Clinical Immunology (2014) 155, 126–135

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness

Hannah H. Walford[a,b], Sean J. Lund[a], Rachel E. Baum[a], Andrew A. White[c], Christopher M. Bergeron[d], Jacob Husseman[e], Kelly J. Bethel[f], David R. Scott[c], Naseem Khorram[a], Marina Miller[a], David H. Broide[a], Taylor A. Doherty[a,*]

[a] Department of Medicine, University of California-San Diego, San Diego, CA, USA
[b] Rady's Children's Hospital of San Diego, Division of Rheumatology, Allergy and Immunology, San Diego, CA, USA
[c] Division of Allergy, Asthma and Immunology, Scripps Clinic, La Jolla, CA, USA
[d] Division of Head and Neck Surgery, Scripps Green Hospital, La Jolla, CA, USA
[e] Department of Surgery, Division of Otolaryngology, University of California-San Diego, San Diego, CA, USA
[f] Division of Surgical Pathology, Scripps Green Hospital, La Jolla, CA, USA

Received 22 May 2014; accepted with revision 9 September 2014
Available online 16 September 2014

KEYWORDS
Alternaria;
Chronic rhinosinusitis;
Nasal polyps;
ILC2;
Group 2 innate lymphoid cells

Abstract Group 2 innate lymphoid cells (ILC2s) have recently been identified in human nasal polyps, but whether numbers of ILC2s differ by polyp endotype or are influenced by corticosteroid use is unknown. Here, we show that eosinophilic nasal polyps contained double the number of ILC2s vs. non-eosinophilic polyps. Polyp ILC2s were also reduced by 50% in patients treated with systemic corticosteroids. Further, using a fungal allergen challenge mouse model, we detected greatly reduced Th2 cytokine-producing and Ki-67+ proliferating lung ILC2s in mice receiving dexamethasone. Finally, ILC2 Annexin V staining revealed extensive apoptosis after corticosteroid treatment *in vivo* and *in vitro*. Thus, ILC2s are elevated in the eosinophilic nasal polyp endotype and systemic corticosteroid treatment correlated with reduced polyp ILC2s. Finally, allergen-challenged mice showed reduced ILC2s and increased ILC2 apoptosis after corticosteroid treatment suggesting that ILC2 may be responsive to corticosteroids in eosinophilic respiratory disease.
© 2014 Elsevier Inc. All rights reserved.

Abbreviations: Dex, Dexamethasone; IL-5, Interleukin-5; IL-13, Interleukin-13; IL-25, Interleukin-25; IL-33, Interleukin-33; ILC2, Group 2 innate lymphoid cell; LTD_4, Leukotriene D4; PBS, Phosphate buffered saline; PGD_2, Prostaglandin D2; TSLP, Thymic stromal lymphopoietin.

* Corresponding author at: Department of Medicine, University of California San Diego, Biomedical Sciences Building, Room 5090, 9500 Gilman Drive, La Jolla, CA 92093–0635. Fax: +1 858 534 2110.
E-mail address: tdoherty@ucsd.edu (T.A. Doherty).

http://dx.doi.org/10.1016/j.clim.2014.09.007
1521-6616/© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Group 2 innate lymphoid cells (ILC2s) are a recently identified population of lineage-negative cells (lacking cell surface markers for T cell, B cell or NK cell lineages) that produce large amounts of Th2 cytokines [1–4]. In humans and mice, several cytokines and inflammatory mediators have been shown to induce ILC2 Th2 cytokine production and/or proliferation including TSLP, IL-25, IL-33, LTD_4, and PGD_2 [2–10]. While a great deal has been learned about the activation of ILC2s in murine models of disease, very few studies have shown connections between tissue ILC2s and human disease. Further, there are limited reports demonstrating negative regulation or pharmacologic inhibition of ILC2s.

ILC2s have been reported as enriched in human nasal polyps from patients with chronic rhinosinusitis (CRS), largely considered to be type 2 inflammatory disease [6]. Epithelial cytokines TSLP and IL-33, as well as leukotrienes, have been detected at higher levels in patients with CRS and are thus available for potent ILC2 stimulation [11–13]. A recent CRS consensus report suggests the importance of "endotypes" within CRSwNP as defined by histopathologic features, cytokine profiles and presence of different cell types [14]. Interestingly, emerging evidence has shown that eosinophilic and non-eosinophilic nasal polyp endotypes have different inflammatory profiles and contrasting responses to corticosteroid treatment [15,16]. However, whether ILC2s are selectively enriched in distinct polyp endotypes or whether use of systemic corticosteroids may affect polyp ILC2 numbers is unknown.

Given the clinical and pathological distinctions between eosinophilic and non-eosinophilic polyposis, our first aim was to determine whether ILC2s, a novel Th2 cytokine producing cell population, are selectively enriched in eosinophilic nasal polyps as compared to non-eosinophilic polyps. Secondly, we sought to determine whether the numbers of ILC2s in human nasal polyps are affected by treatment with systemic corticosteroids. Corticosteroids reduce tissue eosinophilia through induction of cellular apoptosis, inhibition of type 2 cytokine production, and reduction in T-lymphocytes [17–19]. While corticosteroids are the generally first line therapy for rhinosinusitis and asthma, some patients have corticosteroid refractory disease with persistent eosinophilia [20]. A very recent report has shown that TSLP may induce partial corticosteroid resistance in mouse ILC2s *in vitro* as well as during administration of dexamethasone in OVA/alum sensitized and OVA/IL-33 challenged mice [21]. However, whether corticosteroids have an effect on ILC2 *in vivo* during exposure to a naturally encountered aeroallergen is unknown. Thus, our final aim was to determine the effect of corticosteroid treatment on mouse respiratory tissue ILC2 after *in vivo* natural allergen challenge with *Alternaria alternata*.

2. Materials and methods

2.1. Human nasal polyps

Nasal polyps and sinus mucosa samples were collected from 25 human subjects with chronic rhinosinusitis (CRS) undergoing endoscopic sinus surgery after Institutional Review Board approval at UCSD and Scripps Green Hospital. Subjects were consented to have their nasal polyp tissue used for research purposes. The demographic and clinical characteristics of patients were obtained by retrospective chart review and included age, gender, ethnicity, use of topical steroids, antibiotics or systemic steroids at the time of surgery. Physician diagnosis of cystic fibrosis and asthma were obtained by chart review. Aspirin exacerbated respiratory disease (AERD) diagnosis was defined by compelling history involving hypersensitivity reaction within 2–3 hours of ingestion of aspirin or NSAID, and confirmed by aspirin challenge in 2 of 3 patients.

Fresh tissue samples were transported in T cell media consisting of 10% FBS, 100 μg/ml Pen/Strep, 2 mM L-glutamine and 50 μM 2-Mercaptoethanol in RPMI and processed for flow cytometry the same day. Polyps were divided into eosinophilic (n = 8) or non-eosinophilic (n = 10) groups based on eosinophil levels determined by FACS. Eosinophilic polyposis was defined as CCR3 + FcεR1- granulocytes >10% of CD45+ cells or > 40% of granulocytes. Cytospun samples were stained with Giemsa-Wright and independent blinded pathological analysis of hematoxylin and eosin (H&E) stained paraffin sections of polyps was performed to confirm FACS results. Sinus mucosa (n = 7) was collected from patients with chronic sinusitis without nasal polyps (CRSsNP). Finally, ILC2 levels were analyzed from patients treated with a course of systemic steroids (prednisone dose greater than 30 mg daily for a minimum of 5 days, or equivalent methylprednisolone daily dose) within 2 weeks prior to surgery.

2.2. Identification of human nasal polyp ILC2s

Nasal polyp tissue obtained at the time of surgery was digested with Collagenase D (500 μg/ml) (Sigma-Aldrich) or Liberase TM (125 μg/ml) (Roche) in combination with DNase I (100 μg/ml) (Roche), and then filtered into a single cell suspension. To detect ILC2s in human polyps, cells were stained with a FITC lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56; BD, Franklin Lakes NJ), and FITC conjugated TCRγδ (BD, Franklin Lakes NJ, USA), CD4, CD11b, CD235a, FcεR1, (eBioscience San Diego, CA), PerCP conjugated human Anti-CD45 (eBioscience), and PE-conjugated CRTH2 (CD294) (Miltenyi Biotec). The % of ILC2s are reported as the % ILC2s of CD45+ polyp cells. Flow cytometry was performed using a BD Accuri FACS machine and analyzed with FlowJo software (Tree Star, Inc).

2.3. Mice

Studies of ILC2s in mice require sufficient amounts of tissue for FACS analysis, as ILC2s have no unique cell surface markers for immunohistochemistry studies. Given that there are no reported mouse models of nasal polyposis in which sufficient numbers of ILC2s for functional studies are available, we used mouse lung rather than the small amount of mucous membrane in mouse sinuses to investigate the ILC2 responses to corticosteroids *in vivo* and *in vitro*. Female wild-type C57BL/6 mice 6–10 weeks of age (Jackson Laboratories) were challenged intranasal with 25 μg *Alternaria alternata* (Greer, NC) on days 0, 3, and 6, and given oral gavage of either dexamethasone (Abcam) at 3 mg/kg or PBS on

Download English Version:

https://daneshyari.com/en/article/6087364

Download Persian Version:

https://daneshyari.com/article/6087364

Daneshyari.com