Cysteinyl leukotriene E_4 activates human group 2 innate lymphoid cells and enhances the effect of prostaglandin D_2 and epithelial cytokines

Maryam Salimi, DPhil, MD,[a] Linda Stöger, MSc,[b,c] Wei Liu, MSc,[b] Simei Go, BSc,[b] Ian Pavord, F Med Sci,[b] Paul Klenerman, F Med Sci,[d] Graham Ogg, DPhil, FRCP,[a]* and Luzheng Xue, PhD[b,c]* *Oxford, United Kingdom*

Background: Group 2 innate lymphoid cells (ILC2s) are a potential innate source of type 2 cytokines in the pathogenesis of allergic conditions. Epithelial cytokines (IL-33, IL-25, and thymic stromal lymphopoietin [TSLP]) and mast cell mediators (prostaglandin D_2 [PGD_2]) are critical activators of ILC2s. Cysteinyl leukotrienes (cysLTs), including leukotriene (LT) C_4, LTD_4, and LTE_4, are metabolites of arachidonic acid and mediate inflammatory responses. Their role in human ILC2s is still poorly understood.
Objectives: We sought to determine the role of cysLTs and their relationship with other ILC2 stimulators in the activation of human ILC2s.
Methods: For *ex vivo* studies, fresh blood from patients with atopic dermatitis and healthy control subjects was analyzed with flow cytometry. For *in vitro* studies, ILC2s were isolated and cultured. The effects of cysLTs, PGD_2, IL-33, IL-25, TSLP, and IL-2 alone or in combination on ILC2s were defined by using chemotaxis, apoptosis, ELISA, Luminex, quantitative RT-PCR, and flow cytometric assays. The effect of endogenous cysLTs was assessed by using human mast cell supernatants.
Results: Human ILC2s expressed the LT receptor $CysLT_1$, levels of which were increased in atopic subjects. CysLTs, particularly LTE_4, induced migration, reduced apoptosis, and promoted cytokine production in human ILC2s *in vitro*. LTE_4 enhanced the effect of PGD_2, IL-25, IL-33, and TSLP, resulting in increased production of type 2 and other proinflammatory cytokines. The effect of LTE_4 was inhibited by montelukast, a $CysLT_1$ antagonist. Interestingly, addition of IL-2 to LTE_4 and epithelial cytokines significantly amplified ILC2 activation and upregulated expression of the receptors for IL-33 and IL-25.
Conclusion: CysLTs, particularly LTE_4, are important contributors to the triggering of human ILC2s in inflammatory responses, particularly when combined with other ILC2 activators. (J Allergy Clin Immunol 2017;140:1090-100.)

Key words: *Group 2 innate lymphoid cell, leukotriene E_4, prostaglandin D_2, IL-25, IL-33, IL-2, thymic stromal lymphopoietin, atopic dermatitis*

Group 2 innate lymphoid cells (ILC2s) are recognized as an innate source of type 2 cytokines in the pathogenesis of allergic conditions, such as asthma and atopic dermatitis.[1,2] ILC2s are known to be lymphoid effector cells that do not express rearranged antigen-specific receptors but express CD45, IL-7 receptor α, and chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) while lacking lineage markers, including CD3, T-cell receptor (TCR) αβ, TCRγδ, CD14, CD19, CD56, CD11b, and CD11c. Although they can be found in various anatomic locations, their enrichment in mucosal surfaces of the lung, gut, and skin implies a local immunologic role.[2-5] ILC2s contribute to allergic and inflammatory conditions by producing IL-4, IL-5, IL-13, and GM-CSF. In the gut ILC2s are a source of type 2 cytokines for efficient expulsion of the helminth *Nippostrongylus brasiliensis*.[1] In the lungs they induce airway hyperresponsiveness and epithelial repair after influenza infection.[3,6]

ILC2s not only respond to the epithelial cytokines IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) but also respond to the mast cell lipid mediator prostaglandin D_2 (PGD_2).[2,7] It has also been reported that murine ILC2s respond to another group of lipid mediators, cysteinyl leukotrienes (cysLTs), to produce type 2 cytokines, although the effect of cysLTs in human ILC2s is still unclear.[8]

CysLTs, including leukotriene (LT) C_4, LTD_4, and LTE_4, are formed from arachidonic acid metabolism.[9] At the nuclear envelope, cytosolic phospholipase A_2 liberates membrane arachidonic acid, which binds to 5-lipoxygease–activating protein. 5-Lipoxygenase catalyzes the formation of LTA_4 by adding an oxygen moiety to arachidonic acid. Activated inflammatory cells, such as eosinophils, basophils, mast cells, and alveolar macrophages, possessing LTC_4 synthase can synthesize LTC_4 rapidly through conjugation of LTA_4 with reduced glutathione levels.[10] After extracellular export, LTC_4 is

From [a]the MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, NIHR Biomedical Research Centre, John Radcliffe Hospital, University of Oxford; [b]the Respiratory Medicine Unit, Nuffield Department of Medicine, University of Oxford; and [c]the Oxford NIHR Biomedical Research Centre, Core Translational Immunology Laboratory, and [d]the Translational Gastroenterology Unit, Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford.

*These authors contributed equally to this work as joint senior authors.

Supported by the Wellcome Trust (WT109965MA to P.K.), the MRC (CF7720 to G.O.), the NIHR Biomedical Research Centre Programme (to L.X. and G.O.), the NIHR Senior Fellowship (to P.K.), the Oxford Martin School (to P.K.), and the British Medical Association (The James Trust 2011 to G.O., P.K., and L.X.).

Disclosure of potential conflict of interest: I. Pavord has personally received fees from GlaxoSmithKline, AstraZeneca, Almirall, Novartis, Boehringer Ingelheim, Aerocrine, Genentec, Renenеron, Teva, and Roche. P. Klenerman's institution has received a grant from Wellcome Trust for the work under consideration. G. Ogg's institution has received grants from the Medical Research Council, Biomedical Research Centre, and British Medical Association for the work under consideration, and he has personally received consultancy fees from Novartis, Lilly, Orbit Discovery, Grunenthal, UCB, and Johnson and Johnson; patents and royalties from the University of Oxford; and has received stock/stock options from Orbit Discovery. L. Xue's institution has received a grant from the NIHR BRC for the work under consideration. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 20, 2016; revised November 10, 2016; accepted for publication December 12, 2016.

Available online January 20, 2017.

Corresponding author: Luzheng Xue, PhD, Respiratory Medicine Unit, Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, United Kingdom. E-mail: luzheng.xue@ndm.ox.ac.uk.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

http://dx.doi.org/10.1016/j.jaci.2016.12.958

Abbreviations used
CRTH2: Chemoattractant receptor-homologous molecule expressed on T_H2 cells
CSF1: Macrophage colony-stimulating factor
cysLT: Cysteinyl leukotriene
$CysLT_1$: Cysteinyl leukotriene receptor 1
$CysLT_2$: Cysteinyl leukotriene receptor 2
ILC2: Group 2 innate lymphoid cell
LT: Leukotriene
PGD_2: Prostaglandin D_2
TCR: T-cell receptor
TSLP: Thymic stromal lymphopoietin

converted to LTD_4 and then LTE_4 by means of sequential removal of the glutamic acid moiety, followed by the glycine moiety. LTD_4 is the most potent airway muscle contractant with the shortest half-life (in minutes); in contrast, LTE_4 is stable and the dominant LT detected in biological fluids.[11] Monitoring LTE_4 levels in urine, sputum, and exhaled air is an index of the activity of the cysLT synthesis pathway.[12] The surge in cysLT production associates with an increase in microvascular permeability, eosinophil recruitment, mucus hypersecretion, bronchoconstriction, and cell proliferation.[13] The role of cysLTs in the pathogenesis of allergic conditions, such as asthma, allergic rhinitis, urticaria, and other inflammatory conditions, has been well studied.[9] We reported recently that cysLTs potentiated the proinflammatory functions of T_H2 cells in response to PGD_2.[14,15] Two G protein–coupled receptors for cysLTs have been characterized and designated as cysteinyl leukotriene receptor 1 ($CysLT_1$), with high binding affinity for LTD_4, and cysteinyl leukotriene receptor 2 ($CysLT_2$), with similar affinity for LTD_4 and LTC_4, but both receptors have low affinity for LTE_4.[16,17] Other potential receptors for LTE_4 include adenosine diphosphate–reactive purinoceptor $P2Y_{12}$, with the highest homology to $CysLT_1$ (32%) and GPR99.[18,19]

$CysLT_1$ mediates bronchoconstriction and also a range of proinflammatory effects, including activation and migration of leukocytes.[20,21] $CysLT_1$ antagonists, most notably montelukast, are used to control asthma and allergic rhinitis. Definition of the role of cysLTs and their receptors in human ILC2s will improve our understanding of the pathogenic mechanisms of ILC2-mediated allergic inflammation and indicate potential novel therapeutic strategies.

In this study we explored the effect of cysLTs and their receptors on human ILC2s, particularly when combined with other ILC2 stimulators. We investigated whether human ILC2s express functional $CysLT_1$ and whether expression is increased by ILC2s from patients with atopic dermatitis. We assessed the effects of cysLTs on cytokine production, migration, and apoptosis of cells in the presence and absence of the $CysLT_1$ antagonist montelukast. Finally, we assessed whether cysLTs show a synergistic effect with PGD_2 and epithelial cytokines in activating the cells. Our study provides a broad understanding of the role of cysLTs in ILC2-mediated inflammatory responses, particularly in mixed inflammatory environments.

METHODS

ILC2 cell preparation and culture

Human ILC2s were prepared from human blood from healthy donors and cultured by using a modified method, as described previously.[2] Briefly, PBMCs were isolated by using Lymphoprep gradients (Axis-Shield UK, Dundee, United Kingdom). $CD3^+$ T cells were predepleted with CD3 microbeads, and the remaining cells were labeled with an antibody mixture. Lineage (CD3, CD14, CD19, CD56, CD11b, CD11c, TCRαβ, TCRγδ, FcεRI, and CD123)–negative, $CD45^{high}$, $CD127^+$, and $CRTH2^+$ cells were sorted on a FACSAria III cell sorter (BD Biosciences, San Jose, Calif) and cultured for 5 to 6 weeks in RPMI 1640 containing 100 IU/mL IL-2, 10% heat-inactivated human serum, 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin in the presence of gamma-irradiated PBMCs (from 3 healthy volunteers). Half of the medium was refreshed every 2 to 3 days. The cells were changed to fresh medium without IL-2 before treatment. Most ILC2s used in the study were derived from healthy donors, except where indicated specifically.

Adult (30-88 years) patients with atopic dermatitis received a diagnosis based on the United Kingdom refinements of the Hanifin and Rajka diagnostic criteria, and the disease severity score was defined by using SCORAD. None of the patients were receiving systemic therapy at the time of the sample acquisition. Use of human tissue samples was approved ethically by the Oxford Clinical Research Committee.

Human mast cell culture and activation

Human mast cells were cultured from $CD34^+$ progenitor cells and treated with human IgE and goat anti-human IgE in the presence or absence of MK886, as described previously.[14] Cell supernatants were collected and LTE_4 levels were measured with an ELISA, or the supernatants were stored at −80°C until used as mast cell supernatants for the treatment of ILC2s.

Chemotaxis assays

Cell migration assays were conducted, as described previously.[7] Briefly, ILC2s (approximately 5×10^4 cells/well) and treatment reagents were loaded into the upper and lower chambers, respectively, in a 5-μm pore–sized ChemoTx plate (Neuro Probe, Gaithersburg, Md). After incubation for 1 hour, migrated cells in the lower chambers were treated with a Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega, Madison, Wis) and quantified by using a FLUOstar OPTIMA luminescence plate reader (BMG LabTech, Cary, NC).

Apoptosis assay

ILC2s (approximately 5×10^5 cells per condition) were harvested after different treatments and transferred to annexin-binding buffer, followed by incubation with phycoerythrin–Annexin V/propidium iodide at room temperature, according to the manufacturer's instructions (Invitrogen, Carlsbad, Calif). The stained cells were analyzed with an LSR Fortessa flow cytometer (BD Biosciences).

Multiplex bead array

After treatment for 4 hours, concentrations of selected cytokines in the supernatants of ILC2 (approximately 6×10^5 cells/well) cultures were measured with a Human Premixed Multi-Analyte Kit (R&D Systems, Minneapolis, Minn) with magnetic beads, according to the manufacturer's instruction. Results were obtained with a Bio-Plex 200 System (Bio-Rad Laboratories, Hercules, Calif).

Quantitative RT-PCR

Quantitative RT-PCR was conducted, as described previously.[7] Primers and probes (Roche, Mannheim, Germany) are listed in Table E1 in this article's Online Repository at www.jacionline.org.

ELISA

Concentrations of cytokines in the supernatants of ILC2 cultures (approximately 6×10^5 cells/well) were assayed with ELISA kits (R&D Systems). LTE_4 levels in supernatants of mast cells were assayed with an