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Mass cytometry reveals unique subsets of T cells and lymphoid cells in nasal polyps from patients with chronic rhinosinusitis (CRS)

To the Editor:

Chronic rhinosinusitis (CRS), a heterogeneous disease characterized by chronic inflammation in the nasal cavity and sinuses, causes significant morbidity and diminished quality of life while costing the U.S. health system \$22 to 32 billion annually^{1,2}. Aberrant activation of the immune system in nasal and sinus mucosa plays a key role in the etiology and pathophysiology of CRS. Indeed, CRS is associated with increased expression of type 2 and other cytokines in the tissues, and patients have been treated successfully by targeting these molecules³. To better understand the pathophysiologic mechanisms of CRS, characterization of the immune cells that infiltrate nasal and sinus tissues is critical. While conventional tools, such as flow cytometry, have been useful for identifying specific immune cell types, the cellular complexity and functional diversity of the human immune system necessitate the use of high-dimensional tools to characterize heterogeneity of immune cells and to elucidate their roles in disease⁴. Mass cytometry, similar to flow cytometry, is suited to performing single-cell analysis. Spectral overlap between channels limits the number of parameters that can be investigated simultaneously by flow cytometry. Mass cytometry uses monoisotopic heavy metals to tag antibodies and detection by inductively coupled plasma mass spectrometry (ICP-MS)⁴. The

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availability of many different isotopes increases the multiplexing capacity of mass cytometry to, theoretically, 135 separate channels. While mass cytometry supports traditional manual gating, the volume and multi-dimensionality of the data acquired necessitate development of algorithmic analyses that cluster cells by common expression profiles and enable discovery of previously unknown cell populations. This is likely to become a great advantage when analyzing tissue resident cells, as unexpected cell population may be generated in response to the tissue environment. Thus, while mass cytometry is slower than flow cytometry and is unsuitable for cell sorting, it is comparable to flow cytometry in characterizing known cell types and is more robust for discovering new cell types. Here, we performed a proof of concept pilot study applying the mass cytometry technology to CRS research and characterized the T cell and lymphoid cell populations infiltrating into nasal polyps.

Nasal polyps from 18 subjects with CRS with nasal polyps (CRSwNP) and control sphenoid mucosal tissues from 4 subjects without CRS were obtained (see Supplemental Table E1 for subject demographics). Peripheral blood mononuclear cells (PBMCs) from healthy individuals without sinus disease provided another layer of controls. Tissue cells and PBMCs were stained with selected fluorescent-labeled antibodies or with the mass cytometry metal-labeled T cell panel (Supplemental Table E2). We first compared the performance of both methods using conventional biaxial plots (Supplemental Figure E1). The ratio of CD4⁺ to CD8⁺ cells (Figure 1A) and proportions of CD4⁺, CD8⁺, double-negative (DN), and double-positive (DP) cells within the CD3⁺ T cell compartment (Figure 1B) were similar within each cellular source whether analyzed by flow cytometry or by mass cytometry. Indeed, CD3⁺CD4⁺ and CD3⁺CD8⁺ frequencies were not statistically different based on cytometry methods used ($p>0.05$ for both CD4⁺ and CD8⁺ cells in PBMC, control tissue and nasal polyp specimens).

Tissue resident memory T (T_{RM}) cells, which highly express the activation marker and adhesion molecule CD69, likely play a pivotal role in pathophysiology of mucosal organs in immune-mediated diseases⁵. Conventional flow cytometry revealed a significantly greater proportion of CD4⁺ cells co-expressing CD69 in nasal polyps compared to control sinus tissues (Figure 1C, $p<0.05$); few CD4⁺ T cells in PBMCs expressed CD69. Mass cytometry analysis showed comparable findings. Within each tissue source, the frequency of CD4⁺CD69⁺ cells was not significantly different between mass and flow cytometry analysis ($p=0.1560$, 0.6956 and 0.1713 , respectively, for nasal polyps,

control tissues and PBMCs). Furthermore, by mass cytometry, both nasal polyps and control tissues contained similar proportions of CD69⁺ CD8⁺ T cells (Figure 1D). Overall, by using typical cell surface markers for T cells, such as CD4, CD8 and CD69, mass cytometry analysis roughly corroborated flow cytometry findings.

To examine T cell subpopulations in nasal polyps more deeply, we analyzed mass cytometry data using supervised and unsupervised data clustering methods via the Astrolabe platform⁶. Supervised clustering utilized the Human ImmunoPhenotyping Consortium hierarchical guidelines⁷ adapted for the conventional markers included in our T cell panel (Supplemental Table E2). Our panel allowed differentiation of 11 T cell subsets (Supplemental Table E3 and Supplemental Figure E2). Supervised clustering identified several significant differences in subset frequencies among nasal polyps, control sinus tissues and PBMCs. For example, both types of tissues contained significantly lower frequencies of naïve CD4⁺ T cells compared to PBMCs (Figure 1E, $p < 0.01$). Similarly, naïve CD8⁺ T cells were significantly less frequent in nasal polyps compared to PBMCs ($p < 0.05$) or control sinus tissues ($p < 0.01$). In contrast, the proportion of CD8⁺ central memory T (T_{CM}) cells was significantly greater in nasal polyps than in control nasal tissue (Figure 1F, $p < 0.01$). Other canonical T cell subsets were not notably different between the two sources of sinus tissues (data not shown).

We next analyzed the mass cytometry data using two methods for unsupervised clustering. A multidimensional scaling (MDS) map of 21 T cell panel markers (Supplemental Table E2) revealed over 77 different cell subsets, including 27 subsets in the CD4⁺ T cell compartment and 23 subsets in the CD8⁺ T cell compartment (Figure 2A). Examples from a nasal polyp and a control sinus tissue are provided in Supplemental Figure E3. Comparison of multiple nasal polyps and control sinus tissues revealed two notable differences. First, naïve CD8⁺ T cells clustered into 5 subgroups based on CD127 (IL-7R), CXCR3, and CD161 expression (Figure 2A and 2B). Of these 5 subgroups, 4 were found in higher proportions in control nasal tissue than in nasal polyp tissues. One of those four, namely CD127^{hi}CXCR3^{hi}CD161^{lo} naïve CD8⁺ cells, was significantly more prevalent in control sinus tissue than nasal polyps ($p < 0.01$). Second, a subset of CD4⁺ T_{CM} cells, namely CD161^{hi}CXCR3^{lo}CD127^{lo} CD4⁺ T_{CM} cells, was present in 5 of 6 nasal polyp tissues, but was nearly absent in control sinus tissues (Figure 2A and 2C and Supplemental Figure E3). This subset of CD4⁺ T_{CM} cells expressed CCR7, CD28, CD45RO, CD69 and PD-1 but not CXCR5 or CD103

(Supplemental Figure E4A). Because antigen-specific CD161⁺ CD4⁺ T cells vigorously produce IL-5 and IL-13 in response to allergen exposure⁸, this CD4⁺ T_{CM} subset may play a role in the pathophysiology of CRS and warrants further investigation in the future.

We used viSNE, an unsupervised algorithm that generates a 2-dimensional map of multi-dimensional data,⁹ to visualize mass cytometry T cell (CD45⁺CD3⁺CD19⁻ cells) data (Figure 2D). Interestingly, a CD161^{hi}CXCR3^{lo} CD4⁺ T cell population (red box) similar to the CD161^{hi}CXCR3^{lo}CD127^{lo} CD4⁺ T_{CM} cells identified by the MDS map was detected via viSNE. By viSNE, the CD161^{hi}CXCR3^{lo} CD4⁺ population highly expressed CD69 and CD45RO but lacked CD127 or CD103, and was more frequent in nasal polyps than in control sinus tissues (Figure 2E, $p < 0.05$). Heat maps showed comparable molecular expression between CD161^{hi}CXCR3^{lo}CD127^{lo} CD4⁺ T_{CM} cells and CD161^{hi}CXCR3^{lo} CD4⁺ T cells identified by MDS map and viSNE, respectively (Supplemental Figure E4A and E4B). No other apparent differences in the CD4⁺ T cell subsets were detected between nasal polyps and control sinus tissues.

Finally, viSNE analysis of non-B, non-T cells (CD45⁺CD3⁻CD19⁻ cells) revealed a distinct cell population found in nasal polyps, but not in control sinus tissues (Figure 2F, red circle). This innate cell population was positive for CD45RO, CD25, CCR2, and CD69 and expressed low levels of CD127 and ST2 (the IL-33 receptor). Closer examination revealed variable expression of CD25, CCR2, ST2 and CD127 within the population, suggesting that it may consist of several similar but heterogeneous cell types, including group 2 innate lymphoid cells (ILC2s)¹⁰. Further investigation, such as additional cell surface markers, gene expression and functional assays, will be necessary to elucidate the heterogeneity of this innate cell population and corresponding roles in nasal polyp pathobiology.

In summary, our study demonstrates that mass cytometry is comparable to flow cytometry for routine analysis while providing a robust capacity for identifying unique immune cell subsets in mucosal tissues through its high-dimensional resolution. However, several limitations must be considered. First, this study is preliminary due to its small sample size. As it was underpowered, likely several populations differentially represented in nasal polyps versus control sinus tissues were missed. Second, in-depth analysis of cytokine and transcription factor expression and biologic functions of novel subsets, including naïve CD8⁺ cells, CD161^{hi}CD4⁺ T_{CM} cells, and ILC2-like cells,

are warranted. Finally, it will be critical to compare nasal polyps from different CRS endotypes, such as aspirin-exacerbated respiratory disease, to fully decipher the T cell and lymphoid cell involvement in the disease process. Further studies using mass cytometry technology will provide an opportunity to make major progress in clinical studies in CRS and related disorders.

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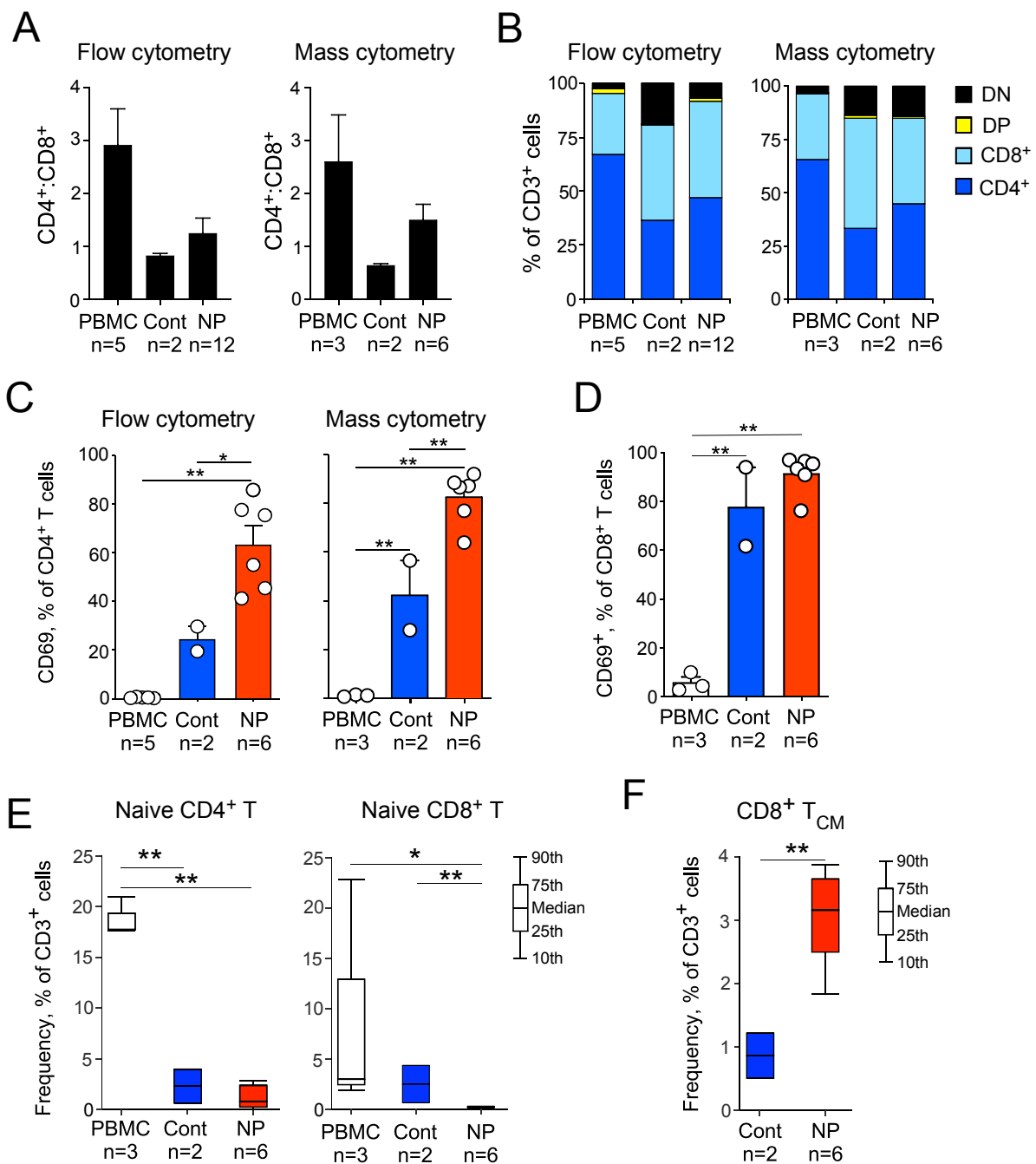
This study was made possible through support of the Immune Monitoring Core, Mayo Clinic. The authors thank Kevin Brown, Ph.D., Fluidigm Corporation and El-ad David Amir, Ph.D., Astrolabe Diagnostics, for their expert assistance in panel design and data analysis and interpretation. This work was supported by grants from the National Institutes of Health (R01 HL117823, R37 AI71106) and the Mayo Foundation. Dr. Bartemes has nothing to disclose. Dr. Choby has nothing to disclose. Dr. O'Brien has nothing to disclose. Dr. Stokken has nothing to disclose. Dr. Pavelko has nothing to disclose. Dr. Kita reports grants from NIAID and a grant from NHLBI, during the conduct of the study.

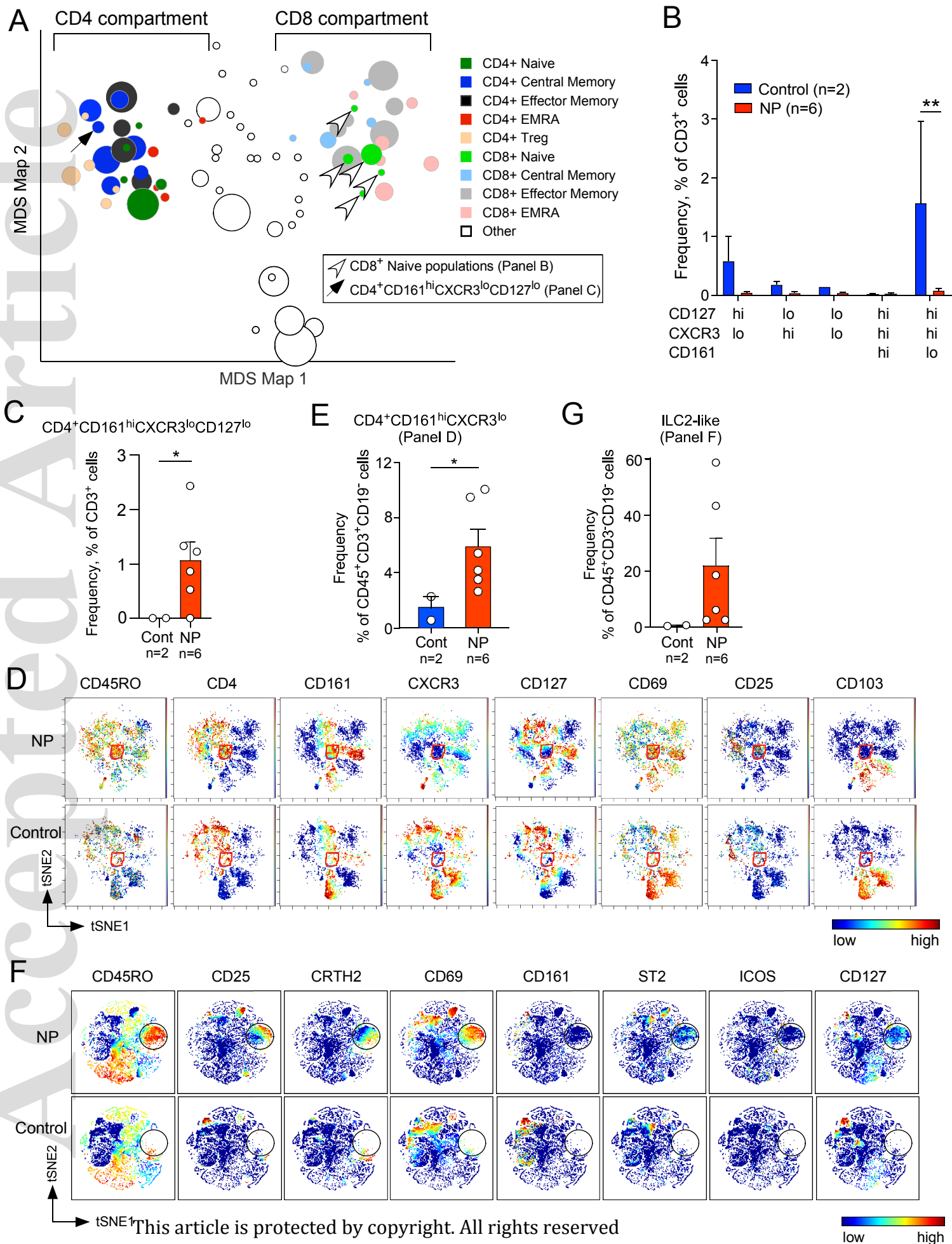
FIGURE LEGENDS

Figure 1. Flow cytometry and mass cytometry identify similar T cell subpopulations. (A) PBMCs and cells from control nasal tissues (Cont) and nasal polyps (NP) were stained for CD3⁺ T cell subsets using flow (left) or mass (right) cytometry. Ratios of CD4⁺ to CD8⁺ cell frequencies within the CD3⁺ T cell population are shown as means $\pm$ SEMs. (B) CD3⁺ cells were analyzed biaxially for CD4⁺ and CD8⁺ expression. Flow (left) and mass (right) cytometry data are depicted as stacked means of CD4⁺CD8⁻ (double negative, DN), CD4⁺CD8⁺ (double positive, DP), CD4⁻CD8⁺ (CD8⁺) and CD4⁺CD8⁻ (CD4⁺). For A and B, Flow cytometry, n=5 PBMC, 2 Cont, and 12 NP; Mass cytometry, n=3 PBMC, 2 Cont, and 6 NP. (C) Proportions of CD69⁺ cells within CD4⁺ T cells determined by flow (left) and mass (right) cytometry are shown as means $\pm$ SEMs. Circles represent individual participants. (D) Proportions of CD69⁺ cells within CD8⁺ T cells determined by mass cytometry are shown as means $\pm$ SEMs. Circles represent individual participants. (E and F) CD45⁺ leukocytes from mass cytometry data were clustered based on expression of canonical markers (see Supplemental Table E3 and Supplemental Figure E2). Frequency of naïve CD4⁺ and CD8⁺ T cells (Panel E) and CD8⁺ central memory T (T_{CM}) cells (Panel F) within the CD3⁺ population are shown as box and whisker plots. For C-F, Flow cytometry, n=5 PBMC, 2 Cont and 6 NP; Mass cytometry, n=3 PBMC, 2 Cont, and 6 NP. *, p<0.05; **, p<0.01 between groups indicated by horizontal lines.

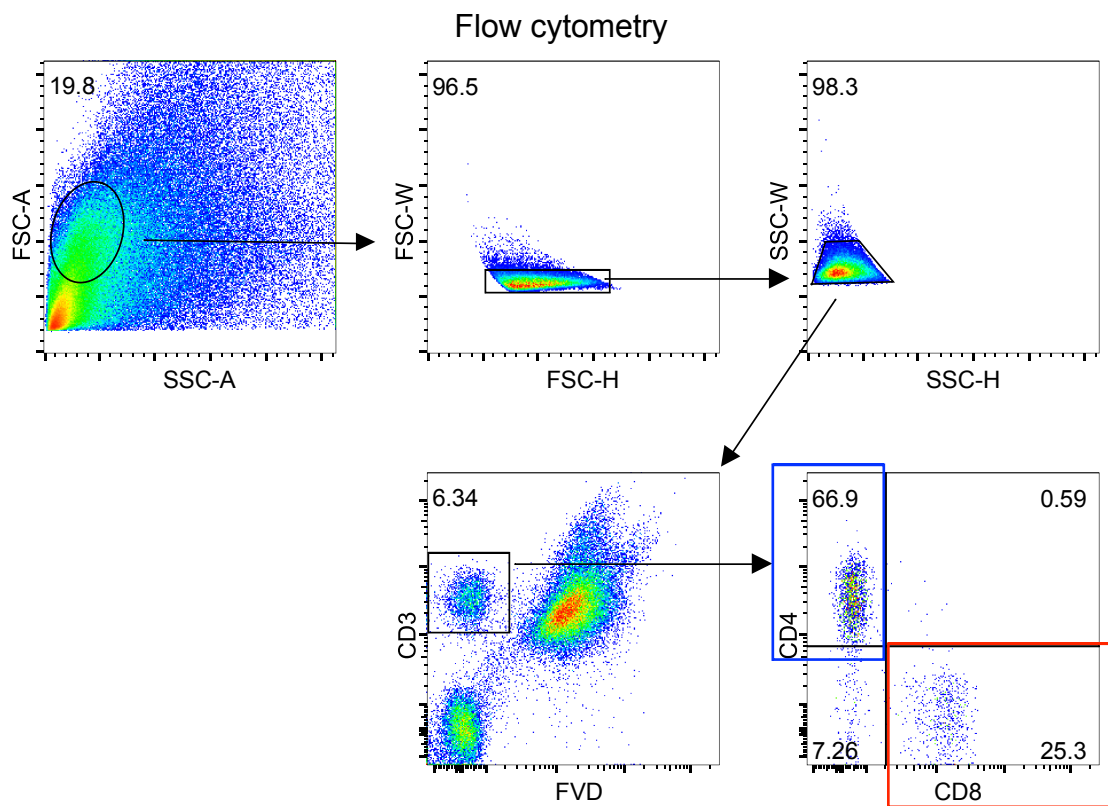
Figure 2. Identification of unique T cell and lymphoid cell populations in nasal polyps by mass cytometry. (A) MDS map of CD45⁺ leukocytes in nasal polyps after unsupervised clustering based on expression of surface markers. (B) Frequency of naïve CD8⁺ T cell subpopulations within CD3⁺ T cells in nasal polyps (NP) and control nasal tissues (Cont) is presented. (C) Frequency of the CD161^{hi}CXCR3^{lo}CD127^{lo} CD4⁺ T_{CM} population within CD3⁺ T cells in nasal polyps (NP) and control nasal tissues (Cont) is presented. (D) viSNE mapping revealed clusters of T cell populations in CD45⁺CD3⁺CD19⁻ cells; representative nasal polyp (top) and control sinus tissue (bottom) are presented. Colors indicate marker expression levels as shown by the color key. Boxed cell population is unique to nasal polyps and minimally represented in control sinus tissues. (E) Frequency of a CD4⁺CD161^{hi}CXCR3^{lo} cell population (Panel D, boxed population) within CD45⁺CD3⁺CD19⁻ cells is shown. (F) viSNE mapping of CD45⁺CD3⁻CD19⁻ cells revealed a cluster of unique lymphoid cells

(red circles) in nasal polyps but not in control sinus tissues; representative nasal polyp (top) and control sinus tissue (bottom) maps are presented. (G) Frequency of ILC2-like CD25⁺CRTH2⁺CD69⁺ cells (Panel F, circled population) within the CD45⁺CD3⁻CD19⁻ cell population is shown. Data in Panels B, C, E, and G are shown as means $\pm$ SEMs, n=6 NP and n=2 Cont. *, p<0.05; **, p<0.01 between groups indicated by horizontal lines. In Panels C, E, and G, each circle represents an individual subject.

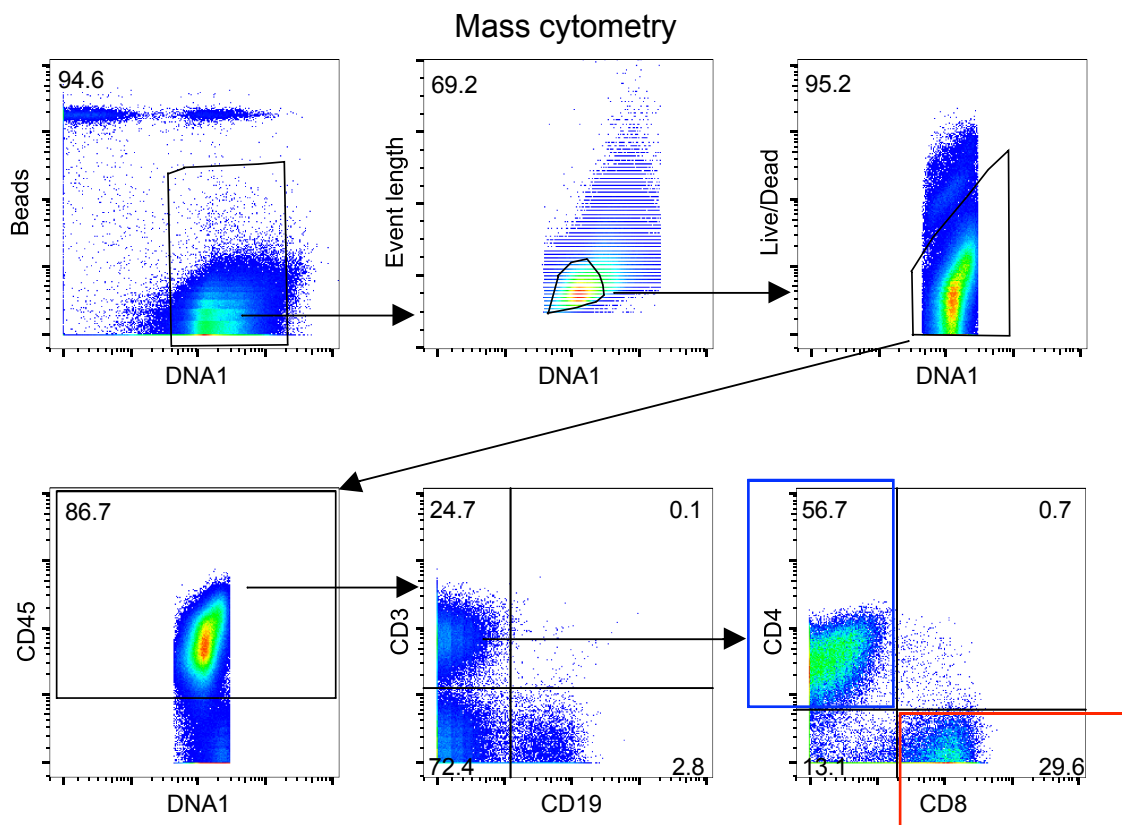


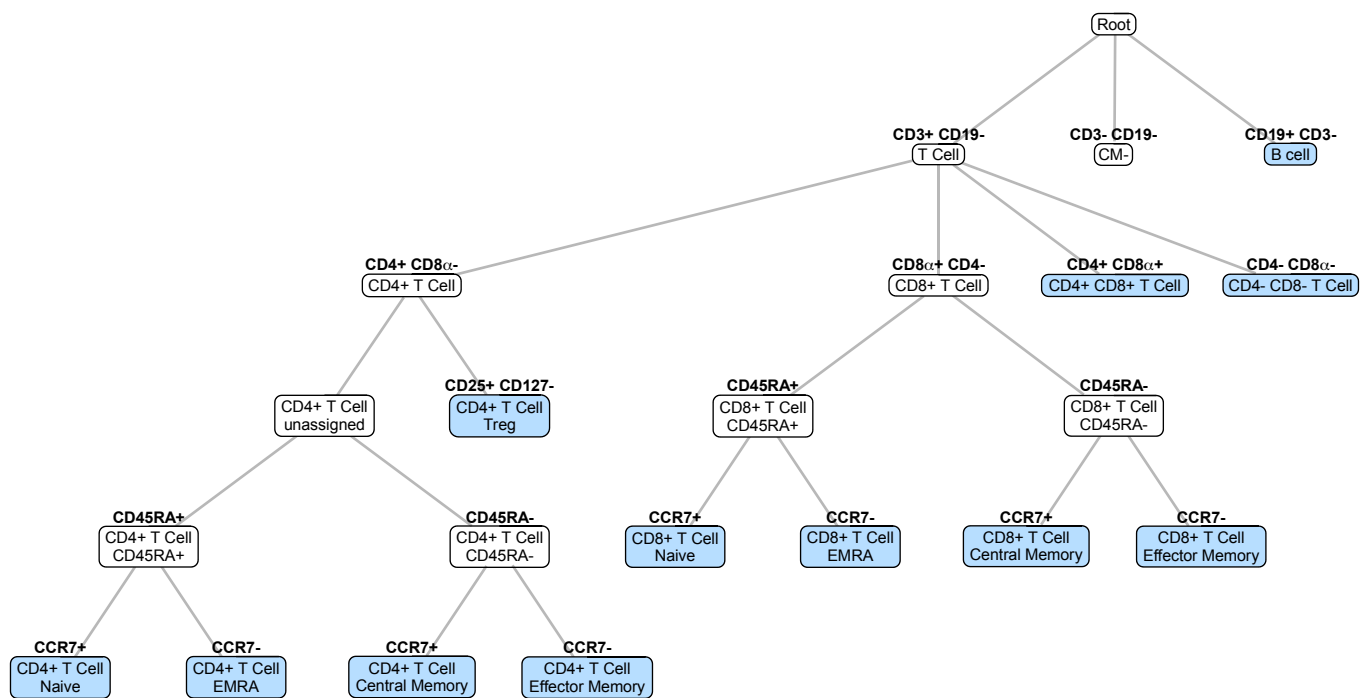


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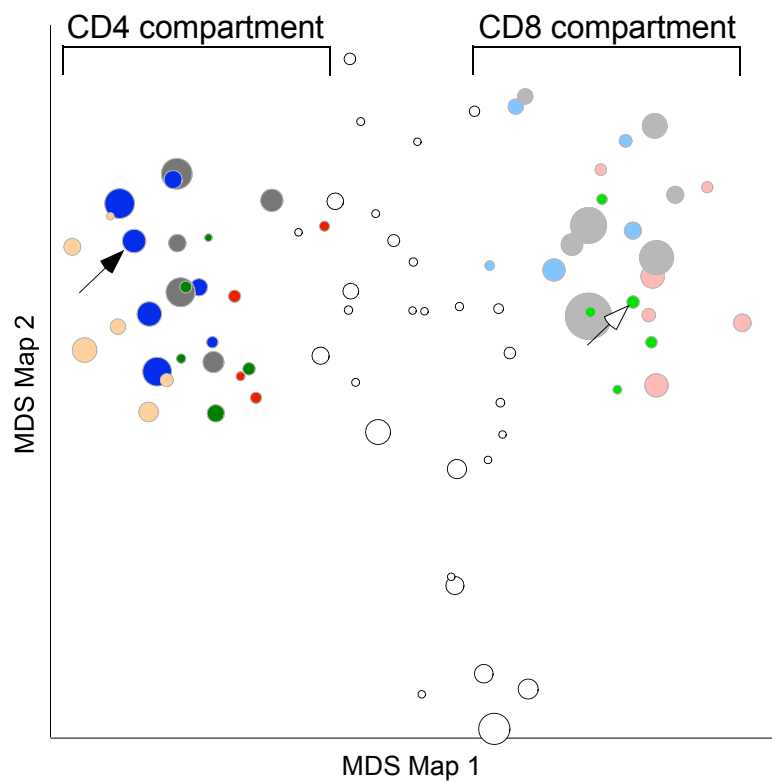
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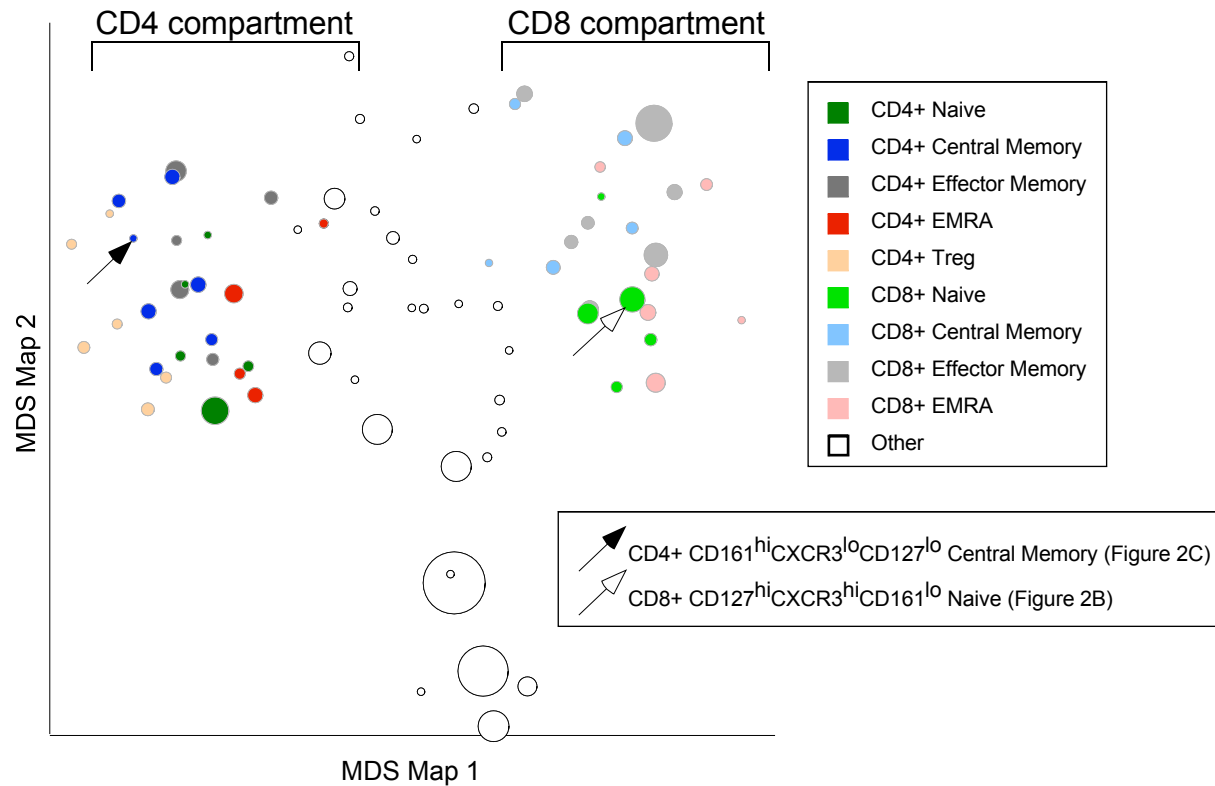


Supplemental Figure E2

NP15

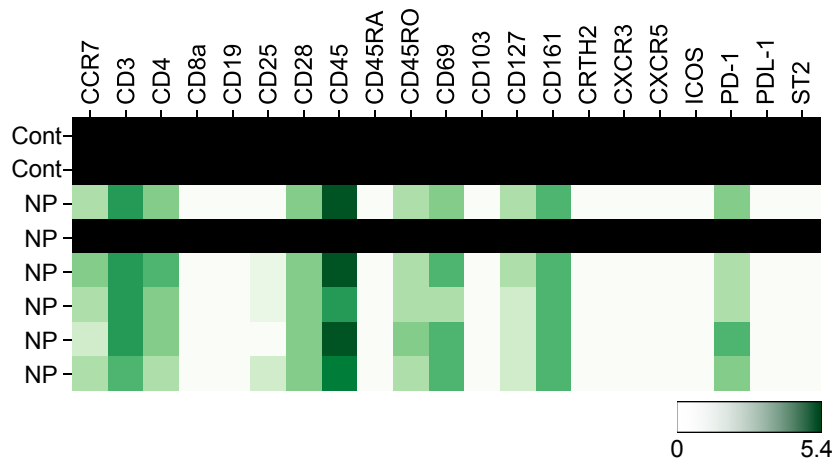


Cont4

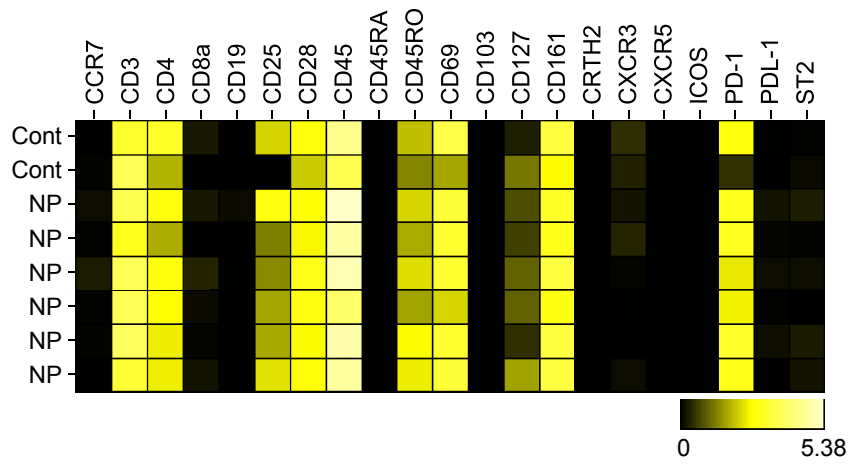


Supplemental Figure E3

A



B



Supplemental Figure E4

ONLINE REPOSITORY

Mass cytometry reveals unique subsets of T cells and lymphoid cells in nasal polyps from patients with chronic rhinosinusitis (CRS)

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METHODS

Study Subjects

Bilateral nasal polyp tissue was collected at the time of endonasal sinus surgery from 18 subjects with chronic rhinosinusitis with nasal polyps (CRSwNP). Diagnosis was made based on the American Academy of Otolaryngology – Head and Neck Surgery Adult Sinusitis Guidelines^{E1}. All patients had undergone appropriate medical therapy according to the American Rhinologic Society International Consensus Statement on Allergy and Rhinology^{E2} prior to being considered for surgical therapy. Individual demographic details are provided in Supplemental Table E1. Control nasal tissues were obtained from the sphenoid mucosa of 4 participants without a history of CRS who were undergoing trans-sphenoidal pituitary surgery. Peripheral blood samples were collected from 7 healthy participants without sinus disease. The Mayo Clinic Institutional Review Board approved this study and written informed consent was obtained from all participants. Staining methods and panels of antibodies that were used for each sample are provided in Supplemental Table E1.

Sample Preparation

To obtain single-cell suspensions from nasal polyps or nasal tissues, tissues were homogenized in RPMI 1640 (ThermoFisher Scientific, Waltham, MA) containing collagenase and thermolysin (Liberase TL; Roche Diagnostics, Indianapolis, IN) and DNase I (StemCell Technologies, Vancouver, BC) using a gentleMACS dissociator (Miltenyi Biotec, San Diego, CA) as described previously^{E3}. Erythrocytes were lysed with an ammonium chloride/potassium (ACK) lysis buffer prior to staining.

To obtain peripheral blood mononuclear cells (PBMCs), heparinized peripheral blood was layered over an equal volume of Histopaque 1077 (Sigma-Aldrich; St Louis, Mo) in SepMate PBMC isolation tubes (StemCell Technologies) and centrifuged per the manufacturer's instructions. The plasma layer was removed by aspiration and PBMCs were poured out and washed with phosphate buffered saline (PBS). Residual erythrocytes were lysed with ACK prior to staining.

Flow cytometry

To determine CD3⁺ T cell subsets, nasal polyp cells, nasal tissue cells and PBMCs (see Supplemental Table E1 for the samples used) were stained with varying combinations of FITC-conjugated anti-CD8 (SK1), PE-conjugated anti-CD4 (RPA-T4) (both from BD Biosciences, San Jose, California, USA), AF700-conjugated anti-CD3 (HIT3a), FITC-conjugated anti-CD4 (RPA-T4), BV421-conjugated anti-CD4 (RPA-T4), and BV510-conjugated anti-CD69 (FN50) (all from BioLegend, San Diego, California, USA). Appropriate isotype control antibodies were purchased from BioLegend, BD Biosciences and eBioscience and used in conjunction with FMO (fluorescence minus one) for gating purposes. Dead cells were excluded by uptake of a fixable viability dye, eFluor-780 (eBioscience). Data were acquired on a FACSCanto X (BD Biosciences) and analyzed using FlowJo (BD Biosciences).

Mass Cytometry

Cells from nasal polyps and nasal tissues and PBMCs from peripheral blood (see Supplemental Table E1 for the samples used) were stained with cisplatin (Fluidigm, South San Francisco, California, USA), washed and resuspended in Cell Staining Buffer (Fluidigm). Cells

were incubated with an antibody cocktail targeted to 21 surface molecules (see Supplemental Table E2) for 30 minutes at room temperature, washed and fixed with 1.6% formaldehyde (Pierce, ThermoFisher Scientific) for 10 minutes at room temperature. Cells were washed, resuspended in Maxpar Fix and Perm buffer containing Intercalator Ir (both from Fluidigm) and incubated for 1 hour at room temperature. Cells were washed and stored at -80°C in RPMI 1640 supplemented with 10% fetal bovine serum and 10% DMSO. Prior to acquisition all samples were spiked with EQ Four Element Calibration Beads containing known concentrations of the metal isotopes 140/142Ce, 151/153Eu, 165Ho and 175/176Lu. These lot-specific beads were used to normalize data across the mass range of the time-of-flight detector. The mathematical correction algorithm in the CyTOF software normalizes the signal variation in all channels that may occur in the mass cytometer over time. Furthermore, a standardized reference sample was tested on each day of operation to ensure the comparability of data that is acquired over multiple days. Data was acquired on a Helios workstation (Fluidigm). Biaxial analyses were performed using FlowJo (BD) and Cytobank (Cytobank, Inc., Santa Clara, California). Cytobank was also used to perform viSNE, an unsupervised single-cell cluster analysis tool that uses a t-Distributed Stochastic Neighbor Embedding (tSNE) algorithm to generate a 2-dimensional map of multi-dimensional data^{E4}.

For analyses performed by Astrolabe, single-cell data was clustered using the FlowSOM R package^{E5} and labeled using the Ek'Balam algorithm^{E6}. Cell subset definitions follow Maecker et al.^{E7} and Finak et al.^{E8}. Cluster labeling, method implementation, and visualization were done through the Astrolabe Cytometry Platform (Astrolabe Diagnostics, Inc.). The MDS map was generated using the cmdscale R function^{E9}. Differential abundance analysis was done using the edgeR R package^{E10, E11} following the method outlined in Lun et al.^{E12}. Differential expression

analysis was done using the limma R package^{E13} following the method outlined in Weber et al^{E14}.

Statistical analyses

Data are presented as the means $\pm$ SEMs for the number of subjects indicated. Statistical analyses were performed with Prism 8 software (GraphPad Software, San Diego, CA) using unpaired Student t tests or 1-way ANOVA, as appropriate for each set of experimental conditions. $P < .05$ was considered statistically significant.

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Table E1. Subject Demographics

<u>Group</u>	<u>ID</u>	<u>Age</u>	<u>Sex</u>	<u>Atopy</u>	<u>Asthma</u>	<u>AERD</u>	<u>Blood eosinophils</u> [†]	<u>Topical corticosteroids</u>	<u>Oral corticosteroids</u> [‡]	<u>Panel</u> [§]
Nasal polyp	1	50	F	No	No	No	0.82	budesonide	prednisone	A
	2	68	M	Yes	Yes	Yes	0.52	budesonide	triamcinolone	A
	3	28	M	Yes	Yes	No	0.41	budesonide	prednisone	A
	4	61	M	Yes	Yes	No	2.14	budesonide	none	A
	5	33	M	Yes	No	No	0.52	budesonide	prednisone	A
	6	79	M	No	Yes	No	0.42	nasacort, budesonide	prednisone	A
	7	61	F	No	Yes	No	0.15	flonase	none	A
	8	22	M	No	No	No	0.19	budesonide	prednisone	A
	9	51	F	No	Yes	Yes	0.66	budesonide	prednisone	A
	10	68	M	No	No	No	0.25	qnasl	prednisone	A
	11	42	F	Yes	Yes	No	0.27	budesonide	none	A
	12	65	F	No	Yes	No	0.45	nasonex	none	A
	13	56	F	Yes	Yes	Yes	0.59	budesonide	none	B
	14	60	M	Yes	Yes	Yes	0.16	budesonide	prednisone [¶]	B
	15	66	F	No	No	No	0.49	budesonide	none	B
	16	28	F	Yes	Yes	Yes	0.72	budesonide	none	B
	17	62	M	Yes	Yes	Yes	0.73	budesonide	none	B
	18	67	M	Yes	No	No	0.13	budesonide	none	B
Control tissue	1	28	F	No	No	No	NA	none	none	A
	2	53	F	No	No	No	NA	none	none	A
	3	55	M	No	No	No	0.22	none	none	B
	4	27	M	Yes	No	No	0.18	flonase	none	B
PBMC	1	61	F	Yes	No	No	NA	NA	NA	A
	2	46	M	Yes	No	No	NA	NA	NA	A,B
	3	48	F	Yes	Yes	No	NA	NA	NA	A
	4	55	F	No	No	No	NA	NA	NA	A
	5	63	F	No	No	No	NA	NA	NA	A
	6	63	F	No	No	No	NA	NA	NA	B
	7	63	F	No	No	No	NA	NA	NA	B

AERD: Aspirin-exacerbated respiratory disease; PBMC: peripheral blood mononuclear cell; NA: not available

[†]highest recent eosinophil count, ($\times 10^9/\text{L}$)

[‡] recent use

[§] Panel A: fluorescent-labeled antibodies, analyzed by flow cytometry; Panel B: metal-labeled antibodies, analyzed by mass cytometry

[¶] 40 mg x 4 days prior to surgery

Table E2. Mass cytometry T cell panel

Metal	Target	Clone	Supplier
089Y	CD45	HI30	Fluidigm
141Pr	CD3	UCHT1	Fluidigm
144Nd	CD69	FN50	Fluidigm
145Nd	CD4	RPA-T4	Fluidigm
146Nd	CD8a	RPA-T8	Fluidigm
148Nd	CD19	HIB19	Biolegend
149Sm	CD25 (IL-2RA)	2A3	Fluidigm
151Eu	CD103	Ber-ACT8	Fluidigm
153Eu	CD45RA	HI100	Fluidigm
155Gd	CD279 (PD-1)	EH12.2H7	Fluidigm
156Gd	CD183 (CXCR3)	G025H7	Fluidigm
159Tb	CD197 (CCR7)	G043H7	Fluidigm
160Gd	CD28	CD28.2	Fluidigm
163Dy	CD294 (CRTH2)	BM16	Fluidigm
165Ho	CD45RO	UCHL1	Fluidigm
167Er	CD161	HP-3G10	Biolegend
168Er	ST2	polyclonal goat	R&D Systems
170Er	CD278 (ICOS)	C398.4A	R&D Systems
171Yb	CD185 (CXCR5)	51505	Fluidigm
175Lu	CD274 (PD-L1)	29E.2A3	Fluidigm
176Yb	CD127 (IL-7R)	A019D5	Fluidigm

Table E3. Canonical marker expression strategy for T cell subset assignment

<u>Compartment</u>	<u>Cell subset</u>	<u>CD3</u>	<u>CD19</u>	<u>CD4</u>	<u>CD8α</u>	<u>CD45RA</u>	<u>CCR7</u>	<u>CD25</u>	<u>CD127</u>
CD4 ⁺ T Cell	Naive	+	-	+	-	+	+		
CD4 ⁺ T Cell	Effector memory	+	-	+	-	-	-		
CD4 ⁺ T Cell	Central memory	+	-	+	-	-	+		
CD4 ⁺ T Cell	EMRA	+	-	+	-	+	-		
CD4 ⁺ T Cell	Treg	+	-	+	-			+	-
CD8 ⁺ T Cell	Naive	+	-	-	+	+	+		
CD8 ⁺ T Cell	Effector memory	+	-	-	+	-	-		
CD8 ⁺ T Cell	Central memory	+	-	-	+	-	+		
CD8 ⁺ T Cell	EMRA	+	-	-	+	+	-		
DN T Cell	CD4 ⁻ CD8 ⁻	+	-	-	-				
DP T Cell	CD4 ⁺ CD8 ⁺	+	-	+	+				
B Cell	B cell	-	+						

EMRA, effector memory re-expressing CD45RA; Treg, T regulatory cell; DN, double negative; DP, double positive

+, presence of marker was required for assignment to cell subset

-, absence of marker was required for assignment to cell subset

Where presence or absence of a marker is not indicated, that marker was not considered in assignment of the cell subset

FIGURE LEGENDS FOR ONLINE REPOSITORY

Figure E1. Representative gating strategy for CD3⁺CD4⁺ and CD3⁺CD8⁺ cells from nasal polyps. (A) Gating of a representative nasal polyp using flow cytometry. FVD, Fixable viability dye. (B) Gating of a representative nasal polyp using mass cytometry.

Figure E2. Canonical gating hierarchy for T cell subset assignment. Using the T cell markers in the mass cytometry T cell antibody panel (Supplemental Table E2), CD3⁺ T cells were assigned to canonically defined subsets as per Supplemental Table E3. Markers used to divide a population into subsets are noted above the new subset. Subset names are noted inside each node box; those highlighted in blue were included in downstream analysis.

Figure E3. Examples of MDS maps for nasal polyp and control sinus tissue. MDS maps of CD45⁺ leukocytes after unsupervised clustering based on expression of surface markers are shown for a representative nasal polyp (A) and control sinus tissue (B). As indicated by the key, light green circles denote CD8⁺ naïve T cells. Open arrows point to CD8⁺ CD127^{hi}CXCR3^{hi}CD161^{lo} naïve T cells and filled arrows point to CD4⁺ CD161^{hi}CXCR3^{lo}CD127^{lo} central memory T cells, which are discussed in Figure 2B and 2C of the main text in detail.

Figure E4. Heat map of molecular expression in subsets of CD4⁺ T_{CM} cells identified by MDS map (A) and viSNE (B). (A) Expression levels of surface markers in the CD161^{hi}CXCR3^{lo}CD127^{lo} CD4⁺ T_{CM} population (indicated by the filled arrow in the MDS map

in Figure 2A) from control nasal tissues (Cont) and nasal polyps (NP) are shown as medians of transformed intensity values (arbitrary units), ranging from 0 (lightest green) to 5.4 (darkest green). Each row indicates an individual subject. Black squares across an entire row indicate the cell population was not found in that sample. (B) Expression levels of surface markers in the $CD4^+CD161^{hi}CXCR3^{lo}$ population from control nasal tissues (Cont) and nasal polyps (NP) (indicated by the red box in viSNE maps in Figure 2D) are shown. Each row indicates an individual subject. Data presented are medians of transformed intensity values, ranging from 0 (black) to 5.4 (darkest yellow).
