

Letter to the Editor

Allergen challenge in allergic rhinitis rapidly induces increased peripheral blood type 2 innate lymphoid cells that express CD84

To the Editor:

Type 2 innate lymphoid cells (ILC2s) have recently been discovered and include nuocytes, natural helper cells, and innate type 2 helper cells.^{1,2} ILC2s do not express known surface markers for T, B, natural killer, or natural killer T cells (lineage-negative) and produce large amounts of T_H2 cytokines in response to cytokines IL-33, IL-25, thymic stromal lymphopoietin, and leukotriene D₄.²⁻⁴ Studies in animal models have shown that ILC2s contribute to allergen-induced airway hyperresponsiveness and type 2 lung inflammatory responses, suggesting that ILC2s may have a role in asthma and allergic disease.² Human ILC2s have been detected in peripheral blood, gastrointestinal tract, lung, bronchoalveolar lavage, and nasal polyps and are defined as lineage-negative lymphocytes that express the chemoattractant receptor homologous molecule expressed on T_H2 lymphocytes (CRTH2).⁵ We have previously reported that peripheral blood ILC2s highly express the master T_H2 cytokine transcription factor GATA-binding protein 3, supporting a role for rapid ILC2 T_H2 cytokine production.⁶

Despite the potential for ILC2s to contribute to human allergic disease, no studies have yet reported whether allergen exposure in sensitized allergic rhinitis subjects has any effect on peripheral blood ILC2 levels. Thus, we hypothesized that CRTH2+ ILC2s may be increased in the peripheral blood of cat-allergic individuals after nasal cat allergen challenge.

To determine whether changes in the levels of peripheral blood ILC2s occurred after nasal cat allergen challenge, we recruited 7 cat-sensitized (skin prick test result positive) adults (age range of 20-27 years old; 4 females; 3 males) with a current history of rhinitis symptoms on cat exposure to undergo nasal challenge with cat allergen extract and diluent control (see Fig E1 in this article's Online Repository at www.jacionline.org). Following approval from the University of California, San Diego Human Subjects Protection Committee and the Food and Drug Administration (Investigational new drug no. 15185), subjects presented for 2 study visits. During the initial visit, subjects underwent a nasal diluent control challenge with lactated ringers solution. Subjects then returned 2 to 12 weeks later for nasal cat allergen challenge, consisting of 3 consecutively increasing concentrations (4 bioequivalent allergy units, 40 bioequivalent allergy units, and 400 bioequivalent allergy units) of Hollister-Stier cat allergen extract administered at 10-minute intervals, in

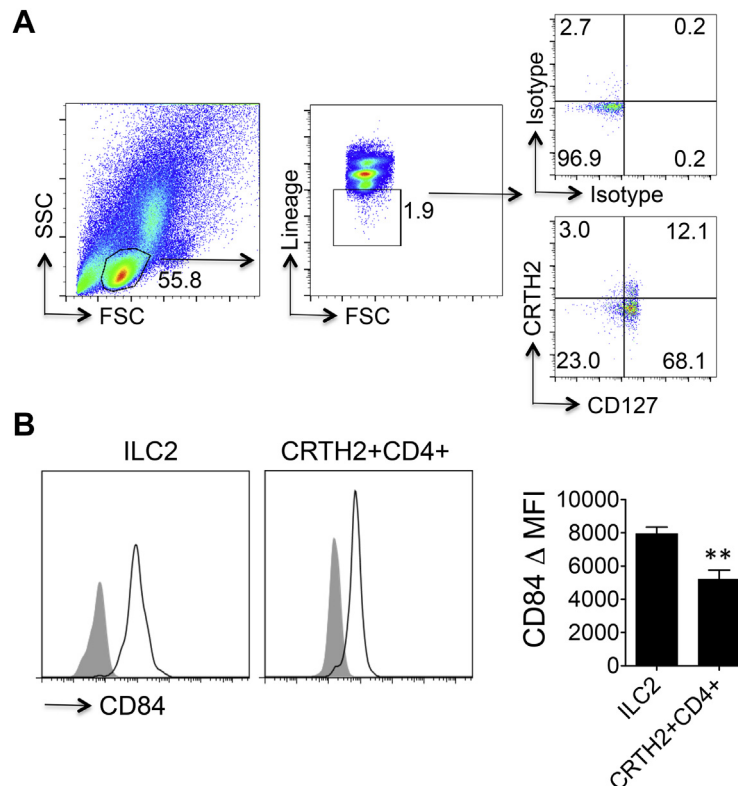


FIG 1. **A**, Lymphocytes were identified from whole PBMCs (*left*) and lineage-negative cells gated (*middle*). Lineage-negative lymphocytes were further assessed for expression of CD127 and CRTH2 or isotype control staining (*right*). ILC2s were identified as lineage-negative CRTH2+ lymphocytes. Example shown is 4 hours after cat allergen exposure in cat-allergic individual. **B**, ILC2s (*left*) and CRTH2+ CD4+ cells (*middle*) were analyzed for baseline CD84 expression in cat-allergic individuals and change in median fluorescent intensity (MFI) compared with respective isotype determined (*right*). FSC, Forward scatter; SSC, side scatter. ** $P < .005$, t test, $n = 5$ individual subjects.

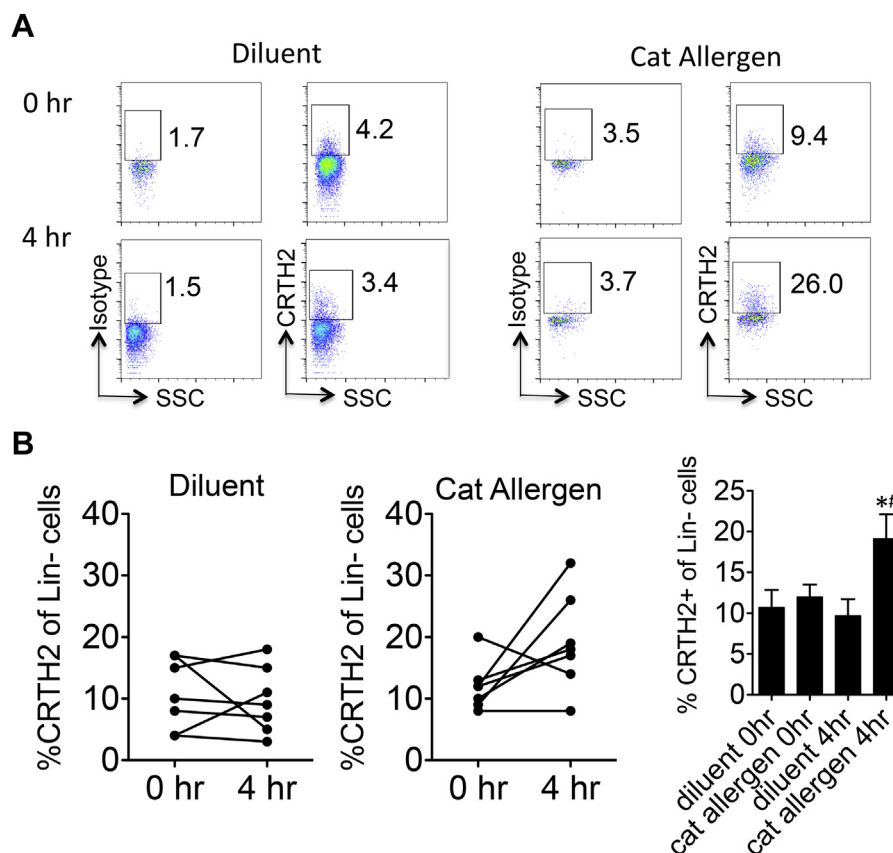


FIG 2. A, ILC2s were analyzed in peripheral blood before (0 hour) and 4 hours after nasal challenge with diluent (left) or cat allergen (right). Lineage-negative lymphocytes gated and CRTH2 expression or isotype staining shown from 1 subject. B, Percentage of CRTH2+ cells of lineage-negative population measured at 0 and 4 hours after diluent (left) and cat allergen challenges (middle). Total percentage of CRTH2+ cells at each time point enumerated (right). SSC, Side scatter. * $P < .05$ compared with diluent at 4 hours, # $P = .05$ compared with cat allergen at 0 hours, t test, $n = 7$ individual subjects.

two 100- μ L sprays in each nostril (SGD North America bottle, MeadWestVaco nozzle). Peripheral venous blood was collected immediately before and 4 hours after the nasal diluent and allergen challenges. Symptoms were scored by using the total nasal symptom score at time points before and after nasal challenges for up to 4 hours (see Fig E2 in this article's Online Repository at www.jacionline.org). The total nasal symptom score area under the curve was calculated to determine the level of immediate response after diluent or cat challenge (see Fig E3 in this article's Online Repository at www.jacionline.org).

PBMCs from each time point were isolated by density gradient centrifugation in sodium citrate Vacutainer Cell Preparation Tubes (BD, Franklin Lakes, NJ). PBMCs were stained with a fluorescein isothiocyanate lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56; BD), TCR $\gamma\delta$ (BD), CD4, CD11b, CD235a, and Fc ϵ RI (eBioscience, San Diego, Calif), which allows for exclusion of B, T, natural killer, and NKT cells as well as mast cells and basophils. Cells were further stained with phycoerythrin-conjugated CRTH2 or biotin-conjugated CRTH2 (Miltenyi, Auburn, Calif) followed by streptavidin allophycocyanin and phycoerythrin-conjugated CD84 (Biolegend, San Diego, Calif). Flow cytometry was performed by using the BD Accuri flow cytometer machine and analyzed with FlowJo software (Tree Star, Inc, Ashland, Ore). The ILC2 population in allergic subjects was defined as lymphocytes that are lineage-negative and express

CRTH2 (Fig 1, A). ILC2s are also known to express the IL-7 receptor alpha subunit (CD127) and nearly all the CRTH2+ cells expressed CD127 as expected (Fig 1, A). Peripheral blood ILC2s were also detected in nonallergic individuals (see Fig E4 in this article's Online Repository at www.jacionline.org).

Human ILC2s are a recently discovered population of cells that are known to express CRTH2 and CD161,⁵ but reported expression of other markers is very limited and we thus sought to identify expression of novel human ILC2 surface molecules. Because mouse ILC2s express molecules involved in T-cell-B-cell interactions including Inducible T-cell Costimulator,² we assessed ILC2s for levels of CD84, a CD2 signaling lymphocyte activation molecule family member with a role in T-cell-B-cell contact.⁷ Interestingly, we detected high levels of CD84 on peripheral blood ILC2s from cat-allergic individuals at baseline (Fig 1, B). CD84 expression has been identified on human T cells, and we compared levels of ILC2 CD84 expression with peripheral blood T_H2 cells (CD4+ CRTH2+ cells; see Fig E5 in this article's Online Repository at www.jacionline.org). ILC2s expressed CD84 at significantly higher levels in allergic subjects than did T_H2 cells based on change in median fluorescence intensity (Fig 1, B). ILC2s from nonallergic individuals also showed increased CD84 expression compared with T_H2 cells (see Fig E6 in this article's Online Repository at www.jacionline.org). Thus, we

demonstrate the novel finding that human ILC2s highly express the signaling lymphocyte activation molecule family member CD84, although this expression is not selective for ILC2s. Allergen challenge did not increase levels of CD84 expression on ILC2s or CD4 cells.

We next assessed the percentage of CRTH2+ ILC2s in the peripheral blood of cat-allergic subjects before and 4 hours after nasal cat allergen or diluent challenges (Fig 2). The baseline percentage of CRTH2+ cells within the lineage-negative population was 10.7 ± 1.9 and 12.0 ± 1.3 at the diluent and cat allergen challenge visit, respectively (Fig 2, B). Four hours after diluent challenge, the percentage of CRTH2+ cells did not change significantly (9.7 ± 1.8) compared with time zero. However, after cat allergen challenge, the percentage of CRTH2+ cells nearly doubled to 19.1 ± 2.6 compared with baseline ($P = .05$) and compared with diluent challenge at 4 hours ($P < .05$) (Fig 2, B). Thus, nasal cat allergen challenge induced an increased percentage of peripheral blood CRTH2+ ILC2s when measured 4 hours after challenge.

ILC2s produce large amounts of IL-5 and IL-13 in response to IL-25, IL-33, TSLP, and LTD4 and could initiate and/or propagate allergic airway inflammation. Our studies demonstrate that the percentage of CRTH2+ ILC2s in the peripheral blood is rapidly increased (within 4 hours) after allergen challenge. Potential mechanisms for the increase in ILC2s in the peripheral blood may be due to enhanced recruitment of ILC2s from the bone marrow triggered by either humoral (cytokine, chemokine, or mediator production in the nose) and/or cellular mechanisms (cells released from the nasal mucosa trafficking to the bone marrow). The human ILC2 marker CRTH2 is the receptor for prostaglandin D2 (PGD2), a lipid mediator that has a known role in chemotaxis and activation of immune cells. Importantly, a previous study demonstrated that high levels of serum $9\alpha,11\beta$ -PGF₂, the major PGD2 metabolite, are induced within 5 minutes after airway allergen challenge, suggesting that PGD2 is rapidly available systemically for the recruitment of CRTH2+ cells after allergen exposure.⁸ We have also recently determined that PGD2 induces chemotaxis of CRTH2+ human blood ILC2s *in vitro*, suggesting that PGD2 may directly regulate the migration of human ILC2s into tissues.⁹ The role of increased peripheral blood ILC2 numbers after allergen challenge is unclear. One hypothesis is that greater ILC2 availability in the blood (within 4 hours after challenge) may result in greater numbers of cytokine-producing nasal mucosa ILC2s at later time points, but this would need to be investigated in future studies. Strategies to inhibit the recruitment of ILC2s in allergic individuals may reduce tissue T_H2 cytokine levels that contribute to allergic inflammation.

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REFERENCES

- Kim BS, Wojno ED, Artis D. Innate lymphoid cells and allergic inflammation. *Curr Opin Immunol* 2013;25:738-44.
- Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 2013;14:536-42.
- Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol* 2013;132:205-13.
- Mjosberg J, Bernink J, Golebski K, Karich JJ, Peters CP, Blom B, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *immunity* 2012;37:649-59.
- Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 2011;12:1055-62.
- Doherty TA, Khorram N, Chang JE, Kim HK, Rosenthal P, Croft M, et al. STAT6 regulates natural helper cell proliferation during lung inflammation initiated by *Alternaria*. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L577-88.
- Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, et al. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* 2010;32:253-65.
- Bochenek G, Nizankowska E, Gielicz A, Swierczynska M, Szczeklik A. Plasma 9alpha,11beta-PGF₂, a PGD2 metabolite, as a sensitive marker of mast cell activation by allergen in bronchial asthma. *Thorax* 2004;59:459-64.
- Chang JE, Doherty TA, Baum R, Broide D. Prostaglandin D2 regulates human type 2 innate lymphoid cell chemotaxis. *J Allergy Clin Immunol* 2013 Nov 5. pii: S0091-S6749(13)01464-4. <http://dx.doi.org/10.1016/j.jaci.2013.09.020>. [Epub ahead of print].

<http://dx.doi.org/10.1016/j.jaci.2013.12.1086>

REFERENCES

- E1. Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy. *Clin Allergy* 1987;17:529-36.
- E2. Scadding GW, Calderon MA, Bellido V, Koed GK, Nielsen NC, Lund K, et al. Optimisation of grass pollen nasal allergen challenge for assessment of clinical and immunological outcomes. *J Immunol Methods* 2012;384: 25-32.

Subject #	Age (years)	M/F	Cat skin test (Wheal/flare in mm)
01	22	F	8/25
02	21	M	8/25
03	20	M	10/30
04	21	M	8/25
05	22	F	8/25
06	27	F	12/30
07	20	F	10/14

FIG E1. Subject characteristics. *F*, Female; *M*, male.

Time Point(min)	Nasal Challenge (Cat or diluent)	TNSS (range 0–12)
–2	–	+
0	+	–
8	–	+
10	+	–
18	–	+
20	+	–
30	–	+
40	–	+
50	–	+
65	–	+
80	–	+
120	–	+
180	–	+
240	–	+

TNSS: Total Nasal Symptom Score

Time Point: Time 0 min is time of first nasal challenge

+ symbol: Indicates time of 3 nasal challenges or time of recording TNSS.

– symbol: Indicates that no nasal challenge or TNSS recording was done at the corresponding time point.

FIG E2. Nasal cat or diluent challenges and total nasal symptom score (TNSS) time points. The TNSS included quantification of 4 symptoms (sneezing, nose running, nasal blockage, and itch) on a 0 to 3 scale scored 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms, and 3 for severe symptoms as described earlier.^{E1,E2} Sneezes were counted over the 10-minute interval preceding the completion of each questionnaire and scored as 0 (no sneezes), 1 (1-2 sneezes), 2 (3-4 sneezes), or 3 (5 or more). The TNSS is derived from these 4 domains (scored 0-3 each) with a maximal score of 12. Time Point: Time 0 minute is time of first nasal challenge. + indicates time of 3 nasal challenges or time of recording TNSS. – indicates that no nasal challenge or TNSS recording was done at the corresponding time point.

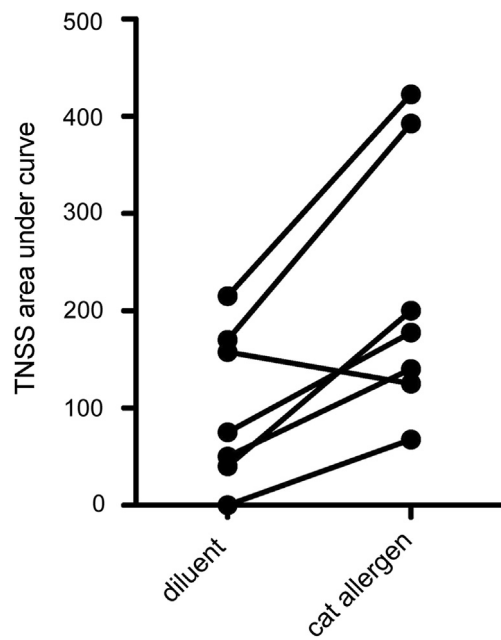


FIG E3. Nasal symptom scores after diluent or cat allergen challenges. Subjects received intranasal diluent (at visit 1) or cat allergen (at visit 2) at 2 separate visits at least 2 weeks apart. Total nasal symptom scores (TNSSs) were recorded, and the area under the curve between time 0 and 65 minutes was calculated for the immediate response. TNSS area under curve values for diluent and cat allergen shown for each patient (N = 7 subjects).

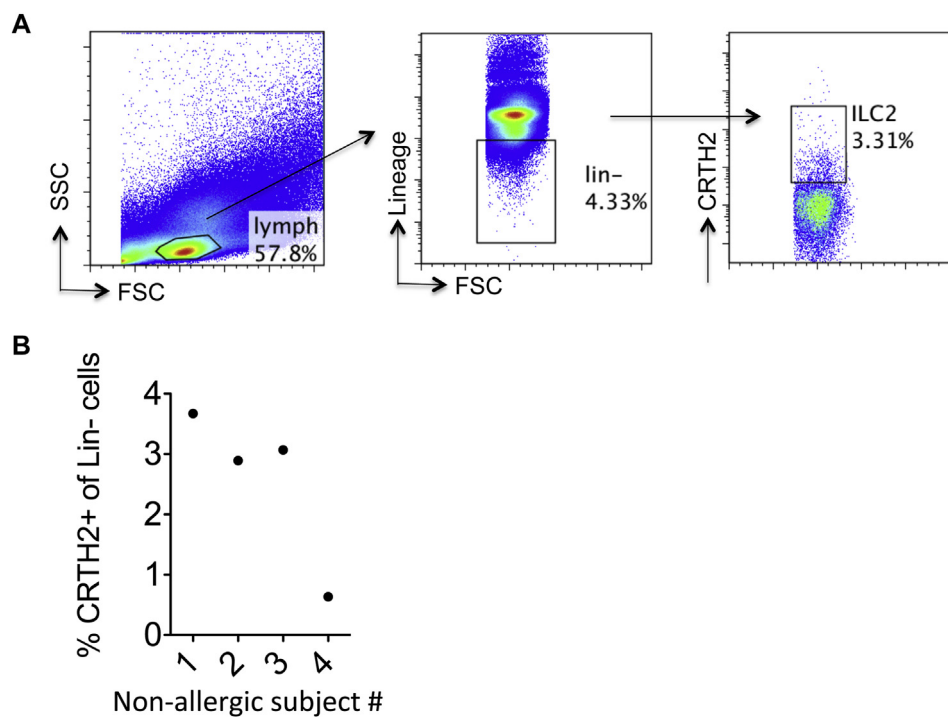


FIG E4. Detection of peripheral blood ILC2s in nonallergic individuals. **A**, PBMCs from nonallergic individuals were isolated. Lymphocytes were gated (*left*), and lineage-negative cells (*middle*) were evaluated for CRTH2 expression. **B**, Percentage of CRTH2+ cells of lineage-negative cells from the PBMC of each nonallergic individual. FSC, Forward scatter; SSC, side scatter.

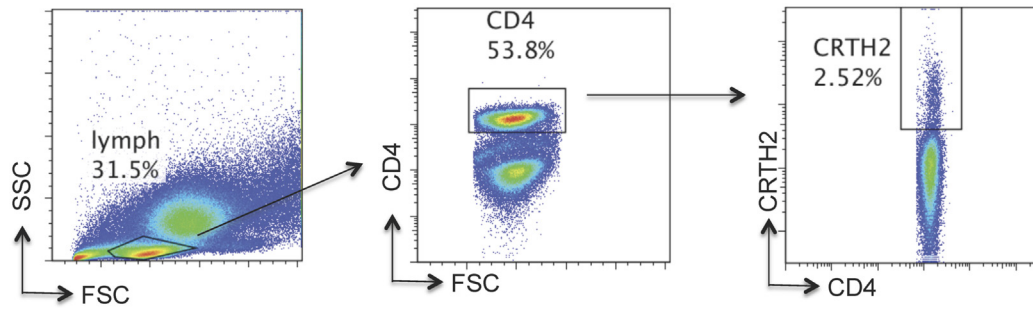


FIG E5. Sequential gating strategy for peripheral blood CD4+ CRTH2+ cells. Lymphocytes were gated from FSC/SSC plot (*left*) and CD4+ cells identified (*middle*). CRTH2+ CD4+ cells (*right*) were then analyzed for levels of CD84 expression shown in [Fig 1](#). FSC, Forward scatter; SSC, side scatter.

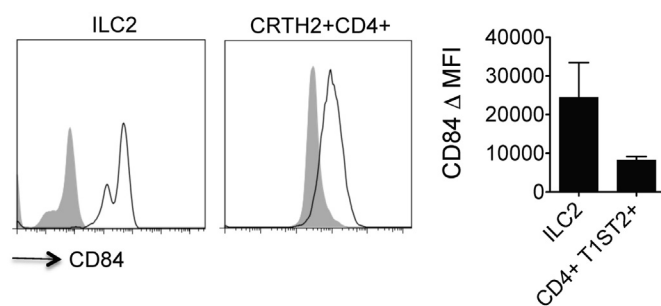


FIG E6. CD84 expression on ILC2s and CRTH2+ CD4+ cells from nonallergic individuals. ILC2s (*left*) and CRTH2+ CD4+ cells (*middle*) were gated and analyzed for levels of CD84 expression. Change in median fluorescent intensity quantified for ILC2s and CRTH2+ CD4+ cells (*right*).