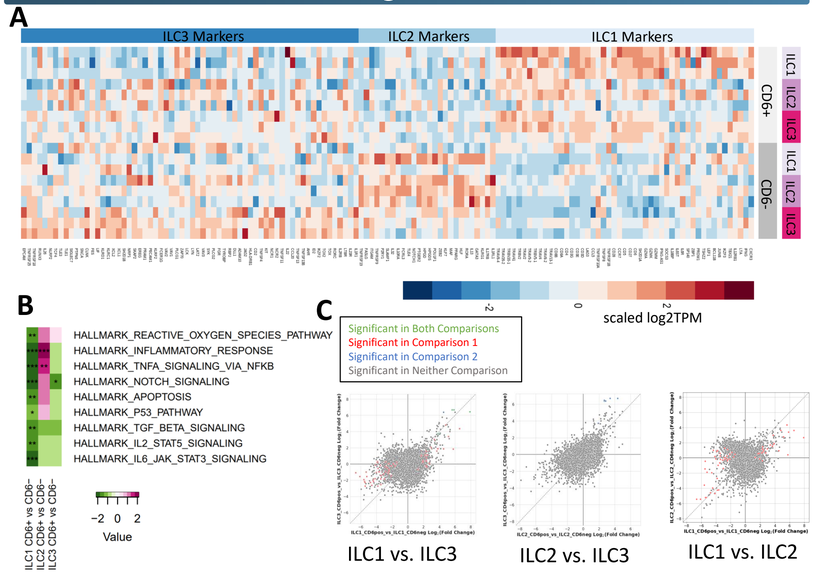
Variable levels of CD6 was expressed on all three ILC subsets from PBMCs, nasal polyps, and lung slices. PBMCs and nasal polyp tissue were collected from the same patient and processed into single-cell suspension before staining. Lung slices were collected from a donor with fatal asthma and processed into single-cell suspension for staining. CD6 expression was compared to a CD6 isotype of the same sample. Donor to donor variability has been observed.

Figure 3



CD6 expression on ILC2s increased after stimulation with IL-33, TSLP, or both IL-33 and TSLP. PBMCs were isolated from whole blood and cultured for 72 hours in media, IL-2, IL-33, TSLP, or a combination. Gated on isotype control.

Figure 4



RNA-seq analysis of CD6+ and CD6- populations of all three ILC subsets demonstrates differential expression of ILC markers. (A) Heatmap of ILC1, ILC2, and ILC3 specific markers[7] for all 6 populations collected. Across all ILC subsets, ILC1 markers were more highly expressed in the CD6+ population; ILC2 markers were more expressed in the CD6- population; ILC3 markers were more expressed in the ILC3 CD6- and CD6+ populations. (B) Heatmap of immune, apoptosis, and signaling pathway gene sets. Gene sets were oppositely regulated between ILC1s and ILC2s. (C) Contrast comparison scatter plots for all ILC subsets. ILC1 and ILC3 contrasts are the most correlated, and ILC2s are more similar to ILC3s than ILC1s.

Conclusions

CD6 is expressed on human airway ILCs and is induced on ILC2s by epithelial cytokines critical to asthma. Given the role of CD6 in driving pathogenic T cell responses, strategies targeting CD6 may be relevant to modulating both ILC- and T cell-driven responses in asthma. These findings are the first to further elucidate the role and significance of CD6 on ILCs.

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