TABLE E1.

Subject demographics

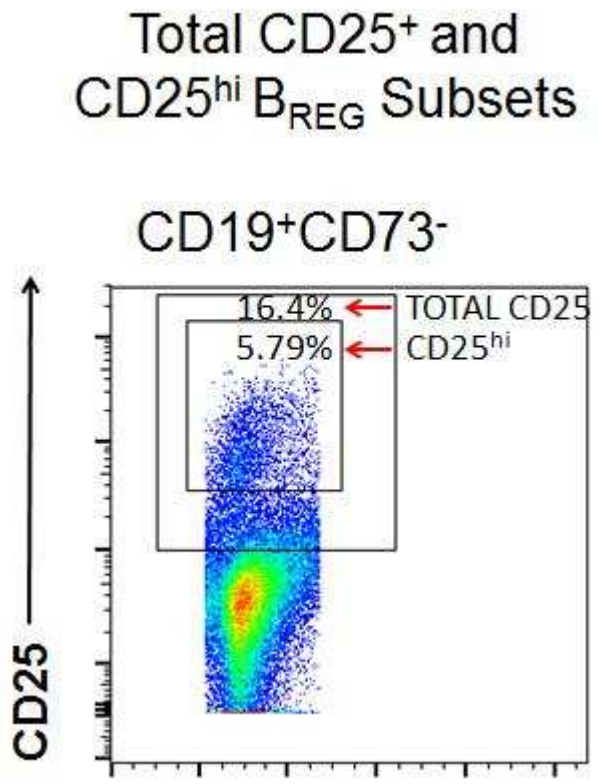
	Peripheral blood		Lymph node
	Allergic	**Non-allergic**	**Asthmatic**
Subjects	10	7	2
Men/women	3/7	2/5	1/1
Age (y)	36 ± 10	35 ± 13	19 ± 1
Race or ethnicity			
White	8	6	1
African American	0	0	0
Hispanic	0	0	1
Asian or Pacific Islander	2	1	0
Total IgE[a]	174 ± 53	33 ± 17	N/A
Specific IgE (number of positive subjects)[b]			
Dermatophagoides farinae	6	0	N/A
Dermatophagoides pteronyssinus	6	0	N/A
Bermuda grass	5	0	N/A
Cat dander	4	0	N/A
Active allergic rhinitis symptoms (at time of study blood draw)	10	0	N/A
Positive skin/ImmunoCap testing	10	0	N/A
On allergy medications (AH, ICS)[c,d]	0	0	N/A

[a] *$P < 0.05$.*

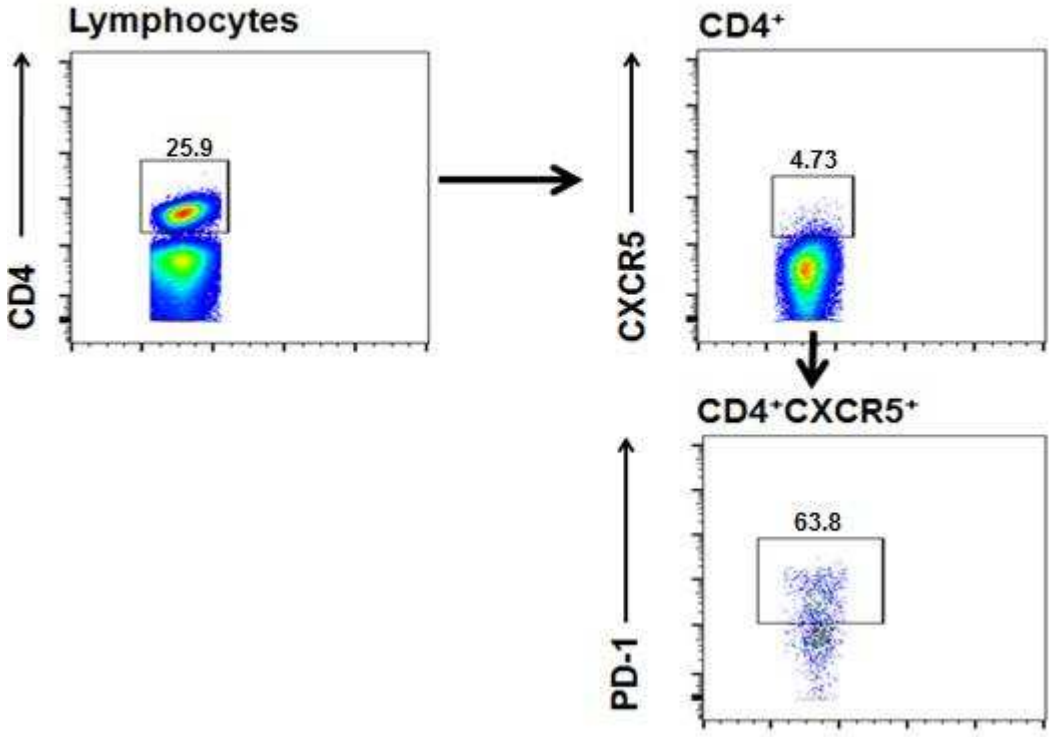
[b] Allergic subjects were sensitized to at least one aero-allergen

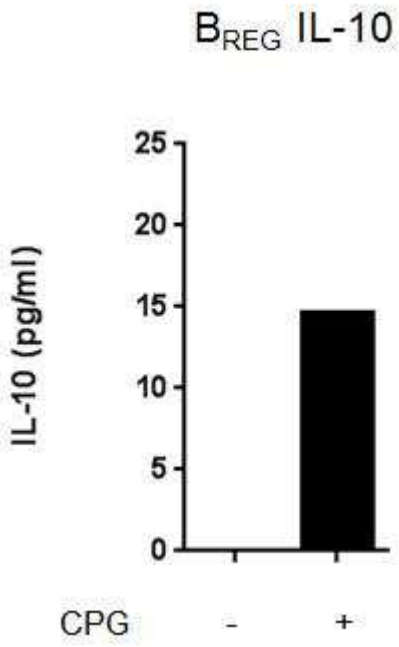
[c] Allergy medication use in one month prior to blood draw. AH = antihistamine, ICS = intranasal corticosteroid

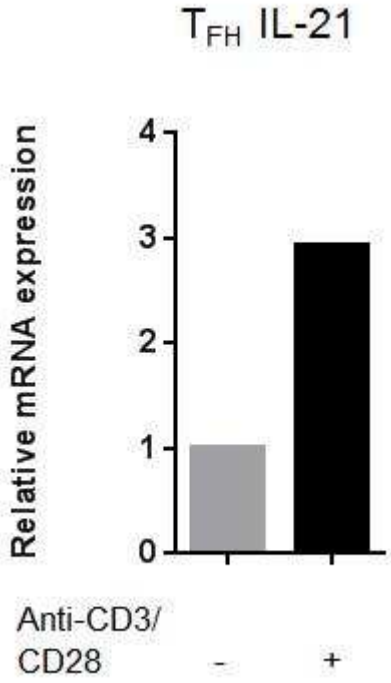
[d] None of the subjects were former or current smokers, on oral corticosteroids or other chronic oral medications, or diagnosed with any chronic medical diseases other than allergic rhinitis (if in allergic group).


Total CD25+ and
CD25hi BREG Subsets
CD19+CD73-
16.4%
TOTAL CD25
5.79%
CD25hi
CD25

Lymph Node T_{FH} Gating Strategy




B_{REG} IL-10
IL-10 (pg/ml)
25
20
15
10
5
0
CPG
-
+


T_{FH} IL-21
Relative mRNA expression
4
3
2
1
0
Anti-CD3/
CD28
-
+

Supplementary Figure Legends:

TABLE E1. Subject demographics.

FIG E1. FACS Gating strategy to detect B_{REGS} and $CD25^{hi}$ B_{REGS} in human lung lymph nodes.

A, Human lung lymph node B_{REGS} were identified from the lung lymph node lymphocyte population as $CD19^{+}CD73^{-}CD25^{+}CD71^{+}$ using the gating strategy shown. **B,** Human lung lymph nodes B_{REGS} from an asthmatic individual were analyzed using isotype controls to analyze their total $CD25^{+}$ and $CD25^{hi}$ subsets.

FIG E2. FACS Gating strategy to detect T_{FH}-like cells in human lung lymph nodes.

T_{FH}-like cells from an asthmatic human lung lymph node were identified from the lung lymph node lymphocyte population as $CD4^{+}CXCR5^{+}PD\text{-}1^{+}$ using the gating strategy shown.

FIG E3. Cytokine production by B_{REGS} and T_{FH} cells.

A, IL-10 protein levels measured by ELISA in supernatants of sorted B_{REGS} from a non-allergic subject. **B,** IL-21 mRNA expression quantitated by qPCR in sorted T_{FH} cells from a non-allergic subject.

Supplementary Methods:

Cell Sorting

Human peripheral whole blood was layered over a ficoll density gradient to isolate PBMCs. B_{REGS} and T_{FH}-like cells were sorted by fluorescently labeling PBMCs to identify B_{REGS} using CD19, CD73, CD25, and CD71 and T_{FH}-like cells using CD4, CXCR5, and PD-1 (eBioscience, San Diego, CA). Labeled PBMCs were sorted into B_{REGS} and T_{FH}-like cells using a FACSAria II cell sorter (BD Biosciences, Franklin Lakes, NJ).

Cell cultures

Sorted B_{REGS} and T_{FH} cells (>99% pure) were cultured in an RPMI-based media, supplemented with 10% FBS, 100 U/ml penicillin/streptomycin, 2mM L-glutamine, 0.1% 2-mercaptoethanol, and 20 ng/ml IL-2 (R&D Systems, Inc., Minneapolis, MN). Cells were plated at $5x10^5$ cells/well in a 96-well plate. B_{REGS} were stimulated with 10 µg/ml CpG ODN 2006 (InvivoGen USA, San Diego, CA) and T_{FH} cells were stimulated with 5 µg/ml anti-CD3 and 2 µg/ml anti-CD28 antibodies (eBioscience) for 72 hours in humidified 5% CO_2 at 37°C. Supernatants from stimulated (and unstimulated controls) were collected for cytokine quantification and cells were lysed for RNA extraction.

IL-10 ELISA

IL-10 was measured using a human IL-10 ELISA kit (GenWay Biotech, Inc., San Diego, CA) from stimulated and unstimulated B_{REG} cell culture supernatants. IL-10 assay sensitivity is < 0.05 pg/ml.

Flow cytometry

Samples were analyzed using either a FACSAria II cell sorter or a Novocyte Flow Cytometer (ACEA Biosciences, Inc., San Diego, CA) and analyzed using FlowJo software (version 10.0.7, Tree Star, Inc., Ashland, OR). The antibodies used in this study include: CD4-APC/Cy7, PD-1-BV 421, CD25 BV605 (BioLegend, San Diego, CA), CXCR5-PE, CD19 PerCP/Cy5.5, CD73 APC, CD71 FitC (eBioscience).

IL-21 quantitative PCR

RNA was isolated from cultured T_{FH} cells using the PicoPure RNA Isolation Kit (Arcturus Therapeutics, San Diego, CA). Synthesis of cDNA was performed using a cDNA kit (Clontech Laboratories, Inc., Mountain View, CA) and qPCR was performed using the Mx3000 qPCR system (Agilent Technologies, Inc., Santa Clara, CA), TaqMan Universal PCR Master Mix (Life Technologies Corporation, Carlsbad, CA), and IL-21 primers (Life Technologies, Carlsbad, CA). The housekeeping gene GAPDH (Life Technologies) was used to normalize quantifications. Relative mRNA expression of IL-21 was calculated by using the $\Delta\Delta$ comparative cycle threshold method.

Supplementary References

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