

Hematopoietic prostaglandin D synthase defines a proeosinophilic pathogenic effector human T_H2 cell subpopulation with enhanced function

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Background: IL-5⁺ pathogenic effector T_H2 (peT_H2) cells are a T_H2 cell subpopulation with enhanced proinflammatory function that has largely been characterized in murine models of allergic inflammation.

Objective: We sought to identify phenotype markers for human peT_H2 cells and characterize their function in patients with allergic eosinophilic inflammatory diseases.

Methods: Patients with eosinophilic gastrointestinal disease (EGID), patients with atopic dermatitis (AD), and nonatopic healthy control (NA) subjects were enrolled. peT_H2 and conventional T_H2 (cT_H2) cell phenotype, function, and cytokine production were analyzed by using flow cytometry.

Confirmatory gene expression was measured by using quantitative RT-PCR. Prostaglandin D₂ levels were measured with ELISA. Gut T_H2 cells were obtained by means of esophagogastroduodenoscopy.

Results: peT_H2 cells were identified as chemoattractant receptor-homologous molecule expressed on T_H2 cells–positive (CRTH2⁺), hematopoietic prostaglandin D synthase–positive CD161^{hi} CD4 T cells. peT_H2 cells expressed significantly greater IL-5 and IL-13 than did hematopoietic prostaglandin D synthase–negative and CD161[−] cT_H2 cells. peT_H2 cells were highly correlated with blood eosinophilia ($r = 0.78$ – 0.98) and were present in 30- to 40-fold greater numbers in subjects with EGID and those with AD versus NA subjects. Relative to cT_H2 cells, peT_H2 cells preferentially expressed receptors for thymic stromal lymphopoietin, IL-25, and IL-33 and demonstrated greater responsiveness to these innate pro-T_H2 cytokines. peT_H2 but not cT_H2 cells produced prostaglandin D₂. In patients with EGID and those with AD, peT_H2 cells expressed gut- and skin-homing receptors, respectively. There were significantly greater numbers of peT_H2 cells in gut tissue from patients with EGID versus NA subjects.

Conclusion: peT_H2 cells are the primary functional proinflammatory human T_H2 cell subpopulation underlying allergic eosinophilic inflammation. The unambiguous phenotypic identification of human peT_H2 cells provides a powerful tool to track these cells in future pathogenesis studies and clinical trials. (J Allergy Clin Immunol 2015;■■■:■■■–■■■.)

Key words: Atopic dermatitis, eosinophilic inflammation, eosinophilic gastrointestinal disease, IL-5, hematopoietic prostaglandin D synthase, chemoattractant receptor-homologous molecule expressed on T_H2 cells, T_H2, CD4, CD161, CD294

Through their expression of the cytokines IL-4, IL-5, and IL-13, T_H2 cells contribute to the pathologic processes associated with allergic diseases, including IgE-mediated hypersensitivity and eosinophilic inflammation. Each T_H2 cytokine has a well-defined and specific function. IL-4 mediates IgE class-switching, alternative macrophage activation, and T_H2 differentiation.¹ IL-5, the major eosinophil-active cytokine, is critical for eosinophil development, bone marrow egress, activation, and survival. IL-13 promotes eosinophil tissue homing, mucus hypersecretion, and airway hyperresponsiveness.

Chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) is a human T_H2 phenotypic marker.^{2,3} On binding of its ligand, prostaglandin D₂ (PGD₂), CRTH2 elicits activation and chemotaxis of T_H2 cells.^{4,5} PGD₂ is synthesized from membrane-liberated arachidonic acid through a 2-step process that requires cyclooxygenase and hematopoietic prostaglandin D synthase (hPGDS).³ hPGDS is preferentially expressed by T_H2 cells.⁶ Furthermore, the hPGDS/PGD₂/CRTH2 axis is highly linked to eosinophilic inflammation in asthmatic patients.⁷ This suggests that T_H2 cell production of and response to PGD₂ is an important component of allergic inflammation.

T_H2 cells are generally regarded as a homogeneous population, but several recent reports demonstrate T_H2 cell heterogeneity based on differential IL-5 expression.^{8–12} IL-5 expression is restricted to antigen-experienced, highly differentiated memory T_H2 cells.^{11,13,14} Notably, IL-5⁺ T_H2 cells demonstrate greater proinflammatory function in murine models^{9,10} and are increased in the blood of patients with eosinophilic gastrointestinal disease (EGID).^{8,12,15} Because of their enhanced function and association with allergic pathology, such IL-5⁺ T_H2 cells have been termed pathogenic effector T_H2 (peT_H2) cells.¹⁴

To better understand human T_H2 cell heterogeneity and its role in allergic eosinophilic inflammatory disease pathogenesis, we examined the coexpression of CRTH2, hPGDS, and CD161 within T_H2 cells and characterized a novel T_H2 subpopulation

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Abbreviations used

AD:	Atopic dermatitis
CD62L:	CD62 ligand
CLA:	Cutaneous lymphocyte-associated antigen
CRTH2:	Chemoattractant receptor–homologous molecule expressed on T _H 2 cells
cT _H 2:	Conventional T _H 2
EGID:	Eosinophilic gastrointestinal disease
HDM:	House dust mite
hPGDS:	Hematopoietic prostaglandin D synthase
ILC2:	Type 2 innate lymphoid cell
MAIT:	Mucosal-associated invariant T
NA:	Nonatopic healthy control
PE:	Phycocerythrin
peT _H 2:	Pathogenic effector T _H 2
PGD ₂ :	Prostaglandin D ₂
PMA:	Phorbol 12-myristate 13-acetate
TSLP:	Thymic stromal lymphopoietin

with innate-like responsiveness and enhanced effector function. These findings identify a pathogenic proeosinophilic human T_H2 cell subpopulation that is highly associated with allergic eosinophilic inflammatory disease.

METHODS**Subjects**

Clinical and immunologic details on patients with allergic EGID, patients with allergic asthma, and nonatopic healthy control (NA) subjects used in this study were previously reported.^{8,11} Patients with atopic dermatitis (AD; median, age 10 years) were diagnosed according to the criteria of Hanifin and Rajka¹⁶; patients with AD with coexisting EGID or receiving systemic immunosuppressive treatment were excluded. The National Institute of Allergy and Infectious Diseases Institutional Review Board approved the clinical protocols used for this study (clinicaltrials.gov identifiers NCT01212016 and NCT01164241). All subjects signed informed consent forms after the nature and possible consequences of the studies were explained. The Department of Laboratory Medicine at the National Institutes of Health Clinical Center performed peripheral blood absolute eosinophil counts.

T_H2 cell lines and cell culture

House dust mite (HDM)–specific T_H2 and respiratory syncytial virus–specific T_H1 cell lines were generated, as previously published.¹¹ T_H2 differentiation was performed according to published methods.^{11,17} Additional details on isolation of cells, lines, and cell culture can be found in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Flow cytometry

Flow cytometric methods for phenotyping (cell surface and intracellular antigens), intracellular cytokine staining, and intranuclear transcription factor staining are detailed in the [Methods](#) section in this article's Online Repository.

Cell sorting and quantitative PCR, chemotaxis assay, and PGD₂ secretion assay

A complete description of cell sorting and quantitative PCR, chemotaxis assay, and PGD₂ secretion assay can be found in the [Methods](#) section in this article's Online Repository.

Statistical analysis

Medians were used as the measure of central tendency. Statistics were calculated with Prism software (GraphPad Software, San Diego, Calif). Paired 2-group and multiple-group comparisons were calculated by using the nonparametric Wilcoxon signed-rank and Friedman tests, respectively. Unpaired 2-group and multiple-group comparisons were calculated by using the nonparametric Mann-Whitney *U* and Kruskal-Wallis tests, respectively. Correlation was determined by using the Spearman rank test. Two-way ANOVA was used to measure the difference in cytokine expression across innate cytokine-simulated conditions in CD161^{hi} versus CD161[−] T_H2 cells. In paired data sets each graphed data point from a given sample or subject is depicted with the same symbol.

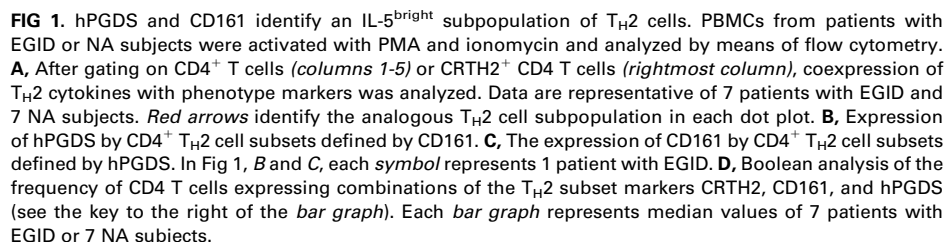
RESULTS**hPGDS and CD161 define an IL-5^{bright} T_H2 subpopulation**

hPGDS is expressed by T_H2 cells.^{6,18} To determine whether hPGDS is differentially expressed within T_H2 cell subpopulations, we investigated hPGDS coexpression with T_H2 cytokines. As previously reported,⁸ there were greater numbers of IL-13⁺IL-5⁺ T_H2 cells in patients with EGID ([Fig 1, A](#), column 1). Notably, both hPGDS and CD161 identified an IL-5^{bright} T_H2 cell subpopulation ([Fig 1, A](#), columns 3 and 5, red arrows). This subpopulation was present in patients with EGID but notably absent in NA subjects. Both hPGDS and CD161 identified a similar IL-13^{bright} T_H2 cell subpopulation (see [Fig E1, A](#), in this article's Online Repository at www.jacionline.org).

As previously established,² CRTH2⁺ CD4 T cells were highly enriched for T_H2 cells and did not express IFN- γ (see [Fig E2, A and B](#), in this article's Online Repository at www.jacionline.org). Coexpression of CRTH2 with either hPGDS or CD161 identified the same subpopulation of T_H2 cells ([Fig 1, A](#), columns 2 and 4, red arrows). Three CRTH2⁺ T_H2 cell subpopulations could be defined: hPGDS[−]CD161[−], hPGDS[−]CD161^{int}, and hPGDS⁺CD161^{hi}, as indicated by quadrants A, B, and C, respectively, in [Fig 1, A](#) (rightmost column). hPGDS and CD161 were highly coexpressed by the same subpopulation of T_H2 cells, and hPGDS expression was restricted to the CD161^{hi} subpopulation ([Fig 1, A](#), column 6, and [B–D](#)). The frequency of CRTH2⁺hPGDS⁺ versus CRTH2⁺CD161^{hi} T cells was highly correlated ($r = 0.92$; see [Fig E1, B](#)). Both hPGDS and CD161 were stably expressed after phorbol 12-myristate 13-acetate (PMA)/ionomycin activation (data not shown). Collectively, these data identify CRTH2⁺hPGDS⁺CD161^{hi} CD4 T cells as a unique human T_H2 cell subpopulation that is highly associated with EGID.

hPGDS⁺CD161^{hi} T_H2 cells have greater T_H2 cytokine expression

Cytokine production by hPGDS⁺CD161^{hi} T_H2 cells was compared with that of other T_H2 cell subpopulations to investigate hPGDS⁺CD161^{hi} T_H2 cell function. There was a stepwise increase in T_H2 cytokine-producing cells in the progression from hPGDS[−]CD161[−] to hPGDS⁺CD161^{hi} T_H2 cells ([Fig 2, A and B](#)). There was a similar stepwise increase in IL-5 and IL-13 fluorescence intensity, indicating greater per-cell cytokine expression ([Fig 2, C and D](#)). IL-4 was highly expressed in the hPGDS⁺CD161^{hi} T_H2 cell subpopulation, although a distinct IL-4[−]IL-5⁺IL-13⁺ population was noted ([Fig 2, E](#); combined results are shown in [Fig E2, C](#)). In conclusion,



To further examine the generalizability of these pT_{H2} markers, their expression was examined in HDM-specific T_{H2} cell lines generated from allergic asthmatic subjects. CRTH2 and hPGDS were expressed by T_{H2} but not T_{H1} cell lines (see Fig E2, D). HDM-specific T_{H2} cell lines were uniformly IL-13⁺ (Fig 2, F). However, there was a stepwise increase in IL-5 expression along the progression from the

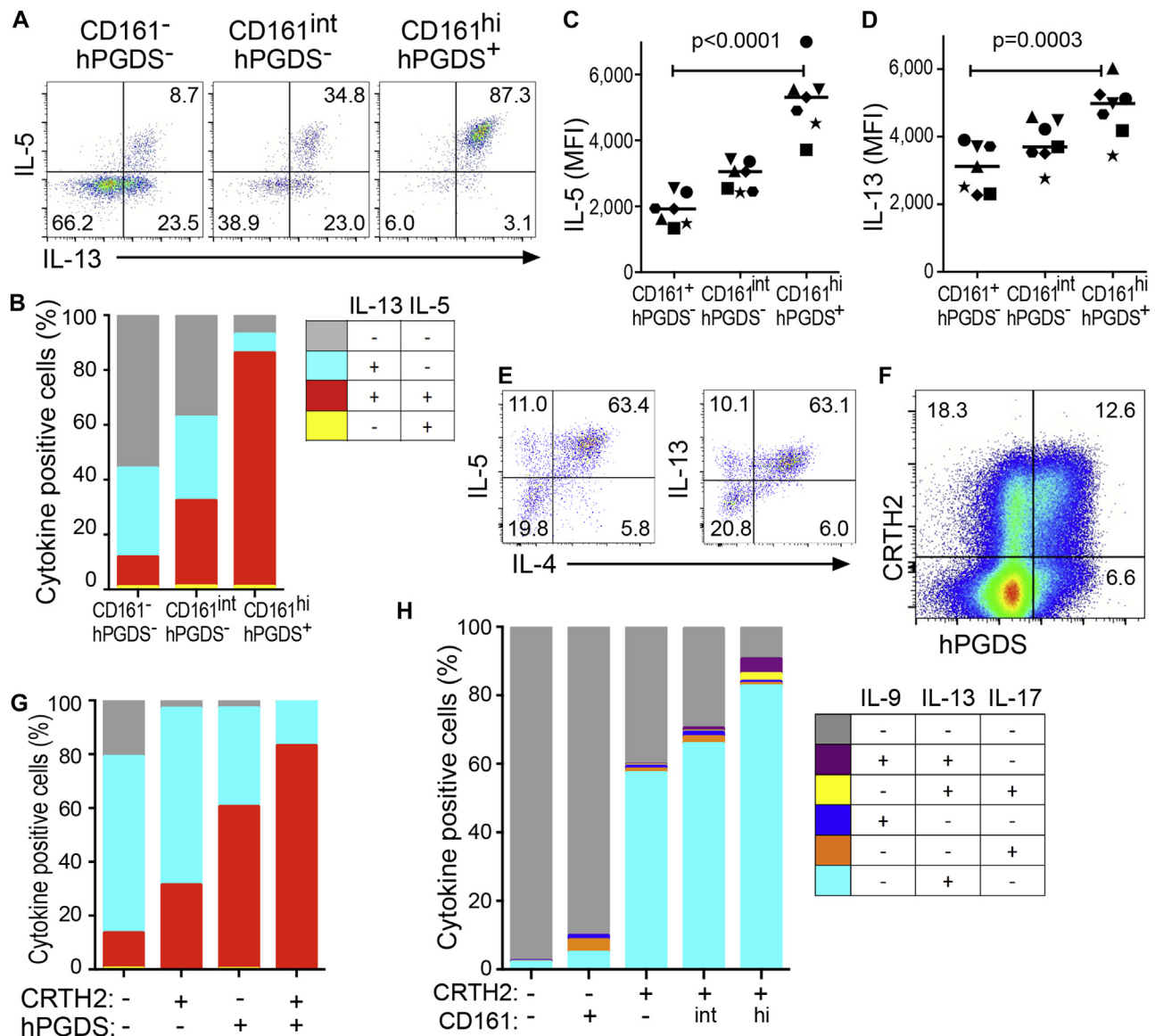


FIG 2. hPGDS⁺CD161^{hi} T_H2 cells have greater T_H2 cytokine expression. PBMCs from patients with EGID were activated as in Fig 1, and cytokine production among CD4 T_H2 cell subpopulations was compared. **A**, After first gating on CRTH2⁺ CD4 T cells, T_H2 cell subpopulations were identified according to the gating scheme in the rightmost column of Fig 1, A, and IL-5 versus IL-13 expression was analyzed. Data are representative of 7 subjects. **B**, Boolean analysis of T_H2 cytokine expression in the above CRTH2⁺ subsets (n = 7 patients with EGID). **C** and **D**, Mean fluorescence intensity (MFI) of IL-5 (Fig 2, C) and IL-13 (Fig 2, D) in the above noted T_H2 cell subsets. Each symbol represents 1 patient with EGID. **E**, T_H2 cytokine expression of CRTH2⁺CD161^{hi}hPGDS⁺ T cells. Data are representative of 5 subjects. **F**, HDM-specific T_H2 cell lines were activated and analyzed for hPGDS and CRTH2 expression. **G**, Boolean analysis of IL-5 and IL-13 expression within T_H2 cell subpopulations of dust mite-specific T_H2 cell lines. The key is the same as in Fig 2, B (n = 3 cell lines). **H**, Boolean analysis of cytokine coexpression within the noted CD4 T-cell subpopulations (n = 3 patients with EGID).

CRTH2⁻hPGDS⁻ to the CRTH2⁺hPGDS⁺ subsets. These results demonstrate that hPGDS identifies peT_H2 cells within the allergen-specific T_H2 response seen in asthmatic patients.

CD161 is expressed by T_H17 cells,¹⁹ and both IL-17 and IL-9 can be coexpressed with T_H2 cytokines.²⁰⁻²² Accordingly, peT_H2 cell coexpression of the inflammatory cytokines IL-9 and IL-17 was examined using Boolean analysis (Fig 2, G). IL-13 was expressed by the majority of cells in all CRTH2⁺ subsets. Small populations of peT_H2 cells coexpressed IL-13 and either IL-9 or IL-17. IL-9⁺ T_H2 cells coexpressed IL-5 (see Fig E2, E).

Collectively, these data demonstrate that the phenotypically defined peT_H2 subset contains smaller subpopulations that coexpress proinflammatory cytokines, such as IL-9 and IL-17.

hPGDS⁺CD161^{hi} T_H2 cells are highly differentiated effector memory CD4 T cells

Multiple T-cell populations express CD161, including natural killer T cells,²³ mucosal-associated invariant T (MAIT) cells,²⁴ and T_H17 cells.¹⁹ peT_H2 cells were uniformly CD4⁺CD8⁻

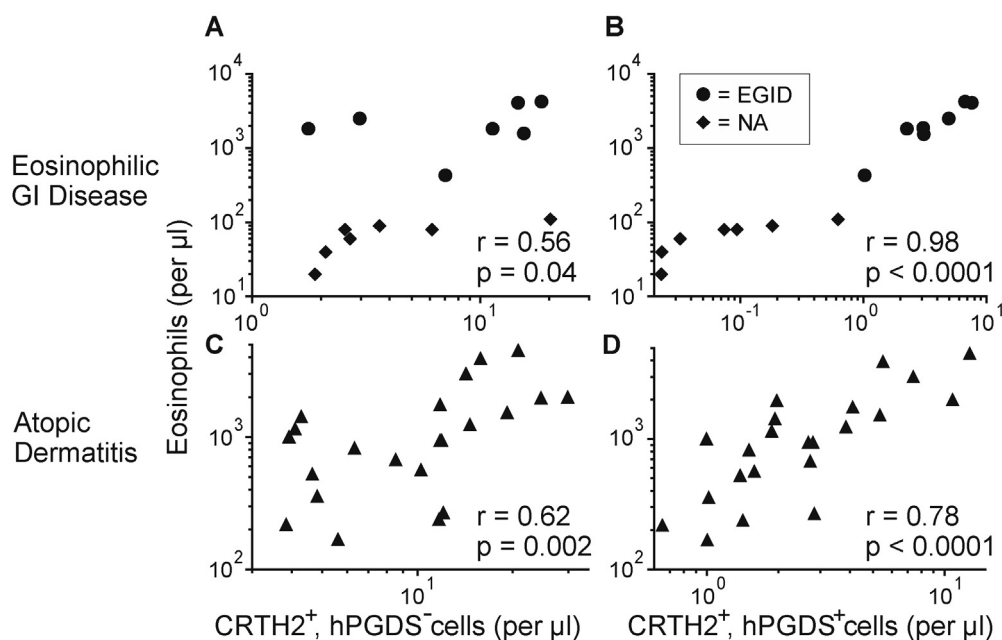


FIG 3. hPGDS⁺ T_H2 cell counts correlate with blood eosinophilia. Correlation of blood eosinophil counts with absolute numbers of blood T_H2 cell subpopulations in adults with EGID (circles) and healthy nonatopic control subjects (diamonds; **A** and **B**) or patients with AD (**C** and **D**). Each symbol represents 1 patient. Spearman rank correlation was used. *GI*, Gastrointestinal.

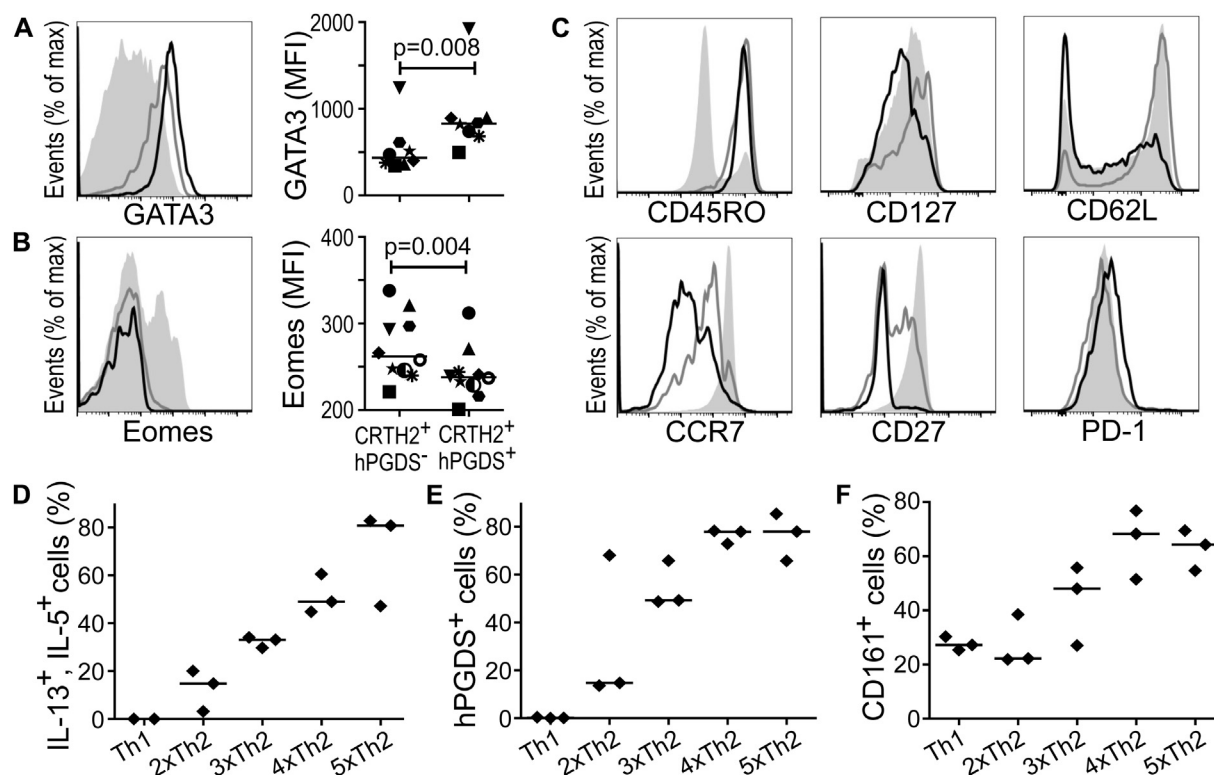


FIG 4. peT_H2 cells are highly differentiated effector memory T cells. **A** and **B**, Flow cytometric analysis of GATA3 (Fig 4, **A**) or eomesodermin (*Eomes*; Fig 4, **B**) expression in hPGDS⁺ T_H2 cells (black line), hPGDS[−] T_H2 cells (gray line), and non-T_H2 CD4 T cells (light gray shading). Combined results of GATA3 and eomesodermin are shown to the right of the histograms (8 and 10 patients with EGID, respectively). *MFI*, Mean fluorescence intensity. **C**, Analysis of lymphocyte memory and differentiation markers of CD161^{hi} T_H2 cells (black line), CD161[−] T_H2 cells (gray line), and non-T_H2 CD4 T cells (light gray shading). Results are representative of 6 to 8 patients with EGID. **D–F**, Naive CD4 T cells underwent serial rounds of *in vitro* T_H2 or T_H1 differentiation and were analyzed for T_H2 cytokine (Fig 4, **D**), hPGDS (Fig 4, **E**), and CD161 (Fig 4, **F**) expression.

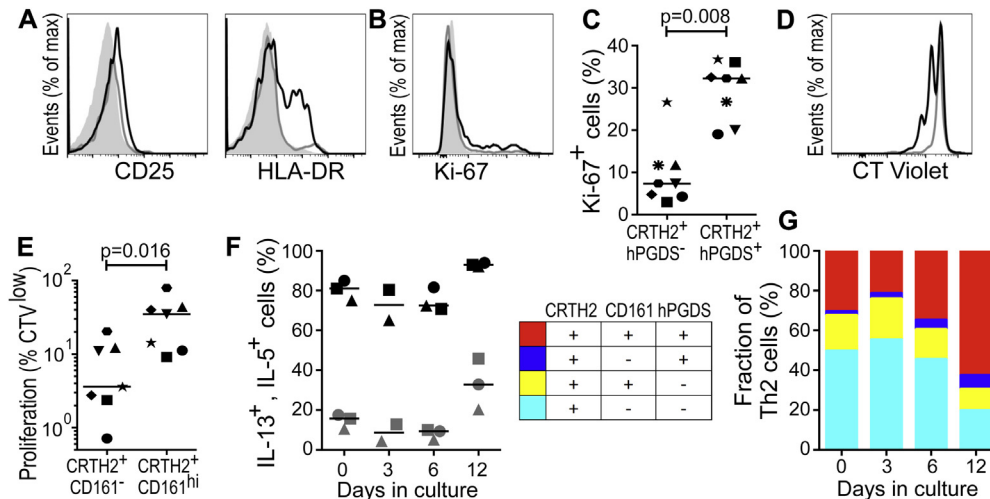


FIG 5. pe_{H2} cells maintain a durable IL-5⁺ phenotype. **A**, *Ex vivo* CD4 T cells were analyzed for CD25 and HLA-DR after gating on CD161^{hi} T_{H2} cells (black line), CD161⁻ T_{H2} cells (gray line), and non-T_{H2} CD4 T cells (light gray shading). Data are representative of 8 patients with EGID. **B**, Parallel samples were analyzed for Ki-67 expression. **C**, Combined Ki-67 data from 8 patients with EGID. **D**, PBMCs from patients with EGID were labeled with Cell Trace Violet (CTV) and cultured in complete medium for 7 days, after which T_{H2} cell subpopulations were assessed for proliferation through dye dilution. **E**, Combined CTV proliferation data from 7 patients with EGID. **F** and **G**, Purified CD4 T cells were cultured for the indicated time in complete medium supplemented with 5 U/mL IL-2 (n = 3 cultures). Fig 5, F, shows T_{H2} cytokine production by hPGDS⁺ (black symbols) and hPGDS⁻ (gray symbols) T_{H2} cells within these cultures after PMA/ionomycin activation. Fig 5, G, shows Boolean analysis of CD161 and hPGDS coexpression within the CRTH2⁺ T_{H2} cell population. In Fig 5, C, E, and F, each symbol represents 1 patient or culture.

(see Fig E3, A and B, in this article's Online Repository at www.jacionline.org), did not express the MAIT or natural killer T-cell invariant T-cell receptor α chains V α 7.2 and V α 24 (see Fig E3, C), and were predominantly IL-17⁻ (Fig 2, G). Because human type 2 innate lymphoid cells (ILC2s) also express CRTH2 and CD161,²⁵ we next examined their lineage. In PBMCs from patients with EGID, 10% or less of circulating CRTH2⁺CD161^{hi} lymphocytes were identified as ILC2s (CRTH2⁺CD161^{hi}Lin⁻CD3⁻), whereas the majority of CRTH2⁺CD161^{hi} lymphocytes were CD3⁺ T cells (see Fig E3, D and E). Intriguingly, hPGDS was highly expressed by ILC2s (see Fig E3, F and G). Both peT_{H2} cells and ILC2s expressed IL-5 and IL-13 comparably (see Fig E3, G). T_{H2} cytokine expression was more apparent in the peT_{H2} cell population because there are greater numbers of peT_{H2} cells in donors with EGID. Collectively, these data confirm that peT_{H2} cells are CD4 T cells and not a more unconventional lymphocyte population.

Because peT_{H2} cells produce greater T_{H2} cytokine levels, their expression of transcription factors implicated in T_{H2} cytokine expression was examined. GATA3 expression was significantly greater in peT_{H2} versus cT_{H2} cells (Fig 4, A). Conversely, eomesodermin, which has been reported to be a negative regulator of IL-5 expression,⁹ was expressed at significantly lower levels in peT_{H2} cells (Fig 4, B).

Both T_{H2} cell subpopulations had a memory phenotype; however, peT_{H2} cells demonstrated significantly reduced expression of CCR7, CD62 ligand (CD62L), and CD127 compared with cT_{H2} cells, which is consistent with an effector memory phenotype (Fig 4, C; combined results are shown in Fig E4, A, in this article's Online Repository at www.jacionline.org).²⁶ peT_{H2} cells were uniformly CD27⁻, a pattern characteristic of highly differentiated memory effector

T cells.²⁶ Programmed cell death 1 (PD-1), a marker of chronically activated or exhausted T cells,²⁶ was greater in peT_{H2} cells (Figs 4, C, and 6, G; combined results are shown in Fig E4, A).

Acquisition of the IL-5⁺ Th2 phenotype requires multiple rounds of T_{H2} differentiation.^{11,12} Accordingly, we examined whether the acquisition of hPGDS and CD161 expression during T_{H2} differentiation displayed similarly delayed kinetics. Expression of hPGDS and CD161 was acquired in a delayed stepwise manner, with kinetics similar to those of IL-5 (Fig 4, D-F). In conclusion, these data establish that peT_{H2} cells are highly differentiated memory effector T_{H2} cells that are the product of multiple rounds of T_{H2} cell differentiation.

peT_{H2} cells maintain a durable IL-5⁺ phenotype

EGID is a food allergen-driven eosinophilic inflammatory gut disease in which chronic food antigen intake results in T_{H2} cell activation. Accordingly, T_{H2} cell activation status was examined. Although CD25 levels were not substantially different, levels of the T-cell activation marker HLA-DR were significantly increased in peT_{H2} cells (Fig 5, A; combined results are shown in Fig E4, B). The proliferation marker Ki-67 was significantly upregulated in peT_{H2} cells (Fig 5, B and C). Similarly, peT_{H2} cells spontaneously proliferated when cultured *in vitro* (Fig 5, D and E). Together, these *ex vivo* data suggest that in patients with EGID, peT_{H2} cells are activated and proliferate *in vivo*.

We next investigated whether the enhanced T_{H2} cytokine expression characteristic of peT_{H2} cells is intrinsic to the cells or is a transient function of their *in vivo* activation state. peT_{H2} cells maintained their prodigious IL-5 and IL-13 production over the course of a 2-week *in vitro* culture (Fig 5, F). During these cultures, peT_{H2} cells also maintained their

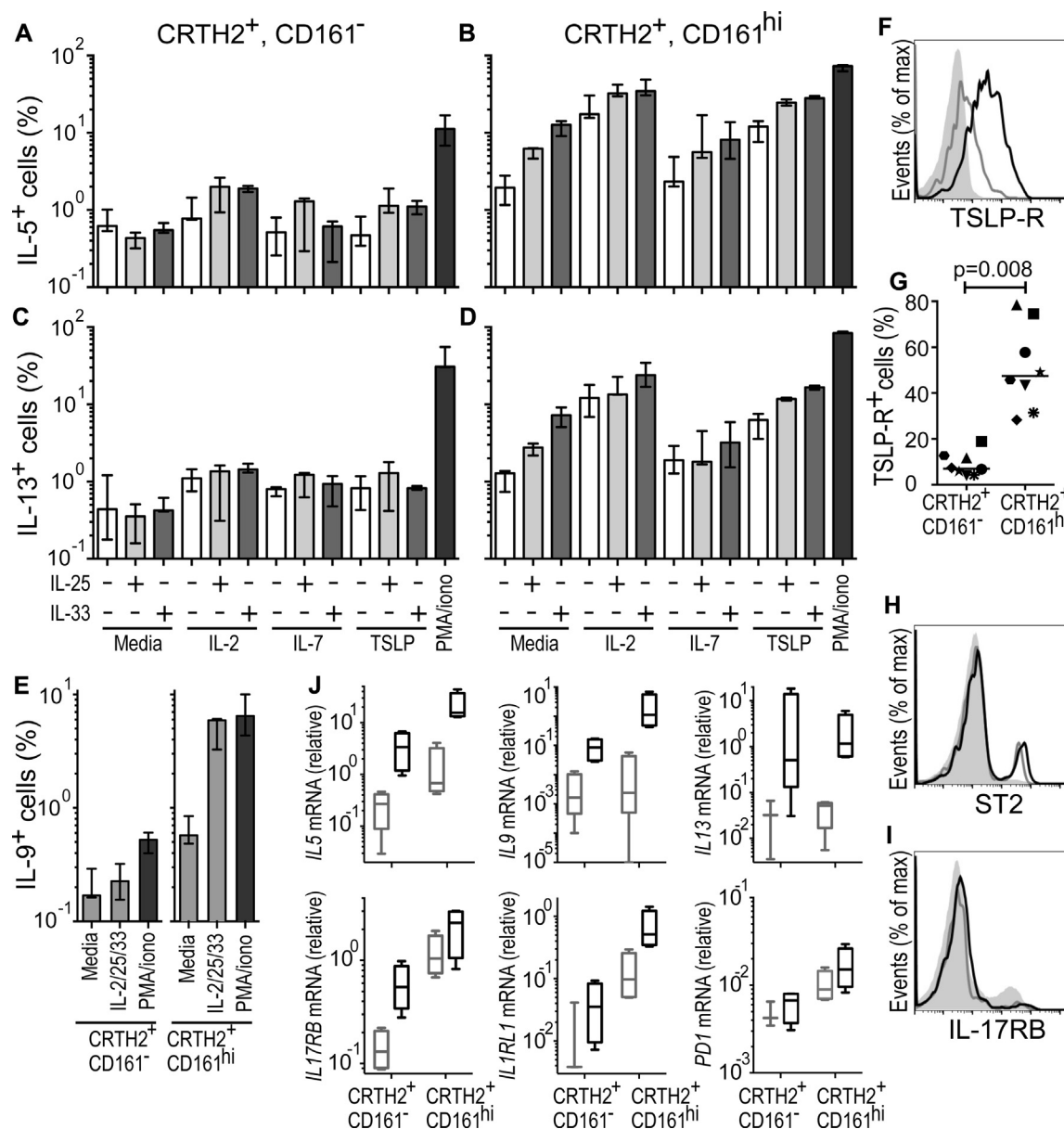


FIG 6. peT_H2 cells have an innate phenotype and function. **A-E**, IL-5 (Fig 6, A and B), IL-13 (Fig 6, C and D), and IL-9 (Fig 6, E) expression within the CD161⁻ and CD161^{hi} T_H2 subsets was measured by using flow cytometry after activation with the indicated cytokine combinations (n = 3 patients with EGID). **F**, **H**, and **I**, Flow cytometric analysis of the indicated cytokine receptors in CD161^{hi} T_H2 cells (black line), CD161⁻ T_H2 cells (gray line), and non-T_H2 CD4 T cells (light gray shading) in *ex vivo* PBMCs. Results are representative of 4 to 8 patients with EGID. **G**, Combined TSLP receptor (TSLP-R) data in T_H2 cell subpopulations (n = 8 patients with EGID). **J**, Sorted CRTH2⁺ CD161^{hi} and CRTH2⁺ CD161⁻ CD4 T cells from 4 patients with EGID were activated with IL-2/IL-25/IL-33 and analyzed by means of quantitative PCR for expression of the indicated genes. Medium control and IL-2/IL-25/IL-33-stimulated T_H2 cells are represented by gray and black bars, respectively.

hPGDS⁺CD161⁺ phenotype (Fig 5, G). These results demonstrate that peT_H2 cells are a phenotypically stable subpopulation with enhanced and durable T_H2 cytokine expression.

peT_H2 cells have an innate-like phenotype and function

IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are epithelial cell-derived cytokines that activate a type 2 innate

immune response.²⁷⁻³¹ We next examined whether peT_H2 cells differentially respond to these innate pro-T_H2 cytokines (Fig 6, A-E). Over all conditions, there was significantly greater cytokine-induced T_H2 cytokine expression in the peT_H2 cell subset (Fig 6, A vs B and C vs D; $P < .0001$; Fig 6, E: $P = .01$). In peT_H2 cells the combination of the signal transducer and activator of transcription 5 activators IL-2 or TSLP with either IL-25 or IL-33 resulted in prodigious T_H2 cytokine expression approaching that of the pharmacologic activators PMA and ionomycin.

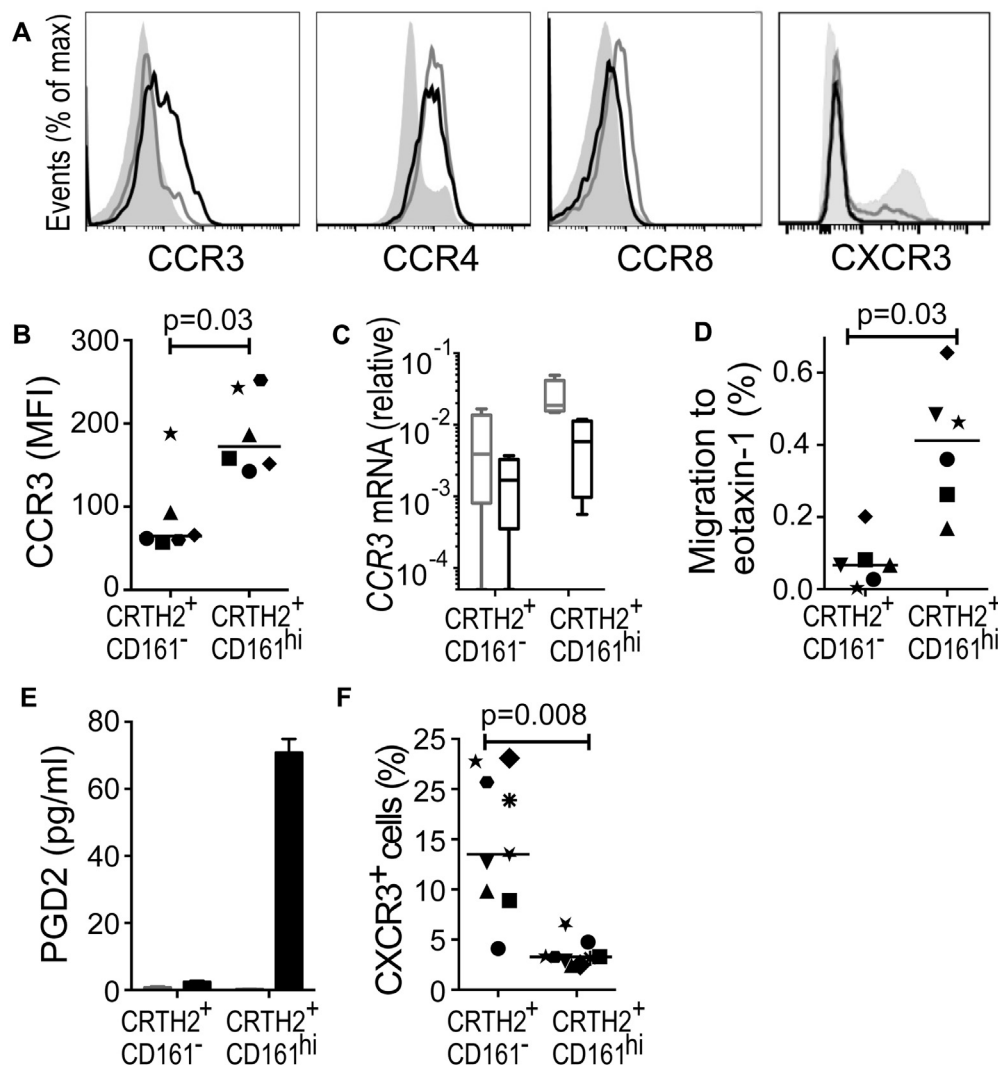


FIG 7. peTH2 cells have an enhanced proallergic chemoattractant ligand and receptor program. **A**, Chemokine receptor expression was analyzed in CD161^{hi} T_H2 cells (black line), CD161[−] T_H2 cells (gray line), and non-T_H2 CD4 T cells (light gray shading). Data are representative of 6 to 9 patients with EGID. **B**, Combined CCR3 data from 6 patients with EGID. MFI, Mean fluorescence intensity. **C**, Sorted CD161[−] and CD161^{hi} T_H2 cells were analyzed for CCR3 by using quantitative PCR as in Fig 6, J. Gray bars, Media; black bars, IL-2/IL-25/IL-33 stimulated. **D**, Chemotaxis of CD161^{hi} versus CD161[−] T_H2 cells in response to eotaxin-1. Each symbol represents the cells from 1 patient. **E**, Sorted CD161^{hi} and CD161[−] T_H2 cells were activated, and supernatant PGD₂ levels were measured. Unactivated and PMA/ionomycin-activated T_H2 cells are represented by gray and black bars, respectively. Results are representative of 4 independent samples. **F**, Combined CXCR3 staining of T_H2 subsets from 9 patients with EGID.

Expression of the corresponding cytokine receptors was examined to determine whether peT_H2 cells have the capacity to directly respond to IL-25, IL-33, and TSLP. TSLP receptor expression was dramatically greater on peT_H2 cells from both patients with EGID and those with AD (Fig 6, F and G, and see Fig E5, B and C, in this article's Online Repository at www.jacionline.org). Transcripts for the receptors for IL-25 and IL-33 (*IL17RB* and *IL1RL1*) were more highly expressed in peT_H2 cells, but differential expression of IL-17RB and ST2 could not be confirmed by using flow cytometry (Fig 6, H-J; combined results are shown in Fig E4, C).

The direct activity of IL-25 and IL-33 on peT_H2 cells was confirmed on sorted T_H2 cell subpopulations (Fig 6, J). Purified peT_H2 cells were highly responsive to IL-25/IL-33 activation,

as evidenced by their induced expression of T_H2 cytokines and associated receptors. In conclusion, these data demonstrate that peT_H2 cells express receptors for and directly respond to innate pro-T_H2 cytokines, suggesting that peT_H2 cells have an innate-like function.

peT_H2 cells have an enhanced proallergic chemoattractant ligand and receptor program

T_H2 cells have been reported to express CCR3, CCR4, and CCR8,³²⁻³⁶ which mediate chemotaxis to sites of inflammation.^{10,37} Thus T_H2 cell subpopulation expression of these chemokine receptors was examined. In both patients with EGID and those with AD, CCR3 expression was substantially

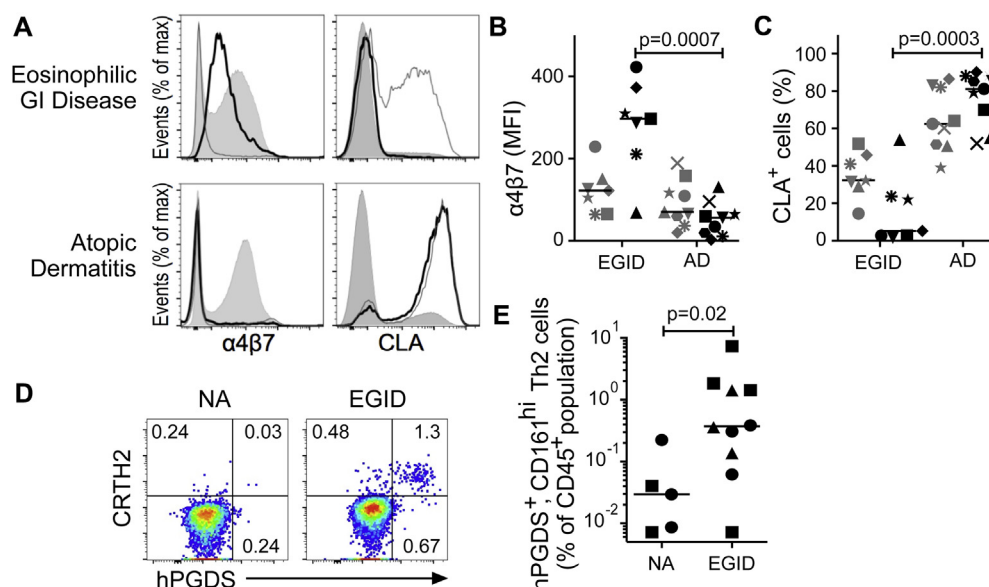


FIG 8. peTH2 cells express tissue-specific homing receptors and localize to sites of eosinophilic inflammation. **A**, Flow cytometric analysis of $\alpha_4\beta_7$ and CLA expression by hPGDS⁺ TH2 cells (black line), hPGDS⁻ TH2 cells (gray line), and non-TH2 CD4 T cells (light gray shading) from patients with EGID and those with AD. **B** and **C**, Combined analysis of $\alpha_4\beta_7$ and CLA expression in hPGDS⁻ TH2 (gray) and hPGDS⁺ TH2 (black) cells. Each symbol represents cells from 1 patient (n = 7 patients with EGID and 9 patients with AD). MFI, Mean fluorescence intensity. **D**, CRTH2 versus hPGDS expression in duodenal CD4 T cells from NA subjects and patients with EGID. **E**, Combined data showing the frequency of hPGDS⁺CD161^{hi} TH2 cells in the antrum (squares), duodenum (circles), and esophagus (triangles) from NA subjects and patients with EGID. Each symbol represents 1 patient.

greater in the peTH2 cell subpopulation (Fig 7, A-C; combined results are shown in Figs E5, B and C). Accordingly, peTH2 cells demonstrated significantly greater chemotaxis to CCL11/eotaxin-1 than did cTH2 cells (Fig 7, D). Collectively, these results demonstrate that peTH2 cells express greater levels of CCR3, expression of which results in enhanced chemotaxis to eotaxins.

Given that hPGDS expression is a defining criterion of peTH2 cells, we next determined whether this resulted in greater PGD₂ generation. Indeed, peTH2 cells produced substantially more PGD₂ than did cTH2 cells (Fig 7, E). These data demonstrate an additional mechanism through enhanced production of PGD₂ whereby peTH2 cells might contribute to allergic inflammation.

Both CCR4 and CCR8 expression was greater in TH2 cells than in non-TH2 CD4 T cells, but neither chemokine receptor was differentially expressed between the 2 TH2 subpopulations (Fig 7, A; combined results are shown in Fig E4, D and E). peTH2 cells expressed significantly less CXCR3 than cTH2 cells (Fig 7, A and F).

peTH2 cells express tissue-specific homing receptors and localize to sites of eosinophilic inflammation

EGID and AD are associated with allergic eosinophilic inflammation of the gastrointestinal tract and skin, respectively. To examine whether specific TH2 subpopulations can differentially localize to these sites, we investigated their expression of the respective gut- and skin-homing receptors $\alpha_4\beta_7$ integrin and cutaneous lymphocyte-associated antigen (CLA). peTH2 cells expressed significantly more $\alpha_4\beta_7$ in patients with EGID relative

to those in patients with AD (Fig 8, A and B). Conversely, peTH2 cells expressed significantly greater CLA levels in patients with AD relative to that in patients with EGID (Fig 8, A and C). Next, gastrointestinal tissue from patients with EGID and NA subjects demonstrated that peTH2 cells were present in significantly greater numbers in gut tissue from patients with EGID relative to that from NA subjects (Fig 8, D and E). In conclusion, these data demonstrate that in patients with organ-specific allergic inflammatory diseases, peTH2 cells express tissue-homing receptors that reflect the site of disease and preferentially home to eosinophilic inflammatory tissue.

DISCUSSION

In this work we identify the human peTH2 cell subpopulation as hPGDS⁺CD161^{hi} TH2 cells and characterize their proeosinophilic inflammatory function. In almost every functional attribute examined, peTH2 cells stood out dramatically from cTH2 cells. peTH2 cells were almost perfectly correlated with peripheral eosinophilia and were present in 30- to 40-fold greater numbers in patients with EGID and those with AD relative to NA subjects. peTH2 cells were almost uniformly IL-5⁺IL-13⁺, strongly supporting a mechanistic link to eosinophilia. peTH2 cells expressed receptors for and produced TH2 cytokines in response to TSLP, IL-25, and IL-33, suggesting that peTH2 cells have an innate-like program that directly responds to epithelial signals. Lastly, peTH2 cells were found in affected EGID tissue and expressed tissue-homing receptors corresponding to the site of disease. These findings demonstrate that human hPGDS⁺CD161^{hi} TH2 cells reiterate the features of the recently described murine peTH2 cell,¹⁴ suggesting they are a major

pathogenic human T_H2 cell subpopulation underlying allergic eosinophilic inflammation.

In this work the human peT_H2 cell subpopulation is defined based on its expression of CRTH2, hPGDS, and CD161. Using these phenotype markers, we investigated T_H2 cell heterogeneity by comparing the function of $hPGDS^+CD161^{hi}$ peT_H2 cells versus $hPGDS^-CD161^-$ cT_H2 cells. Because hPGDS and CD161 were highly coexpressed by peT_H2 cells, either marker could be used interchangeably to distinguish the above T_H2 cell subpopulations (Figs 1 and 2). However, hPGDS is a more specific marker of these pathogenic T_H2 cells because hPGDS expression by T cells is limited to the peT_H2 subset, whereas CD161 is expressed by non- T_H2 cell populations (Fig 1, A). This greater specificity of hPGDS is also reflected in $hPGDS^+T_H2$ cells having the greatest correlation with blood eosinophilia (see Table E2). These data expand on previous knowledge that hPGDS is expressed by T_H2 cells⁶ by the demonstration that hPGDS identifies a minority T_H2 cell subpopulation with dramatically greater effector function and disease correlation. These data support the model proposed by Endo et al¹⁴ wherein a distinct subpopulation of IL-5⁺ peT_H2 cells mediates allergic eosinophilic inflammatory disease. The human peT_H2 cells characterized in the work are notable for their enhanced effector function and high correlation with blood eosinophilia. However, further work is needed to directly demonstrate a pathogenic role for these cells in human disease.

The pro- T_H2 innate epithelium-derived cytokines IL-25, IL-33, and TSLP play a major role in allergic disease pathogenesis.^{29,38,39} Notably, peT_H2 cells demonstrate greater expression of the receptors for and responsiveness to these innate cytokines (Fig 6), indicating that these cells have an innate-like phenotype and function. peT_H2 cells and ILC2s share many phenotypic features, notably CRTH2, CD161, and hPGDS expression (see Fig E3). However, in contrast to ILC2s, peT_H2 cells expressed low CD127 levels and did not respond to IL-7.

Because IL-5 is the major eosinophil-active cytokine, it is unsurprising that counts of peT_H2 cells, which are IL-5^{bright}, are highly correlated with blood eosinophilia (Fig 3). The magnitude of the $hPGDS^+peT_H2$ cell correlation in patients with EGID approached unity and was greater than that for IL-5 itself, strongly suggesting that peT_H2 cells are driving blood eosinophilia (see Table E1). This high correlation is likely due to hPGDS being a specific marker of pathogenic T_H2 cells with proeosinophilic function. Notably, peT_H2 cells were almost nonexistent in the blood of NA subjects (Fig 3).

In patients with EGID and those with AD, peripheral blood peT_H2 cells express tissue-homing receptors that reflect the respective sites of disease (Fig 8). Furthermore, peT_H2 cells are increased in gut tissue from patients with EGID. Collectively, these results indicate that in addition to mediating blood eosinophilia, peT_H2 cells localize to sites of allergic inflammation and mediate tissue eosinophilia.

In this work peT_H2 cells demonstrated prodigious cytokine expression in every assay system used, expressing significantly greater IL-13 and IL-5 levels than cT_H2 cells. Although IL-4 was highly expressed by peT_H2 cells, there was a substantial population of IL-4⁻ peT_H2 cells. Notably, almost all IL-17⁺ T_H2 cells were found within the peT_H2 cell subpopulation (Fig 2, G), extending previous observations of such cells in asthmatic patients.^{20,21} Most IL-9-expressing T_H2 cells were found within a small subpopulation (4%) of peT_H2 cells that

coexpressed IL-13, IL-5, and IL-9 (Figs 2, H, and 6, E, and see Fig E2, E). Both IL-9 and IL-17 have the capacity to augment allergic inflammation through multiple mechanisms. Thus the coexpression of these cytokines by peT_H2 cells provides further evidence for their enhanced proinflammatory function.

Consistent with their expression of hPGDS and previous reports documenting PGD₂ production by T_H2 cell lines,^{6,40} peT_H2 cells generate PGD₂ on activation. Supported by established biology that PGD₂ induces T_H2 cell, eosinophil, and basophil activation,^{3,5,40} PGD₂ production by peT_H2 cells likely contributes to their effector function. Furthermore, PGD₂ ligation of CRTH2 inhibits T_H2 cell apoptosis.⁴¹ In patients with severe asthma, the CRTH2/hPGDS/PGD₂ axis is highly associated with eosinophilic inflammation.⁷ Taken together with the $hPGDS^+peT_H2$ cells found within HDM-specific T cells from allergic asthmatic patients (Fig 2), these findings suggest a potential role for peT_H2 cells in the eosinophilic inflammation seen in asthmatic patients.

Consistent with previous reports,^{10,11} peT_H2 cells have a highly differentiated phenotype that requires multiple rounds of T_H2 differentiation (Fig 4). Furthermore, hPGDS is upregulated when T_H2 cells were stimulated with TSLP-treated dendritic cells.¹⁸ These findings support a model wherein chronic antigen exposure in a TSLP-rich milieu drives peT_H2 cell differentiation and consequent eosinophilic inflammation. This suggests that antigens associated with chronic or repeated exposure, such as cockroach, HDM, or helminth antigens, will most efficiently generate peT_H2 cells. Indeed, cockroach and HDM are among the allergens most highly associated with asthma. Multiple rounds of T_H2 differentiation are required to generate ST2-expressing T_H2 cells.²⁸ Taken together with the current study, this suggests that chronic allergen exposure drives peT_H2 cell differentiation, ultimately resulting in peT_H2 responsiveness to innate signals. This supports a model wherein chronic antigen stimulation and differentiation ultimately results in an adoptive immune response acquiring innate features.⁴²

peT_H2 cells express greater CCR3 and demonstrate enhanced chemotaxis to CCL11, suggesting a mechanism for their trafficking to sites of allergic inflammation. CCR3 was originally characterized as a T_H2 -specific chemokine receptor,^{32,34,43} but its expression by T_H2 cells has been inconsistently reproducible.⁴⁴⁻⁴⁶ The finding that CCR3 expression is limited to the peT_H2 cell subpopulation (Fig 7) provides a simple explanation for this apparent inconsistency. It is likely that these negative studies examined cell or patient populations in which the fraction of peT_H2 cells and hence CCR3⁺ T_H2 cells was low.

The unambiguous phenotypic identification of human peT_H2 cells provides a powerful tool for investigators to track these cells in future pathogenesis studies and clinical trials. We used both hPGDS and CD161 as peT_H2 cell phenotype markers to allow us the greatest versatility in choice of fluorochromes and panel design. Future work is needed to fully define the relative strengths and weaknesses of these peT_H2 markers. Although the current study is limited to flow cytometry, hPGDS is detectable by using immunohistochemistry,⁴⁷ suggesting that hPGDS can be used to identify peT_H2 cells in conventional formalin-fixed tissue.

Human peT_H2 cells share multiple features with 2 recent reports of their murine counterpart. Endo et al^{9,14} have reported a CD62L^{lo}CXCR3^{lo} peT_H2 cell subpopulation that preferentially expresses IL-5 and GATA3 and is negatively regulated by

omesodermin. Notably, the human $\text{peT}_{\text{H}2}$ cell population characterized in this report recapitulates all of those features. Islam et al.^{10,12} have identified a highly polarized IL-5^+ $\text{T}_{\text{H}2}$ cell subpopulation that expresses CCR8 and requires multiple rounds of $\text{T}_{\text{H}2}$ polarization to acquire this phenotype. Similarly, the human $\text{peT}_{\text{H}2}$ cells identified in this study are highly differentiated and require multiple rounds of $\text{T}_{\text{H}2}$ polarization. However, in this work CCR8 was not differentially expressed between the 2 $\text{T}_{\text{H}2}$ subpopulations, despite using 2 different CCR8 mAb clones and a variety of activation conditions. This might be due to the limitations of currently available CCR8 reagents, subtle differences in $\text{T}_{\text{H}2}$ cell activation state, or differences between the *ex vivo* $\text{peT}_{\text{H}2}$ cells used in the current study and the *in vitro*-polarized $\text{T}_{\text{H}2}$ cells used to generate CCR8^+ $\text{T}_{\text{H}2}$ cells in the prior study.

In summary, these findings demonstrate that $\text{hPGDS}^+\text{CD161}^{\text{hi}}$ $\text{peT}_{\text{H}2}$ cells are a pathogenic proeosinophilic human $\text{T}_{\text{H}2}$ cell subpopulation with enhanced effector function and an innate lymphoid program. The distinct phenotype, function, and association of $\text{peT}_{\text{H}2}$ cells with multiple allergic inflammatory disorders, including EGID, AD, and asthma, suggests they play a principal role in allergen-driven $\text{T}_{\text{H}2}$ -mediated eosinophilic inflammation. As such, $\text{peT}_{\text{H}2}$ cells are a potential target in the treatment of allergic eosinophilic inflammation.

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Key messages

- $\text{peT}_{\text{H}2}$ cells are $\text{CRTH2}^+\text{hPGDS}^+\text{CD161}^{\text{hi}}$.
- $\text{peT}_{\text{H}2}$ cells preferentially demonstrated greater responsiveness to innate pro- $\text{T}_{\text{H}2}$ cytokines.
- $\text{peT}_{\text{H}2}$ cell counts correlated highly to blood eosinophilia and associated with eosinophilic gastrointestinal disease and AD, suggesting a potential role in pathogenesis.

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METHODS

Isolation of cells

Patients with EGID, NA subjects, and patients with allergic asthma underwent lymphapheresis (National Institutes of Health Clinical Center, Department of Transfusion Medicine), and PBMCs were isolated by using density gradient separation (Lymphocyte Separation Medium; MP Biomedicals, Santa Ana, Calif), washed twice in HBSS (Mediatech, Manassas, Va), cryopreserved in RPMI (Mediatech) with 10% human serum albumin (Talecris Biotherapeutics, Research Triangle Park, NC), and stored in liquid nitrogen. On thawing, cells were treated with 60 μ g/mL DNase I (Calbiochem, San Diego, Calif) and cultured in complete medium (RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μ g/mL streptomycin [Invitrogen, Carlsbad, Calif], nonessential amino acids, 1 mmol/L sodium pyruvate [Mediatech], and 50 μ mol/L 2-mercaptoethanol [Sigma-Aldrich, St Louis]) at 37°C in a 5% CO₂ incubator.

Human gut single-cell suspensions were obtained from patients with EGID and NA subjects during research and indicated endoscopic biopsy of the esophagus, stomach, and duodenum. Solid tissues were digested in 200 μ g/mL Liberase (Roche Applied Science, Mannheim, Germany) and 0.05% DNase I in RPMI 1640 for 30 to 60 minutes at 37°C. Cell suspensions were filtered twice through a 70- μ m nylon strainer (BD Biosciences, San Jose, Calif), labeled with Live/Dead Fixable Aqua Dead Cell Stain (Invitrogen), fixed with 4% paraformaldehyde (Sigma), and cryopreserved at -80°C for future analysis.

Cell culture

For intracellular cytokine staining, PBMCs were stimulated for 6 hours with 20 ng/mL PMA (Sigma) and 1 μ mol/L ionomycin (Calbiochem) or for 24 hours with various combinations of the following cytokines at 10 ng/mL unless otherwise stated: IL-2 (50 U/mL; Novartis, New York, NY), IL-7 (PeproTech, Rocky Hill, NJ), IL-25, IL-33, and TSLP (all from R&D Systems, Minneapolis, Minn). For intracellular cytokine staining, activated cells were treated with 10 μ g/mL Brefeldin A (Sigma) for the final 6 hours of the above culture conditions. CCR8 was examined after 24 hours of culture with 2 μ g/mL PHA (Murex Biotech, Dartford, United Kingdom).

T_H2 cell lines

T_H1 and T_H2 antigen-specific T_H2 cell lines were generated as previously published.^{E1} Briefly, PBMCs from allergic asthmatic patients were activated for 2 to 4 days with 100 AU/mL HDM antigen (ALK, Round Rock, Tex) or 400 ng/mL respiratory syncytial virus antigen (Bioscience International, Saco, Me), after which they were expanded in 100 U/mL IL-2 for a total of 14 days. Respiratory syncytial virus-specific T_H1 cell lines were generated in the same manner. Selective expression of T_H2 or T_H1 cytokines was confirmed by means of intracellular cytokine staining at day 14. T_H2 differentiation was performed according to published methods.^{E1,E2} Naive CD4⁺ T cells were isolated from PBMCs by using a naive CD4⁺ T-cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany); polarized for 4 days with plate-bound 5 μ g/mL anti-CD3 (clone OKT3; BioLegend, San Diego, Calif), soluble 1 μ g/mL anti-CD28 (clone CK248; in house), 100 U/mL IL-2, 12.5 ng/mL IL-4 (PeproTech), 5 μ g/mL anti-IFN- γ (B133.5; Bio X Cell, West Lebanon, NH), and 5 μ g/mL anti-IL-10 (JES3-9D7; BD Biosciences); and expanded for an additional 3 days in IL-2-containing media. Polarization cycles were repeated weekly. Proliferation dye dilution was measured with 5 μ mol/L Cell Trace Violet (Invitrogen), according to the manufacturer's instructions. Proliferated cells were calculated as the percentage of cells with Cell Trace Violet dye dilution 2 or more generations beyond the unproliferated peak.^{E3,E4}

Flow cytometry

Cells were labeled at 4°C with Live/Dead Fixable Aqua Dead Cell Stain. The following antibodies (clones in parenthesis) were used in various fluorochrome combinations to identify T_H2 cells and ILC2s: CD4 (SK3),

CRTH2 (BM16), and Lin-1 (CD3, CD14, CD16, CD19, CD20, CD56 mAb cocktail; BD Biosciences); CD3 (HIT3A), CD123 (6H6), CD161 (HP-3G10), CD4 (SK3), CD45 (HI30), and Fc ϵ RI (AER-37; all from eBioscience, San Diego, Calif); and CD3 (UCHT1) and CD8 (3B5) (Invitrogen).

Phenotypic analyses were conducted on live cells by using the following antibodies: CD27 (L128), CD45RO (UCHL1), HLA-DR (G46-6), programmed cell death 1 (MIH4; all from BD Biosciences); CCR3 (5E8), CCR7 (G043H7), CCR8 (L263G8; data not shown), CD25 (BC96), CD127 (A019D5), CLA (HECA-452), CXCR3 (G025H7), TSLP receptor (1B4), and T-cell receptor V α 7.2 (3C10) and V α 24 (C15; all from BioLegend); CD62 L (DREG-56) and TSLP receptor (1A6; both from eBioscience); ST2 (C2240) and isotype (CINTO8937; kindly supplied by Janssen Research & Development); CCR4 (205410) and IL-17RB (170220) (all from R&D Systems); unlabeled CCR8 (433H; generously provided by Drs Andrew Luster and Morris Ling, Massachusetts General Hospital; data used in Fig 7 and Fig E4); anti- α 4 β 7 integrin (Act-1; National Institutes of Health AIDS Reagent Program); and mouse IgG₁ (MOPC-21; Sigma). Goat anti-mouse IgG allophycocyanin/Alexa Fluor 750 or phycoerythrin (PE; all from Invitrogen) or goat anti-mouse IgG PE/Cy7 (BioLegend) were used as secondary reagents. For indirect staining, cells were serially stained with primary and secondary antibody, blocked with 2% mouse serum (Invitrogen), and stained with directly labeled mAbs.

The following antibodies were used for intracellular staining: anti-hPGDS (EBC45; BioMedical Laboratories) unlabeled and in house Alexa Fluor 647 conjugate; IL-5 (JES1-39D10) and IL-13 (JES10-5A2; both from BD Biosciences); and IFN- γ (B21), IL-9 (MH9A4), and IL-17 (BL168) (all from BioLegend). Intracellular staining was performed according to previously published methods.^{E1,E5} Transcription factor and nuclear antigen expression was investigated by using the Foxp3/Transcription Factor Staining Buffer Set, according to the manufacturer's protocol, with the following antibodies: GATA3 (TWAJ), eomesodermin (WD1928), and Ki-67 (20Raj1; all from eBioscience).

Multiple combinations of antibodies were used for flow cytometric analysis. The following is an example of cytokine staining for the T_H2 subpopulations used in Figs 1 and 2, A-D. Cryopreserved PBMCs were thawed, recovered overnight in complete medium, and activated with PMA, ionomycin, and Brefeldin A. Cells were stained with Aqua Live/Dead viability dye, fixed with 4% paraformaldehyde, washed, permeabilized in 0.1% saponin, and stained at room temperature (CD3 Qdot 605, CD4 peridinin-chlorophyll-protein complex/Cy5.5, CD8 PE/Texas Red, CRTH2 PE, CD161 BV421, IL-5 fluorescein isothiocyanate, PE/Cy7 IL-13, IFN- γ Alexa Fluor 700, and hPGDS Alexa Fluor 647). Intracellular staining for CD4 with clone SK3 and the use of a CD4 versus CD8 dot plot facilitated the discrimination of CD4⁺ T cells in these PMA/ionomycin-activated cultures.^{E5} For flow cytometry of surface receptor expression, cells were thawed, stained with Aqua Live/Dead, washed, and stained for surface markers; in some cases cells were additionally washed, fixed with 4% paraformaldehyde, washed, permeabilized in 0.1% saponin, and stained for hPGDS.

Data were acquired on an LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar, Ashland, Ore). Lymphocytes were identified by back gating on the forward versus side scatter of CRTH2⁺ T cells to include all lymphocytes and excluded for cell doublets by using forward scatter area versus height. T_H2 subpopulations were analyzed after gating on viable CD3⁺CD4⁺CD8⁻ cells. Unimodal and bimodal histograms were quantitated by using mean fluorescence intensity and percent positive, respectively. Boolean analysis was performed with FlowJo software and exported to Prism software; figures using Boolean analysis include a key adjacent to the figure panel. Bar graphs of Boolean analyses represent the median values normalized to 100%.

Cell sorting and quantitative PCR

CRTH2⁺ T_H2 cells were enriched from PBMCs by staining with anti-CRTH2 PE and positively selecting with anti-PE immunomagnetic beads on an LS column (Miltenyi Biotec). Cells were labeled with 7AAD (eBioscience) and mAbs to CD3, CD4, CD123, CD161, and CRTH2. Viable

CD3⁺CD4⁺CD123[−]CRTH2⁺ cells were sorted into CD161^{hi} and CD161[−] fractions on a FACSaria (BD Biosciences). Sorted populations were 93% to 97% pure.

Each fraction was cultured for 6 hours in complete medium with or without IL-2, IL-25, and IL-33. RNA was extracted from cell pellets by using TRIzol reagent, precipitated with 10 μ g of glycogen, and treated with DNase I (all from Invitrogen) to remove genomic DNA. cDNA was synthesized from 250 ng ($n = 3$) or 180 ng ($n = 1$) of RNA by using the RT² First Strand Kit, and RT-PCR was carried out with the RT² SYBR Green Mastermix and Human Allergy & Asthma RT² PCR Profiler (all from Qiagen, Hilden, Germany), according to the manufacturer's protocol. The StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, Calif) was used for thermal cycling and fluorescence detection. All samples were normalized to the average expression of the housekeeping genes *ACTB*, *B2M*, *GAPDH*, *HPRT1*, and *RPL13A*; results are presented as $2^{-\Delta C_t}$.^{E6}

Chemotaxis assay

CCL11 (PeproTech) was diluted in chemotaxis medium (RPMI 1640, 0.5% BSA [Sigma], and 25 mmol/L HEPES [Mediatech]) and added at 500 ng/mL to the lower wells of a 24-well Transwell chemotaxis plate (3- μ m pore size, polycarbonate membrane; Corning, Corning, NY). CD4⁺ T cells were isolated from PBMCs from patients with EGID by using a human CD4⁺ T cell isolation kit II (Miltenyi Biotec) and seeded at 8 to 10 $\times 10^5$ cells per chamber. Loaded chambers were preincubated in chemotaxis medium for 30 minutes at 37°C in 5% CO₂ and transferred to wells with chemokine. After 3 hours, migrated cells were harvested from lower wells and stained for the appropriate markers. CD161[−] and CD161^{hi} T_H2 cells were enumerated by using flow cytometry. Percentage migration was calculated by dividing the number of migrated cells of each T_H2 cell subset (positive events in the

medium control subtracted from the test well) by the total number of cells of each T_H2 subset contained within the CD4⁺ T cells initially seeded into the transwell.

PGD₂ secretion assay

Sorted CD161[−] and CD161^{hi} T_H2 cells were seeded at 4 $\times 10^4$ cells/mL into RPMI 1640 medium supplemented with 2% FBS, 100 U/mL penicillin, 100 μ g/mL streptomycin, nonessential amino acids, 1 mmol/L sodium pyruvate, and 50 μ mol/L 2-mercaptoethanol and cultured for 10 hours in the indicated conditions. Concentrations of PGD₂ in culture supernatants were measured with the Prostaglandin D₂-MOX Enzyme Immunoassay kit (Cayman Chemical, Ann Arbor, Mich).

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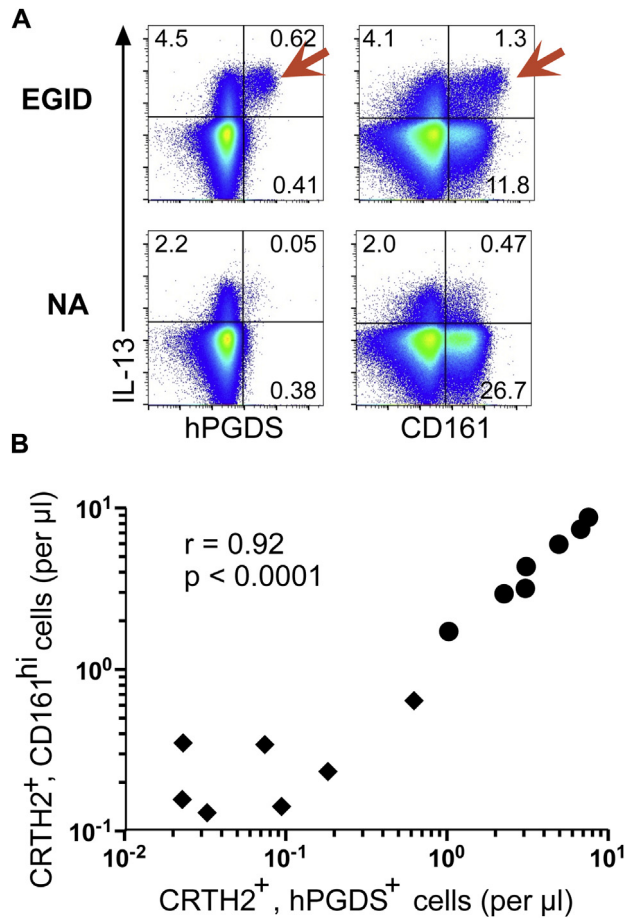


FIG E1. CD161 and hPGDS define a T_H2 cell subpopulation. **A**, PBMCs from the indicated subject groups were activated and stained as in Fig 1 to examine IL-13, hPGDS, and CD161 coexpression by CD4 T cells. Data are representative of 7 patients with EGID and 7 NA subjects. The red arrows represent the analogous T_H2 cell subpopulation in each dot plot. **B**, Correlation of the absolute peripheral blood cell count for the indicated T_H2 cell subpopulations for patients with EGID (circles) and NA subjects (diamonds).

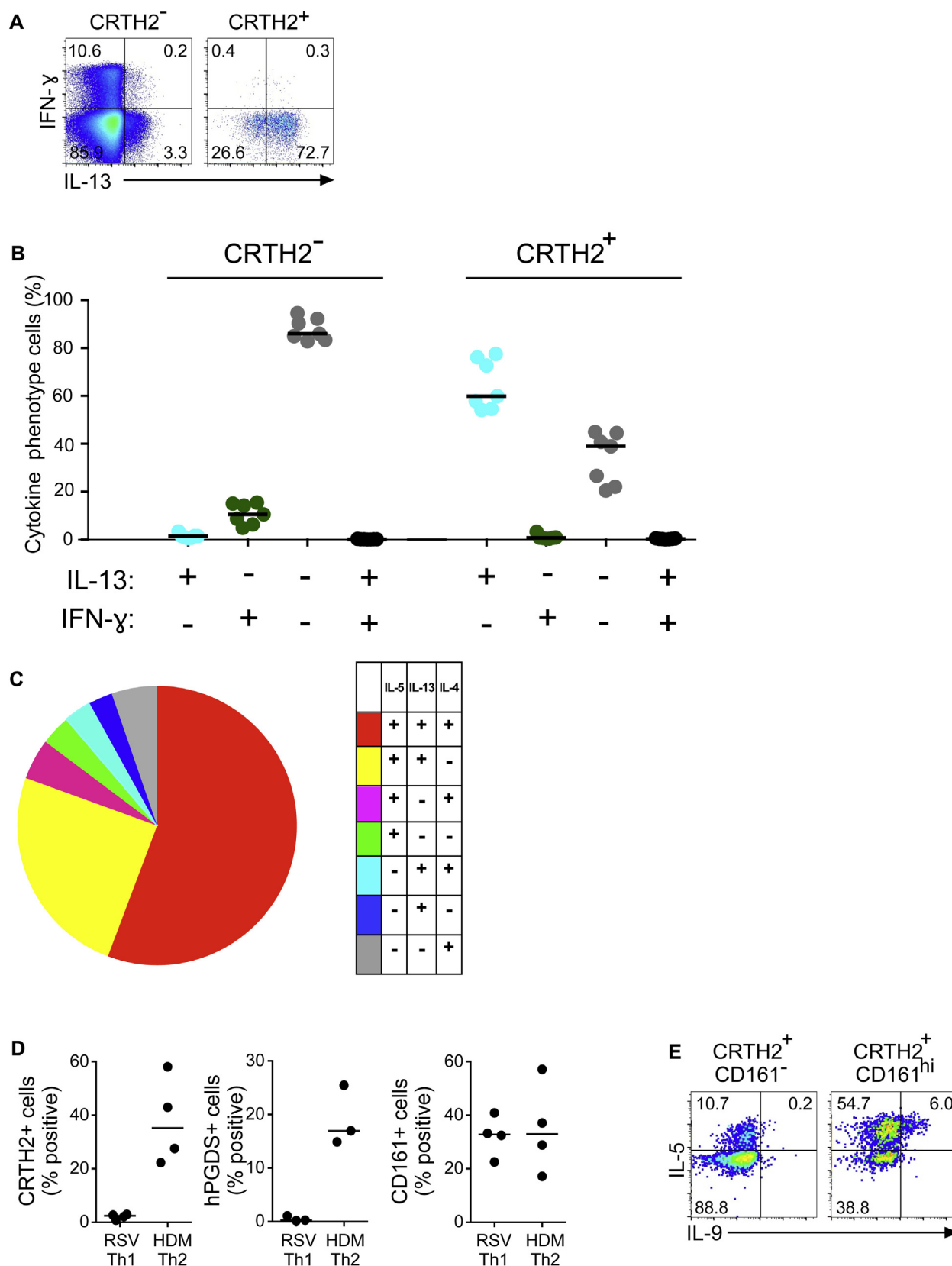


FIG E2. Cytokine expression by T_H2 cell subpopulations. **A**, PBMCs from patients with EGID were activated and stained to examine coexpression of IFN- γ and IL-13 in CRTH2 $^-$ and CRTH2 $^+$ CD4 T-cell subpopulations. Data are representative of 7 subjects. **B**, Boolean analysis of the above showing combined results of 7 patients with EGID. **C**, Boolean analysis of IL-4/IL-5/IL-13 coexpression within the CRTH2 $^+$ CD161 ^{hi} hPGDS $^+$ CD4 T-cell subpopulation. The key is to the right of the pie chart. Each pie segment represents the median values of 5 patients with EGID. **D**, Respiratory syncytial virus (RSV)-specific T_H1 and HDM-specific T_H2 cell lines were analyzed for expression of CRTH2, hPGDS, and CD161 ($n = 3$ -4 cell lines). **E**, IL-5 versus IL-9 dot plots in the noted T_H2 cell subpopulations. Data are representative of 3 patients with EGID.

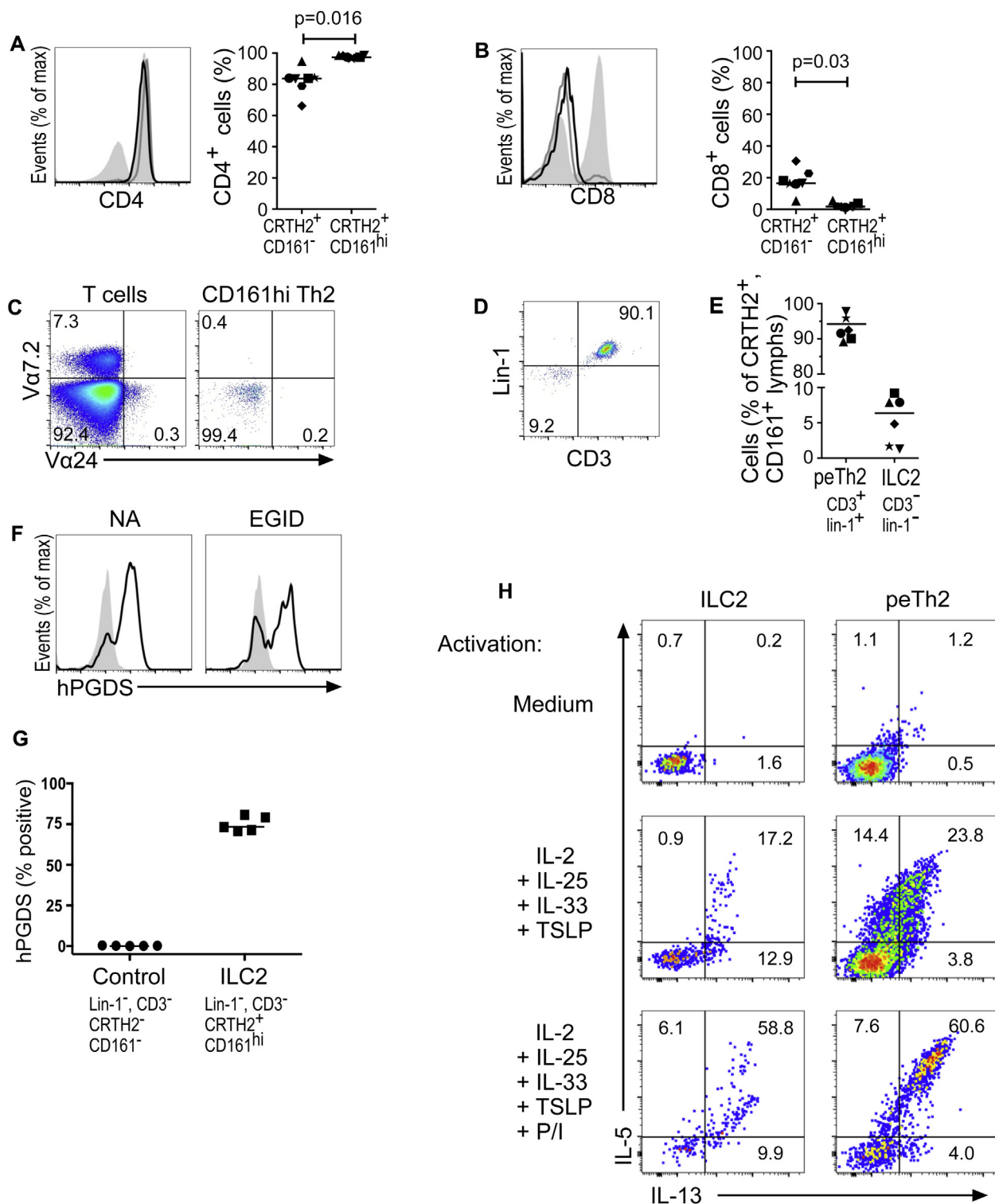


FIG E3. peTh2 cells are effector memory CD4 T_H2 cells. **A** and **B**, CRTH2⁺CD161^{hi} (black line), CRTH2⁺CD161^{lo} (gray line), and CRTH2⁺CD161^{lo} (light gray shading) T cells from PBMCs of patients with EGID were analyzed for expression of CD4 (Fig E3, A) and CD8 (Fig E3, B). **C**, CD161^{hi} T_H2 cells were examined for expression of the MAIT and natural killer T (NKT) cell invariant T-cell receptor (TCR) α chains Vα7.2 and Vα24. **D**, CD3 versus Lin-1 panel staining of CRTH2⁺CD161^{hi} lymphocytes from PBMCs of patients with EGID. **E**, Combined data showing the relative percentage of peTh2 cells (CD3⁺Lin-1⁺) versus ILC2s (CD3⁺Lin-1⁺) within the CRTH2⁺CD161^{hi} lymphocyte gate. **F**, Expression of hPGDS by ILC2s, defined as Lin-1⁺CD3⁺CRTH2⁺CD161^{hi} lymphocytes in PBMCs of patients with EGID. Black line, Anti-hPGDS antibody; light gray shading, isotype control. **G**, Combined data showing hPGDS expression in ILC2s and a Lin-1⁺CD3⁺CRTH2⁺CD161^{lo} control lineage-negative cell population. **H**, PBMCs were activated with the noted stimuli, and cytokine production was measured after gating on either ILC2s or peTh2 cells. Data are representative of 5 patients with EGID. P/I, Phorbol myristate acetate, ionomycin.

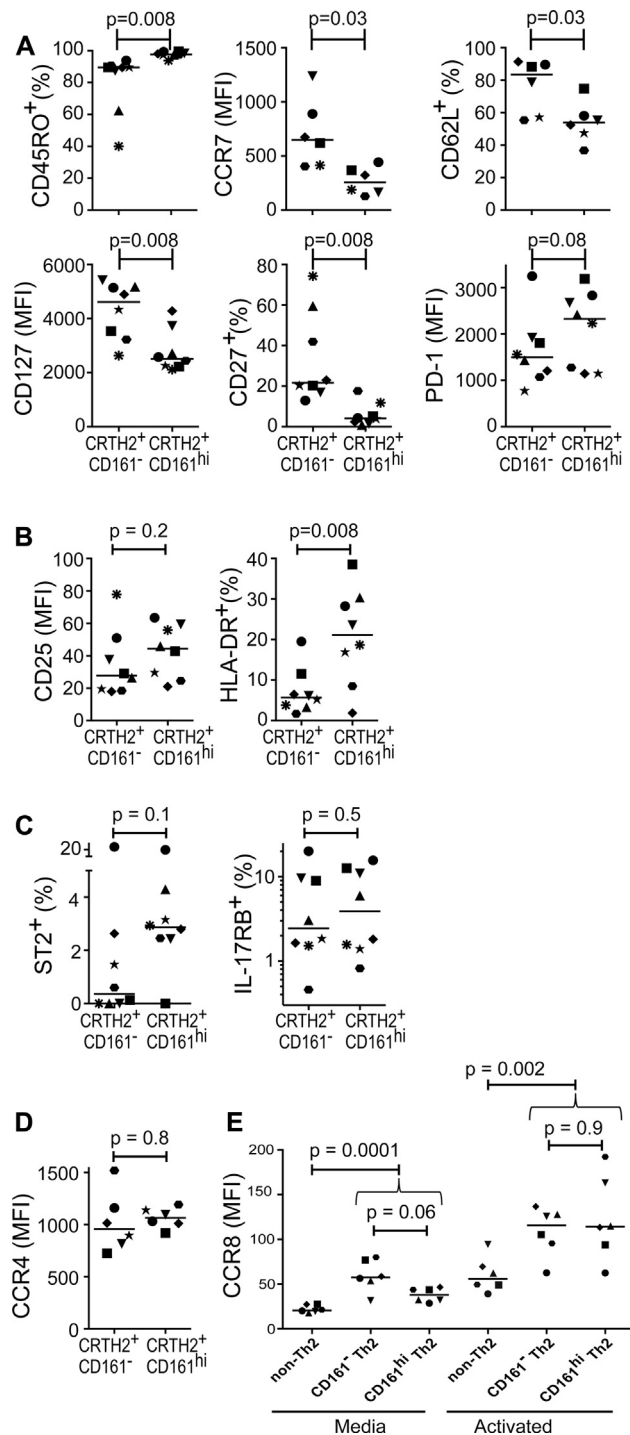


FIG E4. *Ex vivo* phenotype of peT_H2 cells. **A-D**, Combined results of lymphocyte memory (CD45RO, CCR7, CD62 L, and CD127) and differentiation (CD27 and programmed cell death 1 [PD-1]) markers (Fig E4, A), CD25 and HLA-DR (Fig E4, B), IL-17RB and ST2 (Fig E4, C), and CCR4 (Fig E4, D) in CD161^{hi} and CD161⁻ T_H2 cells from donors with EGID. **E**, CCR8 (using clone 433H) expression by T_H2 subpopulations after 24 hours of culture in medium or PHA (n = 6-8 different donors with EGID per experiment). *MFI*, Mean fluorescence intensity.

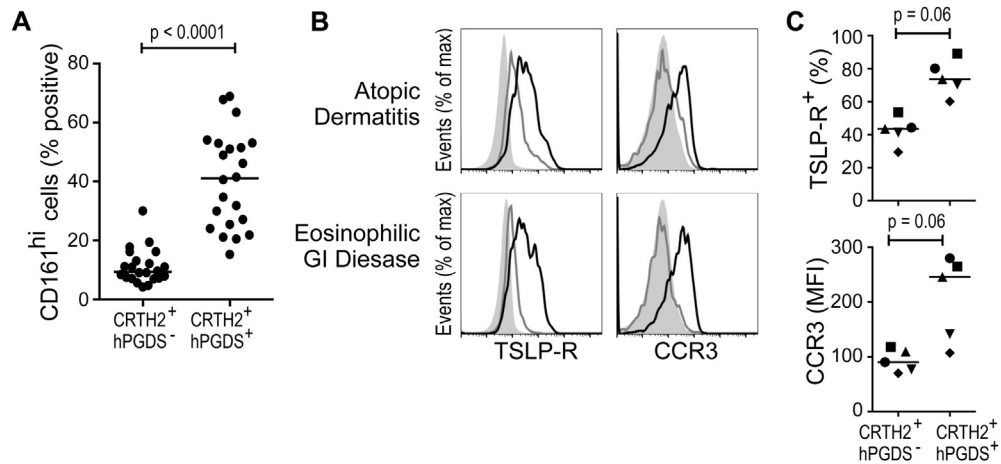


FIG E5. Characterization of T_H2 cell heterogeneity in patients with AD. **A**, CD161 expression by hPGDS⁺ and hPGDS⁻ T_H2 cells in patients with AD (n = 22). **B**, Flow cytometric analysis of TSLP receptor and CCR3 expression by hPGDS⁺ T_H2 cells (black line), hPGDS⁻ T_H2 cells (gray line), and non-T_H2 cells (light gray shading) in CD4 T cells from the indicated subject groups. *GI*, Gastrointestinal. **C**, Combined phenotype data of T_H2 cell subsets from patients with AD. Each symbol represents 1 patient. *MFI*, Mean fluorescence intensity.

TABLE E1. Correlation of absolute T_H2 cell subpopulation counts versus absolute blood eosinophil counts in patients with EGID and nonatopic subjects (Spearman rank correlation)

Absolute T _H 2 cell count vs absolute eosinophil count		
Cell population	<i>r</i>	<i>P</i> value
CRTH2 ⁺ hPGDS ⁺	0.98	<.0001
CRTH2 ⁺ CD161 ^{hi}	0.91	<.0001
CRTH2 ⁺ hPGDS ⁻	0.56	.04
CRTH2 ⁺ CD161 ⁻	0.52	.06
CRTH2 ⁺	0.82	.0005
IL-5 ⁺	0.81	.0008

TABLE E2. Correlation of absolute T_H2 cell subpopulation counts versus absolute blood eosinophil counts in patients with AD (Spearman rank correlation)

Cell population	Absolute T _H 2 cell count vs absolute eosinophil count	
	<i>r</i>	<i>P</i> value
CRTH2 ⁺ hPGDS ⁺	0.78	<.0001
CRTH2 ⁺ CD161 ^{hi}	0.4	.06
CRTH2 ⁺ hPGDS [−]	0.62	.002
CRTH2 ⁺ CD161 [−]	0.58	.004
CRTH2 ⁺	0.68	.0005