AWARD NUMBER: W81XWH-17-1-0527

TITLE: Role of the Leukotriene E4 Receptor GPR99 in Asthma

PRINCIPAL INVESTIGATOR: Nora Barrett

CONTRACTING ORGANIZATION: Brigham and Women's Hospital, Inc., Boston, MA

REPORT DATE: January 2022

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| January 2022 | Final | 15Sep2017 -14Sep2021 |

**4. TITLE AND SUBTITLE**

Role of the Leukotriene E4 Receptor GPR99 in Asthma

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-17-1-0527

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Nora Barrett, MD

E-Mail: nbarrett@bwh.harvard.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Brigham and Women's Hospital, Inc.

Boston Massachusetts 02115-6110

**8. PERFORMING ORGANIZATION REPORT**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

We previously demonstrated that inhalation of common aeroallergens such as the house dust mite *Dermatophagoides pteronyssinus* and the mold *Alternaria alternata* elicits the generation of a potent proinflammatory lipid mediator, leukotriene E4 (LTE4), which is part of the cysteinyl leukotriene (CysLT) family. LTE4 promotes lung inflammation, epithelial remodeling, and release of mucus into the airway through its action on a G protein-coupled receptor called CysLT3R or GPR99 or OXGR1. During this funding period,

1. We discovered that GPR99 is expressed on alpha smooth muscle actin-positive myoepithelial cells in the airway submucosa. GPR99 activation promotes myoepithelial cells to migrate to the surface of regenerating epithelium and make more brush cells. This is a feed forward loop which promotes airway remodeling.
2. We discovered that mast cell (MC) generation of LTE4 is an adjuvant signal for the development of Th1 and Th17 immunity in part through the expansion and activation of lung-draining lymph node T cells during sensitization. This likely occurs via CysLT1 action on T cells.

**15. SUBJECT TERMS**

Lipid mediators, Leukotrienes, G protein-coupled receptors, Lung, Inflammation, Epithelial cell, Brush cell, Mast cell

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| | | | | | USAMRDC |
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | **19b. TELEPHONE NUMBER** *(include area code)* |
| Unclassified | Unclassified | Unclassified | Unclassified | 14 | |

## Table of Contents

## 1. INTRODUCTION

Cysteinyl leukotrienes (CysLTs), leukotriene $C_4$ ($LTC_4$), $LTD_4$, and $LTE_4$, are lipid mediators that elicit lung inflammation and bronchoconstriction in asthma. CysLTs are not normally detected in biologic fluids, but are generated from membrane lipids through the 5-lipoxygenase/$LTC_4$ synthase ($LTC_4S$) pathway when leukocytes are activated. $LTC_4$, the terminal product of intracellular CysLT generation, is exported extracellularly, and rapidly metabolized to $LTD_4$ and to $LTE_4$, the stable CysLT detected in the bronchoalveolar lavage and the urine of patients with active asthma. CysLTs act at three receptors, $CysLT_1R$, $CysLT_2R$, and $CysLT_3R$ (also known as GPR99 or Oxgr1). Our group previously defined $CysLT_3R$ as the high affinity receptor for $LTE_4$ (1), and we found that $CysLT_3R$ mediated the development of airway goblet cells and their release of mucus (2). In subsequent studies, we have demonstrated that $CysLT_3R$ can control the generation of IL-25 and type 2 inflammation through a distinct epithelial pathway (3). This proposal dissected how $CysLT_3R$ controls type 2 inflammation and epithelial plasticity and secretory function in mouse models of allergic asthma using null strains for each CysLT receptor.

## 2. KEYWORDS

Lipid mediators, Leukotrienes, G protein-coupled receptors, Inflammation, Epithelial cells, Lung

## 3. ACCOMPLISHMENTS

### A. Table 1: Research Accomplishment Summary

| RESEARCH SPECIFIC TASKS (AS PROPOSED IN SOW) | ACCOMPLISHMENTS IN THIS REPORTING PERIOD |
|---|---|
| | |
| **Major Task 1: Mouse studies on epithelial cell function and development** | |
| Subtask 1: Submit documents for ACURO approvals | Done |
| *Milestone(s) Achieved: Obtain ACURO approval* | Done |
| Subtask 2: Define how GPR99 regulates secretory epithelial cell function in the nasal and bronchial mucosa of mice | 90% accomplished.<br>• Unexpectedly, we found that GPR99 is expressed on submucosal myoepithelial cells in murine trachea and nose. In the setting of tissue damage from protease allergen (Alternaria), GPR99 controls an epithelial repair pathway, allowing myoepithelial cells to migrate from glands to the surface of the airway epithelium and act as accessory stem cells. The epithelial cells that result have enhanced secretory function, with upregulated expression of cell surface receptors that allow mucus secretion. This work is a manuscript in preparation. See Research Narrative. |
| Subtask 3: Examine the role of the mast cell/cysteinyl leukotriene/GPR99 axis in mucin release elicited by several secretogogues | 100% accomplished.<br>• We hypothesized that activated mast cells generated CysLTs which triggered mucus release in response to several agonists. We found instead |

| | |
|---|---|
| | that a rare epithelial cell called a brush cell was the source of CysLTs (not mast cells). Brush cell CysLT generation can be activated by several secretogogues, including ATP. This work was published in Science Immunology in 2020. |
| Subtask 4: Examine how GPR99 controls Alternaria-induced goblet cell metaplasia in the lung | 100% accomplished. This work will be the subject of future studies.<br>• We found that cysLTs generated from brush cells drive proliferation and differentiation of basal stem like epithelial progenitor cells into goblet cells. This happens in a STAT6-independent fashion. Subsequent work has found that the stem cells are derived from myoepithelial cells in submucosal glands, see subtask 2 and Research Studies. |
| *Milestone(s) Achieved: Presentation of project data at a national meeting* | • Dr. Barrett spoke about these findings at the annual American Academy of Asthma, Allergy, and Immunology (AAAAI) meeting plenary in 2019 and virtual plenary in 2020. She also spoke about them at the Asthma Keystone meeting (virtual symposium) and Penn-CHOP Lung Biology Institute (virtual guest lecture) in 2021. |
| *Milestone(s) Achieved: Determination of the role of GPR99 in epithelial function and development; publication of 1-2 peer reviewed papers* | • This work was published in the manuscript *Airway brush cells generate cysteinyl leukotrienes through the ATP sensor P2Y2* by Ualiyeva et al, Science Immunology in January 2020 and is part of a manuscript in process. |
| **Major Task 2: Mouse studies on *Alternaria*-elicited type 2 pulmonary inflammation** | |
| Subtask 1: Submit documents for ACURO approvals | Done |
| *Milestone(s) Achieved: Obtain ACURO approval* | Done |
| Subtask 2: Generation of adaptive immunity in Alternaria-sensitized and challenged mice. Comparison between wild-type and null strains | 90% accomplished.<br>• Here we have defined that CysLTs derived from mast cells also act on tissue dendritic cells causing them to migrate to regional lymph nodes, promoting Th2, Th2, and Th17 responses to aeroallergen. See Research Narrative. |
| Subtask 3: Examine innate type 2 pulmonary inflammation in the first week after a single Alternaria exposure in mice. Comparison between wild-type and null. | 100% accomplished.<br>• Done in prior reporting period. |
| Subtask 4: Examine ILC2 expansion and type 2 inflammation elicited by repeated doses of intranasal LTE4 in mice | 100% accomplished.<br>• Done in prior reporting period. |
| *Milestone(s) Achieved: Presentation of project data at a national meeting* | • As noted in prior progress reports, this work was presented at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2019 by Dr. Sachin Samuchiwal in a talk entitled "MC-dependent adjuvant activity is a key component of the respiratory immune response to inhaled Alternaria."<br>• As noted in prior progress reports, this work was presented at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2019 by Dr. Lora Bankova in a talk entitled |

| | |
|---|---|
| | "Leukotriene-Dependent Regulation of Epithelial-Dependent Innate Type 2 Immunity". |
| *Milestone(s) Achieved; publication of 1-2 peer reviewed papers* | • As noted in prior progress reports, this work was published in the manuscript *The cysteinyl leukotriene 3 receptor regulates expansion of IL-25–producing airway brush cells leading to type 2 inflammation* by Bankova et al in Science Immunology October 2018 |

## B. Research Accomplishment Narrative

**Specific Aim 1. To define the role of MCs and the LTE4 receptor GPR99 in respiratory EpC secretory function and differentiation in the murine nasal and lung mucosa.**

Part of this work was previously published (4) so it is summarized briefly. Using MC-deficient mice, we demonstrated that MCs were not the source of CysLTs driving innate (early) lung inflammation. After assessment of macrophage and dendritic cell-deficient strains (which also had intact CysLT generation), we turned to epithelial cells (EpCs). We isolated several EpC subtypes from the nasal and lung mucosa. We noted that the transcriptional profile of olfactory, nasal and tracheal brush cells (BrCs) was enriched for the enzymes that generate CysLTs. We then flow cytometrically isolated these cells from the nasal mucosa and stimulated them with the calcium ionophore A23187 to show that they generated robust levels of CysLTs. **These results demonstrate that BrCs generate CysLTs.**

After assessment of candidate cell surface receptors that may mediate CysLT generation, we found that BrCs from most sites expressed several members of the P2Y receptor family including P2Y2, which recognizes ATP (not shown). Ex-vivo stimulation of isolated nasal BrCs with the stable ATP analogue ATPyS elicited dose-dependent CysLT generation, which was inhibited by the P2Y2 inhibitor (AR-C118925) and by MK886. Finally, brush cell-deficient mice had impaired CysLT generation in response to intranasal allergen challenge with Alternaria. **These results demonstrate that P2Y2 is a novel BrC receptor regulating CysLT generation. Activation of these cells controls goblet cell mucin release in a paracrine fashion. These results were published in Science Immunology (4).**

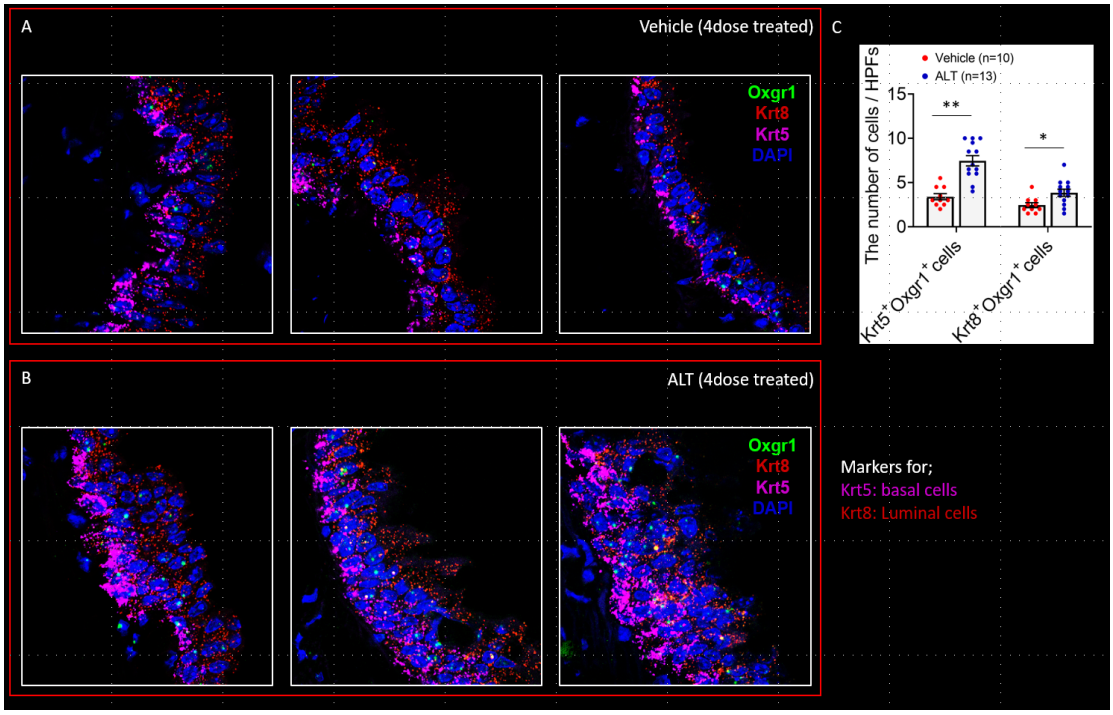


**Fig. 1. Krt5+ and Krt8+ basal cells express GPR99 (Oxgr1) and are increased after Alternaria.** WT mice were sensitized and challenged with inhaled Alternaria or vehicle control for 2 weeks. Tracheas were harvested and stained for the probes as detailed above. (**A-B**) Representative images of RNA in situ hybridization. (**C**) Quantification. *, p = 0.01. **, p= 0.001.

In these studies, we found that GPR99 regulated BrC development, but that it was not expressed on BrCs themselves. After establishing that available antibodies lacked specificity (stain tissues from GPR99-null mice), we pursued in situ RNA-hybridization to define GPR99 expressing cells (**Fig. 1**). In murine trachea, we found that GPR99 was expressed on basal stem cell subsets that are that are distinguished by expression of KRT5 or KRT8 (**Fig. 1A-B**). These cells were increased after Alternaria (**Fig. 1C**). Unexpectedly, we also detected GPR99 transcript in the submucosa (**Fig. 2**). Here it was localized to submucosal glands that are lined by alpha smooth muscle actin (α-SMA) expressing myoepithelial cells (**Fig. 2A, left**). Sox9 staining, marking the nuclei of myoepithelial progenitor cells, confirmed that they were the cells expressing GPR99 (**Fig. 2A, right**). Remarkably, while α-SMA+ EpCs are not present in the naïve murine trachea (not shown), they are detected after Alternaria in WT but not GPR99KO mice (**Fig. 2B**). **These findings suggest that GPR99 regulates airway epithelial remodeling by eliciting a well-recognized epithelial repair pathway(5, 6)**. This hypothesis will be tested using SOX9 reporter mice and cultured WT and GPR99KO myoepithelial cells stimulated with and without LTE4 to conclude this study.

Notably, in studies funded by other grants, we did verify that GPR99 was localized to the same cells in human respiratory tissue. Staining of resected turbinate tissue from patients who had undergone sinus surgery for resection of concha bullosa showed that GPR99 was indeed expressed on KRT5+ basal cells in the superficial airway epithelium and on SOX9+ epithelial cells in the submucosal glands (**Fig. 3**). These findings suggest that the work done in mice may translate to human tissues.

**In summary, we have had to revise our hypotheses that 1) MCs were the primary source of CysLTs regulating epithelial biology and that 2) they were the primary responders to alarmins such as ATP. We instead have found that the ATP/P2Y2 axis that regulates CysLT generation from Brush cells and that this pathway is operative in allergen recognition in vivo. Additionally we discovered that the CysLT receptor GPR99 regulates airway remodeling and potentiates brush cell (3) and myoepithelial cell (Fig. 2) expansion.**

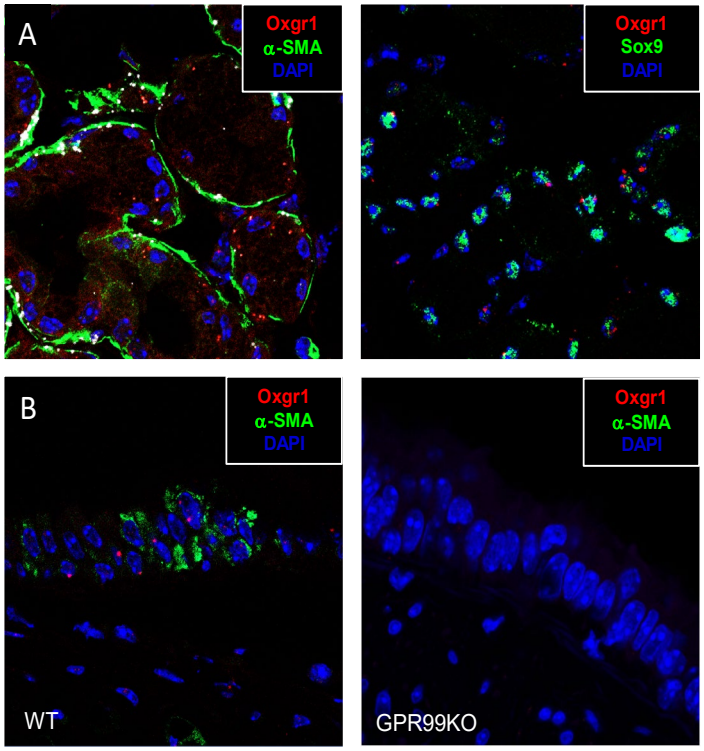


**Fig 2. SOX9 and aSMA+ myoepithelial cells express GPR99 (Oxgr1) which regulates the myoepithelial repair pathway in the damaged surface airway epithelium.** (**A**) Oxgr1 staining on α-SMA+ and SOX9+ myoepithelial cells in the naïve tracheal submucosa. (**B**) After inhaled *Alternaria* α-SMA+ myoepithelial cells appear in the surface airway mucosa of the trachea in WT but not GPR99KO mice.

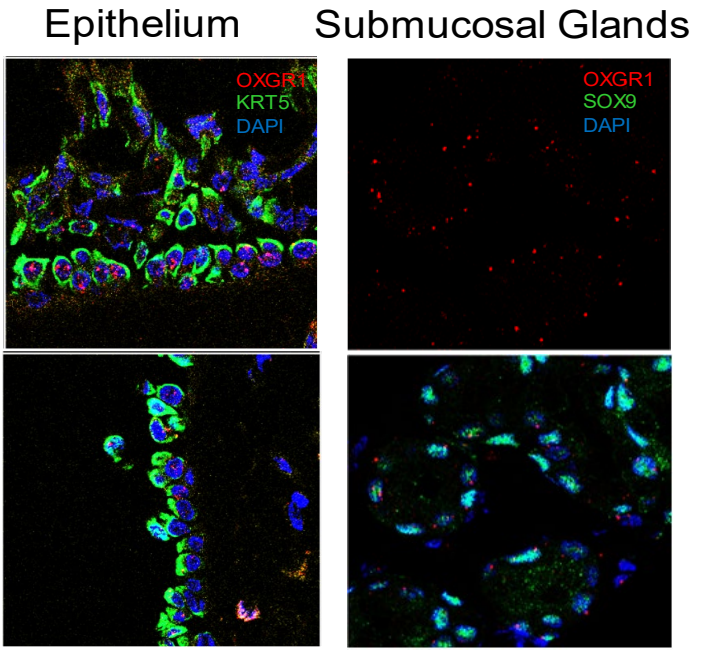


**Fig. 3. In resected human nasal epithelium, in situ RNA hybridization demonstrates that GPR99 is localized to KRT5+ basal epithelial cells in the epithelium (left) and to SOX9+ myoepithelial cells in submucosal glands (right).**

**Specific Aim 2. To define the role of MCs, GPR99, and other CysLT receptors in driving type 2 pulmonary inflammation elicited by *Alternaria alternata*.**

To define the role of MCs and CysLTs in allergen-elicited adaptive lung inflammation, we sensitized and challenged mice with inhaled *Alternaria.* WT mice developed robust lung inflammation, including increased CD45+ cells, eosinophilia, lung DC recruitment, ILC2 expansion, and Th1, Th2, and Th17 infiltration, as assessed by flow cytometry (**Fig. 4**). MC-deficient mice had decreased total CD45+ cell counts, decreased lung DCs, and decreased numbers of pulmonary Th1 and Th17 cells. By contrast, Mcpt5/DTA mice had no reduction in lung eosinophilia, Th2 cells, or lung ILC2 numbers. To understand whether this reflected a defect at sensitization, we treated mice with a single dose of *Alternaria* and assessed dendritic cell (DC) activation in the lung and migration to the lung-draining lymph nodes (LNs) 24h later. WT mice treated with *Alternaria* had a robust migration of DCs to the lung-draining LNs and recruitment of CD4 and CD8 T cells (**Fig. 5**). By contrast, MC-deficient Mcpt5/DTA mice had dramatically reduced DC migration and T cell recruitment. **These results indicate that MCs control early events in *Alternaria* sensitization**.

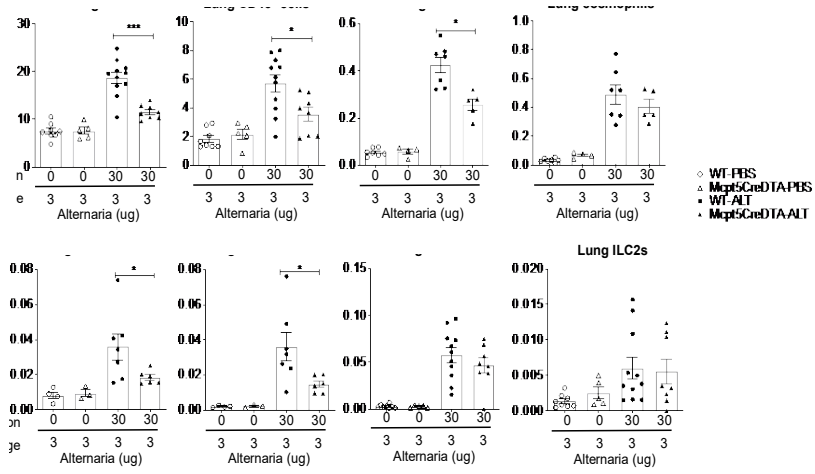


**Fig. 4. MC Regulation of *Alternaria*-elicited Th1 and Th17 lung inflammation.** WT (circles) and MC-deficient Mcpt5/DTA mice (triangles) were sensitized with inhaled *Alternaria* at 0 or 30 ug/ml, challenged with 3 ug of inhaled *Alternaria* on days 9 and 10 and euthanized on day 12. Flow cytometric enumeration of the lung T cell infiltrate demonstrating: (**A**) reduced total lung infiltrate (left), CD45+ cells (middle left), and lung DCs (middle right) in the lungs of MC-deficient mice, as compared to WT. There is no difference in eosinophilia. (**B**) Reduced Th1 and Th17 cells without a difference in Th2 cells or ILC2 expansion. Data are means ± SEM, across 3 experiments. * p = 0.01, ***p= 0.001.

To understand whether the failure of DCs to migrate to the regional LN reflected a baseline DC abnormality or a failure to be activated, we assessed lung DC activation (CD80, CD86, OX40L expression) at 0, 6, and 24 h after Alternaria inhalation. We found no difference in the lung DC response between WT and Mcpt5/DTA at baseline or after *Alternaria* (not shown). To use a more sensitive technique to assess DC activation, lung DCs were isolated from each genotype after *Alternaria* challenge and sent for sequencing. This demonstrated marginal changes in gene expression (not shown).

We next focused on mediators that can drive DC migration and T cell LN recruitment that are reported to be generated in MCs. To understand whether the reduction in DC migration seen in MC-deficient mice might be due to MC-derived CysLTs we performed *Alternaria* inhalation in WT and CysLT-deficient ($LTC_4S$-null) mice (**Fig. 6**). Here we found that DC migration and T cell

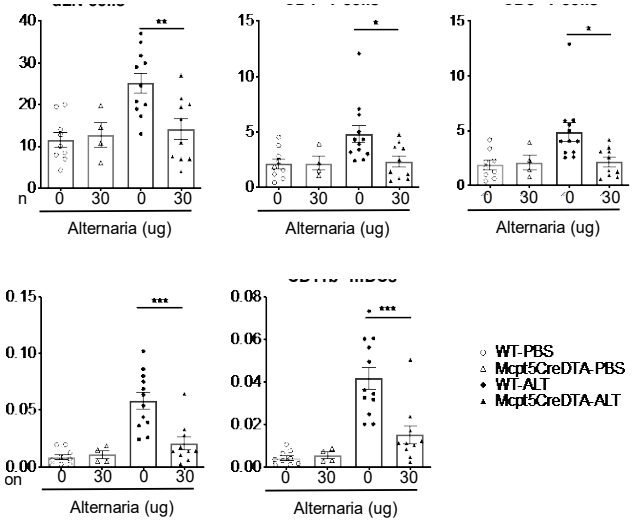


**Fig. 5. MC-dependent Recruitment of DCs and T cells to Regional LNs.** WT (circles) and MC-deficient Mcpt5/DTA mice (triangles) were treated with inhaled *Alternaria* for a single dose. 24 h later, the lung draining LNs were evaluated flow cytometrically for (**A**) total LN cell count (left), CD4+ T cells (middle), and CD8+ T cells (right). DC cell count (left), CD11b+ myeloid DCs (middle), and PDL2+CD301b+ DCs (right). Data are means ± SEM, across 3 experiments. ** p<0.01, *** p<0.001.

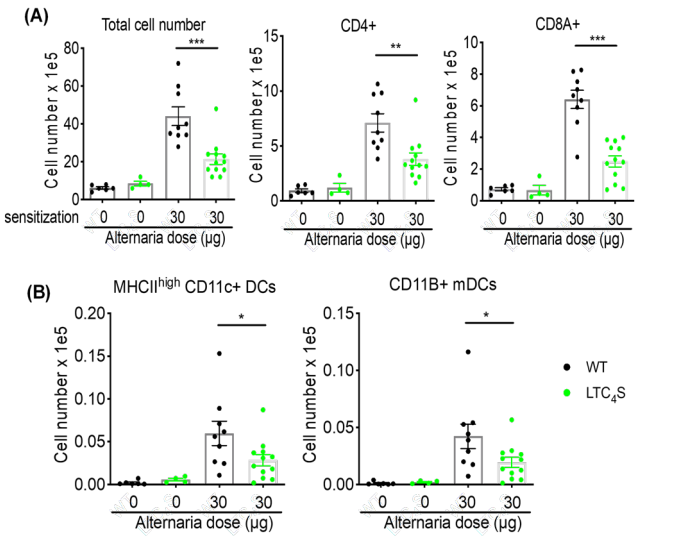


**Fig. 6. CysLT-dependent Recruitment of DCs and T cells to Regional LNs.** WT and CysLT-deficient $LTC_4S$-null mice were treated with inhaled *Alternaria* for a single dose. 24 h later, the lung draining LN were evaluated for total cell number, CD4 and CD8 T cell numbers (A) and migration of $MHCII^{hi}$ and $CD11b^{+}$ myeloid DCs (B). Data are means ± SEM, across 3 experiments. *p = 0.05, ** p = 0.01, *** p = 0.001.

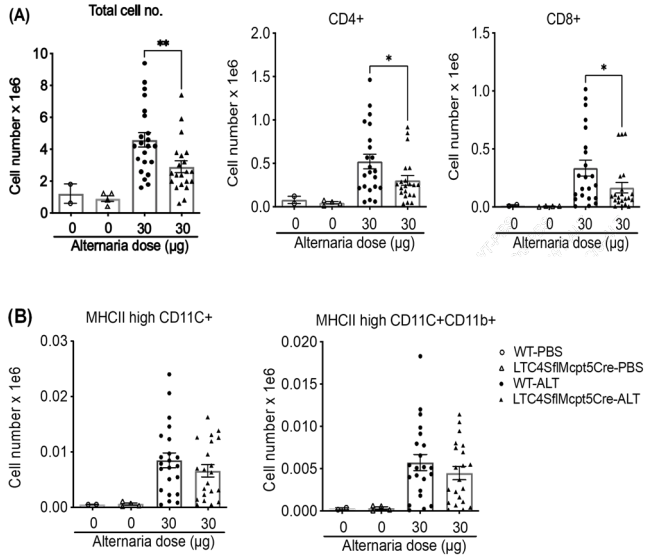


**Fig. 7. MC CysLT-dependent Recruitment of DCs and T cells to Regional LNs.** WT and MC CysLT-deficient $Mcpt5^{CRE}xLTC_4S^{fl/fl}$ mice were treated with inhaled *Alternaria* for a single dose. 24 h later, the lung draining LN were evaluated for total cell number, CD4 and CD8 T cell numbers (A) and migration of $MHCII^{hi}$ and $CD11b^{+}$ myeloid DCs (B). Data are means ± SEM, across 3 experiments. *p = 0.05, ** p = 0.01.

recruitment was significantly reduced. Thus, we crossed the Mcpt5CRE strain to a novel $LTC4S^{fl/fl}$ mouse from a collaborator to find that T cell recruitment was reduced (**Fig. 7**). Finally, to understand the result of this pathway we sensitized and challenged this novel strain with Alternaria. Here we saw that deletion of MC CysLTs did indeed lead to reduced Th1 and Th17 responses in the lung (**Fig. 8**) and LN (not shown). Notably, it also resulted in reduced ILC2, lung eos, and lung Th2 cells (not shown), likely reflecting the role of MCs in augmenting Th2 immunity at the effector phase. Thus, these studies demostrated a proximal role for MCs in the generation of Th1 and Th17 responses to inhaled Alternaria.

**Conclusions.**

In sum, our findings demonstrate that: 1) Several cells in the naïve respiratory mucosa can generate CysLTs including MCs and BrCs. 2) BrC CysLT generation can be elicited by *Alternaria* and by danger signals such as ATP. This contributes to the CysLT-dependent mucus release we have previously reported. 3) The CysLT $LTE_4$ acts on GPR99 expressed on myoepithelial cells in submucosal glands and promotes a myoepithelial repair pathway whereby

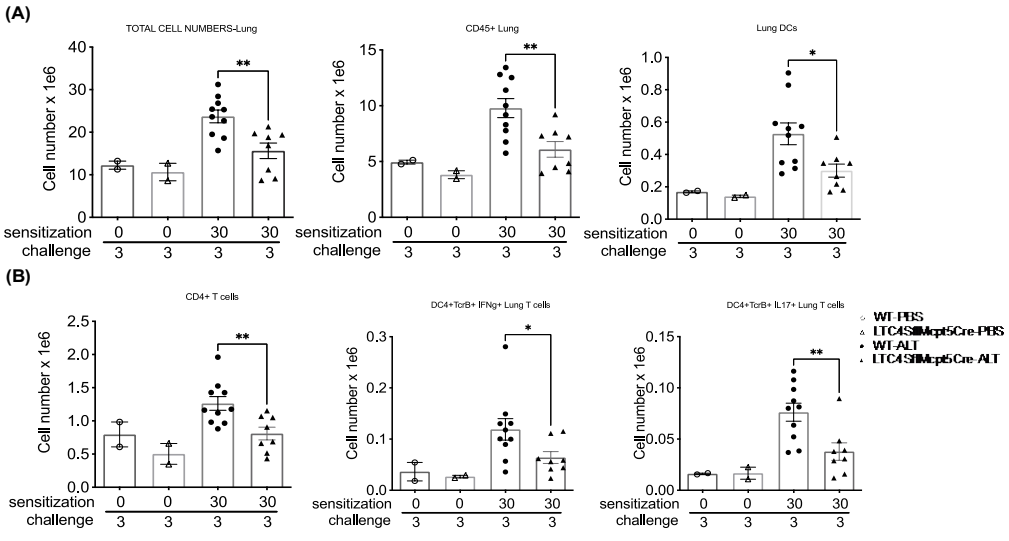


**Figure 8. MC CysLT-dependent Adaptive Immunity.** WT (circles) and MC-CysLT-deficient $Mcpt5^{CRE}xLTC_4S^{fl/fl}$ mice (triangles) were sensitized with inhaled *Alternaria* at 0 or 30 ug/ml, challenged with 3 ug of inhaled *Alternaria* on days 9 and 10 and euthanized on day 12. Flow cytometric enumeration of the lung T cell infiltrate demonstrating: (**A**) reduced total lung infiltrate (left), CD45+ cells (middle), and DCs (right) in the lungs of MC-deficient mice, as compared to WT. (**B**) Reduced CD4+ T cells, Th1 and Th17 cells. Data are means ± SEM, across 3 experiments. * p = 0.05, **p= 0.01.

these cells migrate to the surface of damaged airway epithelium. These studies are part of a manuscript in preparation. 4) Additionally, we find that MC CysLT generation in the submucosa can also be elicited by *Alternaria*. This contributes to the CysLT-dependent migration of DCs from the lung to the LN and to priming for Th1 and Th17 adaptive immune responses. These studies are part of a manuscript in preparation.

**C. Opportunities for training and professional development**

While this grant is not specifically designed to provide professional development, it did support work that allowed investigators to present at national meetings.

- This work was presented at the annual meeting of the American Academy of Asthma, Allergy, and Immunology (AAAAI) in 2019 by Dr. Sachin Samuchiwal in a talk entitled "MC-dependent adjuvant activity is a key component of the respiratory immune response to inhaled Alternaria".

- Some of this work was presented at the annual meeting of the AAAAI in 2019 by Dr. Lora Bankova in a talk entitled "Leukotriene-Dependent Regulation of Epithelial-Dependent Innate Type 2 Immunity".

- Dr. Barrett spoke about these findings at the annual meeting of the AAAAI in 2019 in a talk entitled "Type 2 Immunity Elicited By Airway Allergens"

- Dr. Barrett spoke about these finding at Northwestern University Feinberg School of Medicine in a talk entitled "Airway Epithelial Remodeling"

- Dr. Lora Bankova was invited to give at talk at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2020. This was cancelled due to COVID19.

- Dr. Nora Barrett was invited to give a plenary talk at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2020. Her plenary talk was recorded for a virtual seminar in July 2020.

- Dr. Nora Barrett spoke about this work at the Asthma Keystone meeting (virtual symposium) in 2020 in a talk entitled "Revisiting Airway Epithelial Remodeling in T2 Inflammation"

- Dr. Nora Barrett spoke about this work at the University of Pennsylvania-Children's Hospital of Pennsylvania Lung Biology Institute (virtual guest lecture) in 2021 in a talk entitled "Brush Cell Development and Function".

**D. Dissemination to communities of interest**

- Nothing to report

**E. Plans for the Next Reporting Period**

- N/A

**4. IMPACT**

- Impact on the principal discipline. We have made three important discoveries in this work.
  - The first is that the $LTE_4$ receptor GPR99 promotes the excessive release of airway mucus from goblet cells. **This can contribute to airflow obstruction in asthma**. This occurs in part through the action of $LTE_4$ in driving myoepithelial stem cells in the airway submucosa

to move to the overlying surface epithelium and differentiate into brush cells. When activated, brush cells then promote mucus release by goblet cells in a paracrine fashion.
    - The second discovery is that brush cells generated in response to $LTE_4$ can also make $LTE_4$ at high levels. Thus, activation of GPR99 leads to a feedforward loop promoting the generation of brush cells that make more ligand. This circuit is likely designed to stabilize the remodeling of the airway epithelium and will **endow it with enhanced proinflammatory function** due to the established proinflammatory functions of $LTE_4$ and other brush cell products such as IL-25.
    - Mast cells in the airway submucosa generate CysLTs which serve to mobilize T cells to the lung draining lymph node and thus play a key sentinel role in shaping adaptive Th1 and Th17 immune responses. **Future studies will examine whether this is a steroid resistant pathway that may be implicated in severe neutrophilic asthma**.

- Impact on additional disciplines, technology transfer, society and behavior. Nothing to report.

## 5. CHANGES/PROBLEMS

- There was no change in approach over this reporting period.
- There was a delay in performing animal experiments due to COVID 19, leading to the no cost extension of this award that has just completed.
- There were no changes in the use of vertebrate animals, biohazards, or select agents.
- This grant does not include human subjects.

## 6. PRODUCTS

- Journal publications.
    - Ualiyeva S**, Yoshimoto E, **Barrett NA**, Bankova LG. Isolation and Quantitative Evaluation of Brush Cells from Mouse Tracheas. J Vis Exp. 2019; (148), e59496, doi:10.3791/59496 PMID: 31259891
    - **Barrett NA**, Shalek AK. Revisiting airway epithelial remodeling in type 2 immunity: Beyond goblet cell metaplasia. J Allergy Clin Immunol. 2019; 144 (5): 1158–60. PMID: 31600548
    - Ualiyeva S**, Hallen N, Kanaoka Y, Ledderose C, Matsumoto I, Junger W, **Barrett NA**, Bankova LG. Airway Brush Cells Generate Cysteinyl Leukotrienes Through the ATP Sensor P2Y2. Sci_Immunol, 2020; 5 (43):eaax7224, doi:10.1126/scciimmunol.aax7224. PMID: 31953256.
    - McGinty JW, Ting H, Billipp TE, Nadjsombati MS, Khan DM, **Barrett NA**, Liang H, Matsumoto I, von Moltke J. Tuft-Cell-Derived Leukotrienes Drive Rapid Anti-helminth Immunity in the Small Intestine but Are Dispensable for Anti-protist Immunity. Immunity, 2020.
    - Bankova LG, **Barrett NA**. Epithelial cell function and remodeling in nasal polyposis. Ann All Asthma Immunol. 2020; 124(4):333-341. PMID: 32007569
    - Buchheit KM, Dwyer DF, Ordovas-Montanes J, Katz HR, Lewis E, Vukovic M, Lai J, Bankova L, Bhattacharyya N, Shalek AK, **Barrett NA**, Boyce JA, Laidlaw TM. IL-5Rα marks nasal polyp

IgG4 and IgE-1 expressing cells in aspirin-exacerbated respiratory disease. J Allergy Clin Immunol, 2020; 145(6):1574-1584. doi: 10.1016/j.jaci.2020.02.035. PMID:32199912.

- Derakhshan T, Samuchiwal SK, Hallen N, Bankova L, Boyce JA, **Barrett NA**, Austen KF, Dwyer DF. Lineage-specific transcriptomes define differential expansion and development of inducible and constitutive mast cells. J Exp Med, 2021; 218 (1): doi: 10.1084/jem.20200321. PMID: 32946563

- Kohanski MA, Cohen NA, **Barrett NA**. Epithelial Dysregulation in Chronic Rhinosinusitis with Nasal Polyposis (CRSwNP) and Aspirin-Exacerbated Respiratory Disease (AERD). J Allergy Clin Immunol. 2021 *accepted*

- Ualiyeva S, Boyd AA, **Barrett NA**, Bankova LG. Isolation of Nasal Brush Cells for Single-cell Preparations. Bio Protoc. 2021 Sep 20; 11(18):e4163. PMID: 34692913.

- Ualiyeva S, Lemire E, Aviles E, Wong C, Boyd AA, Lai J, Liu T, Matsumoto I, **Barrett NA**, Boyce JA, Haber AL, Bankova LG. Tuft cell-produced cysteinyl leukotrienes and IL-25 synergistically initiate lung type 2 inflammation. Sci Immunol 2021 *accepted*

- Book publications.
  - Bankova L**, **Barrett NA**. Chapter 1: Innate Immunity. In: Burks AW, Holgate ST, O'Hehir RE, Bacharier LB, Broide DH, Khurana Hershey G, Peebles RS, eds. Middleton's Allergy, 9th Edition. Amsterdam, Netherlands: Elsevier; 2020.

- Websites.
  - We have developed a laboratory website to help disseminate research to the community. https://barrettlab.bwh.harvard.edu/

- Presentations.
  - "MC-dependent adjuvant activity is a key component of the respiratory immune response to inhaled Alternaria" was presented by Dr. Sachin Samuchiwal at the American Academy of Allergy Asthma and Immunology in 2019.

  - "Leukotriene-Dependent Regulation of Epithelial-Dependent Innate Type 2 Immunity" was presented by Dr. Lora Bankova at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2019.

  - "Nasal epithelial brush cells generate cysteinyl leukotrienes in response to aeroallergens and stress signals" was by Dr. Saltanat Ualiyeva at the annual meeting of the American Academy of Allergy Asthma and Immunology in 2019.

  - "Airway Epithelial Remodeling" was presented at the Northwestern University Allergy and Immunology seminar series, Division of Allergy and Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL in 2019.

  - "Epithelial remodeling in the airway mucosa: from form to function" was presented by Dr. Nora Barrett at the annual immunology retreat at the Washington University Immunology Program, St. Louis MO in 2019.

  - "A Single-Cell Expression Atlas of Epithelial Cells – Mechanistic Insights into Inflammatory and Allergic Airway Diseases" was presented as an online plenary for the annual meeting of the AAAAI in a recorded session due to COVID19.

- "Revisiting Airway Epithelial Remodeling in T2 Inflammation" was presented by Dr. Nora Barrett at the Keystone Symposia 2020, Asthma: Making New Discoveries into Better Therapies. *this was rescheduled due to COVID19 to December 2, 2020.

- "Brush Cell Development and Function" was presented by Dr. Nora Barrett at the Penn-CHOP Lung Biology Institute, Philadelphia, PA. *this was held virtually due to COVID19

- No technologies, inventions, patents, licenses, or other reportable outcomes resulted from this research.

## 7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

| | |
|---|---|
| Name: | Nora Barrett |
| Project Role: | PI |
| Researcher Identifier: | 0000-0003-2211-8811 |
| Nearest person month worked: | 1.1 mos |
| Contribution to the Project: | Dr. Barrett is responsible for the design and conduct of all experiments. |
| Funding Support: | R01 AI134989, U19 AI095219, R01 AI078908 |

| | |
|---|---|
| Name: | Sachin Samuchiwal |
| Project Role: | Associate Scientist |
| Researcher Identifier: | 0000-0001-6232-5650 |
| Nearest person month worked: | 3.5 mos |
| Contribution to the Project: | Dr. Samuchiwal is spearheading our mouse mast cell work to define the mechanism by which they regulate the initiation of adaptive immunity. |
| Funding Support: | No other grants |

| | |
|---|---|
| Name: | Minkyu Lee |
| Project Role: | Associate Scientist |
| Researcher Identifier: | |
| Nearest person month worked: | 3.5 mos |
| Contribution to the Project: | Dr. Lee is responsible for spearheading work on GPR99/OXGR1 regulation of goblet cell metaplasia and submucosal gland function. |
| Funding Support: | R01 AI134989 |

- There are no other organizations involved as a partner.

## 8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

## 9. APPENDICES

**Nothing to report**

## 10. REFERENCES


1. Kanaoka Y, Maekawa A, Austen KF. 2013. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. *J Biol Chem* 288: 10967-72
2. Bankova LG, Lai J, Yoshimoto E, Boyce JA, Austen KF, Kanaoka Y, Barrett NA. 2016. Leukotriene E4 elicits respiratory epithelial cell mucin release through the G-protein-coupled receptor, GPR99. *Proc Natl Acad Sci U S A* 113: 6242-7
3. Bankova LG, Dwyer DF, Yoshimoto E, Ualiyeva S, McGinty JW, Raff H, von Moltke J, Kanaoka Y, Frank Austen K, Barrett NA. 2018. The cysteinyl leukotriene 3 receptor regulates expansion of IL-25-producing airway brush cells leading to type 2 inflammation. *Sci Immunol* 3
4. Ualiyeva S, Hallen N, Kanaoka Y, Ledderose C, Matsumoto I, Junger WG, Barrett NA, Bankova LG. 2020. Airway brush cells generate cysteinyl leukotrienes through the ATP sensor P2Y2. *Sci Immunol* 5
5. Lynch TJ, Anderson PJ, Rotti PG, Tyler SR, Crooke AK, Choi SH, Montoro DT, Silverman CL, Shahin W, Zhao R, Jensen-Cody CW, Adamcakova-Dodd A, Evans TIA, Xie W, Zhang Y, Mou H, Herring BP, Thorne PS, Rajagopal J, Yeaman C, Parekh KR, Engelhardt JF. 2018. Submucosal Gland Myoepithelial Cells Are Reserve Stem Cells That Can Regenerate Mouse Tracheal Epithelium. *Cell Stem Cell* 22: 779
6. Anderson PJ, Lynch TJ, Engelhardt JF. 2017. Multipotent Myoepithelial Progenitor Cells Are Born Early during Airway Submucosal Gland Development. *Am J Respir Cell Mol Biol* 56: 716-26