Dissecting mechanisms of immune tolerance with single cell multiomic analysis

Researchers at the Geisel School of Medicine at Dartmouth (Lebanon, NH, USA) provide evidence that V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) serves as the earliest checkpoint regulator of T-cell tolerance. VISTA promotes T-cell quiescence and balances cell types and states within the naïve CD4$^+$ T-cell compartment. By examining changes in transcriptome, T-cell repertoire, and open chromatin, the Chromium Single Cell Immune Profiling and Chromium Single Cell ATAC Solutions helped reveal the role of VISTA in immune tolerance. MA ElTanbouly et al., *Science*. (2020).

Snapshot	10x Genomics product
Research area: Autoimmunity **Organism:** Mouse **Sample type:** Flow-sorted CD4$^+$ T cells **Research questions:** How is naïve T-cell quiescence maintained? What role does VISTA play in immune tolerance? How does VISTA influence negative checkpoint regulation of the immune system?	**Chromium Single Cell Immune Profiling Solution** • Chromium Single Cell 5′ Library and Gel Bead Kit • Chromium Single Cell V(D)J Enrichment Kit, Mouse T Cell • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines **Chromium Single Cell ATAC Solution** • Chromium Single Cell ATAC Library and Gel Bead Kit • Chromium Chip E Single Cell ATAC Kit • Chromium i7 Multiplex Kit N, Set A • Cell Ranger ATAC Analysis Pipelines

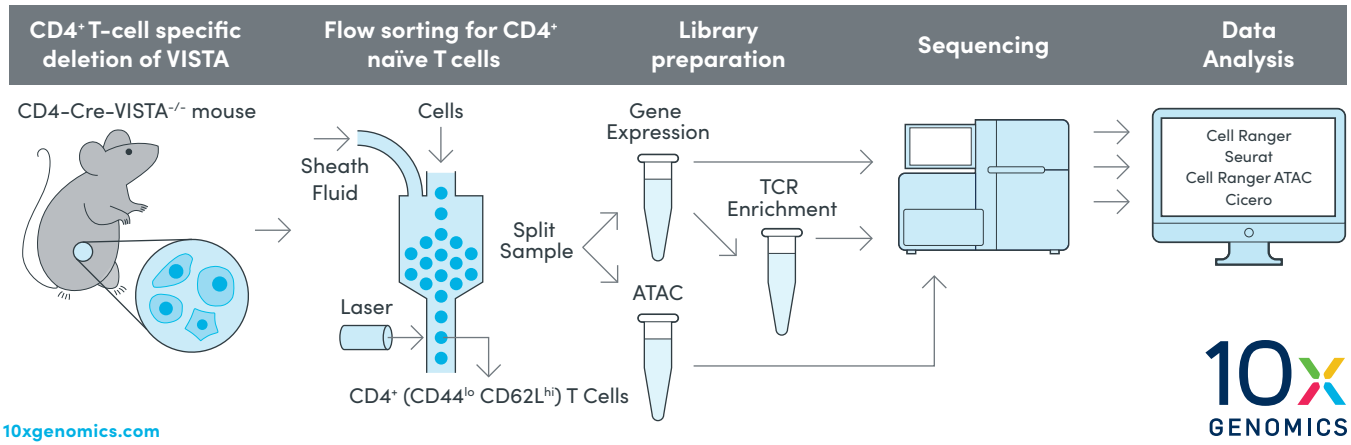
Experiment overview

Deletion or engagement of VISTA in mouse CD4$^+$ T cells

- Conditional deletion of VISTA using Cre-lox system with CD4-Cre mice
- Treatment of mice with anti-VISTA antibody antagonists and agonists

Single cell multiomics characterization of VISTA-deficient immune cells

- Flow-sorting of naïve T cells based on expression of CD4, CD44, and CD62L
- Single cell RNA-seq, sequencing of full-length, paired T-cell receptors, and single cell ATAC-seq of naïve T cells



Why single cell?

Phenotyping of naïve T cells via transcriptomics and epigenomics revealed that within a specific immune cell population, surprising cellular heterogeneity exists. Multiple T-cell populations were represented within $CD4^+$ ($CD44^{lo}$ $CD62L^{hi}$) T cells, along with unique chromatin states that depended on the presence or absence of VISTA. Loss of VISTA predisposed naïve cells to react more strongly to TCR stimulation, reducing tolerance.

Computational analysis

To ascertain the changing cell populations in mice lacking VISTA, Seurat was used to perform unsupervised clustering of single cell gene expression data. Only genes with high expression levels and genotypic variance were used to inform principal component analysis (Figure 1). If genes are too lowly expressed, their signal in a single cell is likely due more to chance than biological differences. Genes expressed similarly across samples provide little information about differences between samples.

Results

Loss of VISTA disrupts quiescence

Deletion of VISTA from $CD4^+$ T cells resulted in an altered balance of naïve T cells, with expansion of memory-like naïve T cells at the expense of quiescent T cells. Single cell ATAC data demonstrated that memory-like T cells in VISTA-deficient mice had increased accessibility for several TCR effector genes, suggesting they may be primed for enhanced T-cell activation. Comparison of TCR sequences from VISTA-deficient and wild-type mice revealed no difference in autoreactivity.

Opposing impacts on tolerance by VISTA agonism or antagonism

Engagement of VISTA with an agonistic antibody led to enhanced tolerogenic T-cell death in the absence of inflammation, thereby promoting T-cell tolerance. Likewise, VISTA antagonism blocked T-cell death in tolerogenic conditions, but had no impact under inflammatory conditions.

VISTA occupies a unique space in immune checkpoint regulation, as it is expressed exclusively on naïve T cells and exerts its action upstream of more canonical immunotherapy targets, such as CTLA-4 and PD-1, currently being used to treat cancers (Figure 2). Understanding the role VISTA plays in T-cell heterogeneity, immune tolerance, and the balance between T-cell quiescence and activation provides yet another window into the mechanisms enabling immunotherapy.

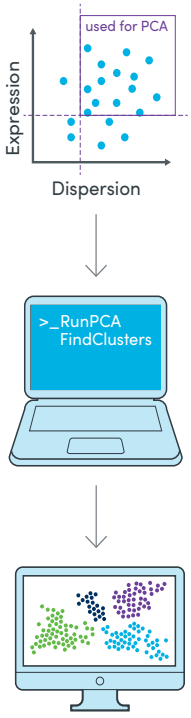


Figure 1. Steps for cluster analysis. Of all genes present in the dataset, only those with dispersion and expression values above a threshold were used for principal component analysis, which reduces the dimensionality of a complex dataset. Using the top 15 components, unsupervised clustering by the *FindClusters* function in Seurat determined cell clusters.

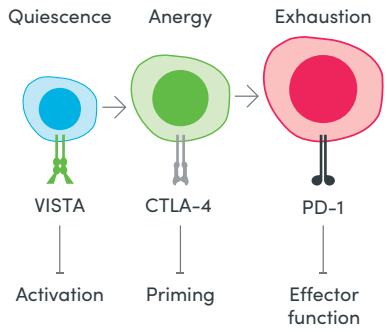


Figure 2. VISTA promotes immune regulation alongside other immune checkpoint regulators. As T cells mature, immune checkpoint regulators block immune activation at different stages. VISTA, expressed on naïve T cells, promotes quiescence. CTLA-4 is expressed on activated T cells, and blocks costimulation of T cells at the priming stage. PD-1 is expressed later and inhibits the effector function of exhausted T cells.

References

1. MA ElTanbouly et al., VISTA is a checkpoint regulator for naïve T cell quiescence and peripheral tolerance. *Science*. 367, eaay0524 (2020).

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