12/15-Lipoxygenase deficiency protects mice from allergic airways inflammation and increases secretory IgA levels

Amanda R. Hajek, BS, Alexa R. Lindley, BS, Silvio Favoreto, Jr, PhD, Roderick Carter, BS, Robert P. Schleimer, PhD, and Douglas A. Kuperman, PhD *Chicago, Ill*

Background: Induction of 15-lipoxygenase-1 (15-LO-1) has been observed in the airways of subjects with asthma, although its physiologic role in the airways has remained largely undefined.
Objectives: We sought to test the hypothesis that the mouse 15-LO-1 ortholog 12/15-LO contributes to the development of allergic airways inflammation.
Methods: Two models were used to evaluate wild-type and 12/15-LO–deficient mice. The systemic model involved intraperitoneal injections of allergen, and the mucosal model involved allergen exposures occurring exclusively in the airways. The systemic and mucosal-specific contributions of 12/15-LO to allergic sensitization and airways inflammation were determined by comparing the results obtained in the 2 models.
Results: In the mucosal model 12/15-LO knockout mice were protected from the development of allergic sensitization and airways inflammation, as evidenced by circulating levels of allergen-specific IgE, IgG1, and IgG2a; the profile of inflammatory cells in bronchoalveolar lavage fluid; and the expression of cytokines and mediators in lung tissue. In the systemic model 12/15-LO knockout mice were not protected. This suggested the presence of a lung-restricted protective role for 12/15-LO deficiency that was potentially accounted for by increased activation of mucosal B cells and increased production of the known mucosal-specific protective mediator secretory IgA.
Conclusions: Induction of 15-LO-1 in asthma might contribute to allergic sensitization and airways inflammation, potentially by causing suppression of secretory IgA. (J Allergy Clin Immunol 2008;122:633-9.)

Key words: *Asthma, allergy, inflammation, lipoxygenase, IL-13, IgA, B cells, airway, lung*

Abbreviations used
AA: Arachidonic acid
APRIL: A proliferation-inducing ligand
BAFF: B cell–activating factor from the TNF superfamily
BAFF-R: BAFF receptor
BALF: Bronchoalveolar lavage fluid
BCMA: B-cell maturation antigen
CysLT: Cysteinyl leukotriene
HETE: Hydroxyeicosatetraenoic acid
HODE: Hydroxyoctadecadienoic acid
J chain: Joining polypeptide
LO: Lipoxygenase
LT: Leukotriene
PG: Prostaglandin
pIgR: Polymeric immunoglobulin receptor
SC: Secretory component
SIgA: Secretory IgA
STAT6: Signal transducer and activator of transcription 6
TACI: Transmembrane activator and calcium modulator and cyclophilin ligand interactor

Allergen exposures at the airway mucosal surface are well tolerated by most individuals but often trigger asthma exacerbations.[1] This suggests a disease-related failure of first-line airway mucosal defenses, which might help to explain the high levels of circulating antigen-specific IgE that often develop in asthmatic subjects after aeroallergen exposures.[2] One critical first-line mucosal defense is mediated by IgA produced by plasma cells underlying mucosal surfaces.[3] The locally produced IgA is transported by the polymeric immunoglobulin receptor (pIgR) onto mucosal surfaces as secretory IgA (SIgA).[4] SIgA is a noninflammatory protective mediator that functions through a spectrum of low-affinity and high-affinity interactions with allergens, toxins, and microbes and by binding to specific receptors on host cells.[3] Decreases of SIgA levels in bronchoalveolar lavage fluid (BALF) from subjects with asthma correlate with worsening symptoms.[5] Therefore suppression of SIgA could result in failure of a first-line mucosal immune defense in patients with asthma.

In previous studies 12/15-lipoxygenase (12/15-LO) was identified as an allergen- and IL-13–induced gene in the lungs of mice.[6] Its human ortholog (15-LO-1) is highly expressed in the airways of subjects with severe asthma.[7] The 15-LO-1 and 12/15-LO enzymes insert molecular oxygen into arachidonic acid (AA), resulting in formation of 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] and 15(S)-HETE.[8] The hypothesis was tested that 12/15-LO contributes to the severity of allergic inflammation by comparing allergen-induced systemic antibody responses and inflammatory responses in the lungs of wild-type and 12/15-LO knockout mice that were exposed to allergen either by means of intraperitoneal injection or by means of repeated airways exposure. The approach was used to identify systemic and mucosal-specific functions of 12/15-LO. The results point to 12/15-LO as a mucosal-specific inhibitor of SIgA and a contributor to the development of allergic sensitization and airways inflammation in mice.

From the Department of Medicine, Allergy-Immunology Division, Northwestern University Feinberg School of Medicine.
Dr Kuperman was supported by the Respiratory Health Association of Metropolitan Chicago and the Ernest S. Bazley Trust.
Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication December 18, 2007; revised June 17, 2008; accepted for publication June 18, 2008.
Available online August 11, 2008.
Reprint requests: Douglas A. Kuperman, PhD, Division of Allergy-Immunology, 240 E Huron, Room M-317, Chicago, IL. E-mail: d-kuperman@northwestern.edu.
0091-6749/$34.00

doi:10.1016/j.jaci.2008.06.021

METHODS

Mice

The experiments were approved by the Northwestern University Animal Care and Use Committee and complied with the "Guide for the care and use of laboratory animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996). 12/15-LO$^{-/-}$ and strain-matched wild-type mice (Jackson Laboratories, Bar Harbor, Me) were interbred to generate in-house C57Bl/6 wild-type and 12/15-LO$^{-/-}$ littermates. AKR/J, C57Bl/6, BALB/c, and 129/SvJ mice and BALB/c wild-type and signal transducer and activator of transcription 6 (STAT6)–deficient mice were also evaluated.

Protocols

Mice were sensitized by means of intraperitoneal injection of 10 μg of grade V ovalbumin in 200 μL of PBS with adjuvant (alum) twice, 1 week apart, or an equivalent volume of PBS with alum alone (control animals). A small volume of blood was collected from behind the eye immediately before each injection. One week after the second intraperitoneal injection, a different set of mice also received a total of 2 airway challenges, 1 day apart, with 1.5% ovalbumin in 50 μL of PBS or an equivalent volume of PBS (control animals) through the intratracheal route and were harvested 2 days later. A different group of mice received 1.5% ovalbumin (50 μL of PBS) through the intratracheal route once every 4 days or an equivalent volume of PBS (control animals) for a total of 4 airway challenges. A small sample of blood was collected from these mice immediately before the second or third airway challenge. Mice were harvested 2 days after the fourth airway challenge. In the lung permeability study *Escherichia coli* LPS (InvivoGen, San Diego, Calif) in 50 μL of PBS or PBS (control animals) was delivered through the intratracheal route, and then 1.5 mg of BSA was delivered through the retro-orbital route. Mice were harvested 1 day later. C57Bl/6 wild-type mice were treated with baicalein (Caymen Chemicals, Ann Arbor, Mich) dissolved in Cremophor EL (Sigma, St Louis, Mo) by means of subcutaneous injection once per day for 7 days, and the mice were harvested 1 day after the last treatment.

Samples

Blood was collected after excision of a kidney, or a small volume was sampled by inserting a capillary tube behind the eye. BALF was collected through a tracheotomy with aliquots of 0.9 mL of PBS. The BALF cells were counted and stained for identification by means of light microscopy. The right atrium was cannulated with a 20-gauge needle and perfused with 30 mL of PBS to clear lungs of blood. The right lung was homogenized in 1.0 mL of PBS. The left lung was homogenized in Trizol (Sigma).

Immunoglobulins

ELISA kits for IgA, IgG, albumin, and BSA were obtained (Bethyl Laboratories, Montgomery, Tex). An ELISA kit for IgA was also obtained (Immunology Consultants Laboratories, Inc, Newberg, Ore), as was an ELISA kit for ovalbumin-specific IgE (AbD Serotec, Raleigh, NC). Ovalbumin-specific IgG1 and ovalbumin-specific IgG2a were detected as previously described,[9] except the primary antibody used to detect IgG2a was clone LO-MG2a-9 (AbD Serotec). Serum was diluted 4-fold starting at 1:500 for IgG1 and 3-fold starting at 1:100 for IgG2a. BALF and supernatants from blood-free lung tissue were assayed neat. Reagents were not available for ovalbumin-specific IgG1 and IgG2a standard curves. Therefore optical density values from serially diluted samples were compared to estimate fold differences between groups.

Secretory component

Two aliquots of BALF were combined to allow sufficient volume for the generation of standard curves. Ninety-six-well plates were incubated overnight with 100 μL of BALF and then 1:200 goat anti-mouse pIgR (R&D systems, Minneapolis, Minn), followed by 1:1000 horseradish peroxidase–conjugated donkey anti-goat IgG and TMB substrate (BD Biosciences, San Jose, Calif). Concentrations in samples were determined by means of comparison with optical density values generated by BALF from wild-type and 12/15-LO$^{-/-}$ mice to which known amounts of recombinant mouse free secretory component (SC; R&D systems) were added.

Flow cytometry

Lungs were placed in 2.5 mg/mL collagenase D and 0.25 mg/mL DNaseI for 1 hour at 37°C, passaged though a 200-μm nylon mesh, and suspended in red blood cell lysis buffer and then in media (RPMI with 1% BSA) with 2.4G2 anti-FcγRI/III mAb (BD PharMingen, San Diego, Calif) to block nonspecific binding. The cells were incubated with no antibodies, concentration-matched isotype control antibodies, or allophycocyanin–anti-CD19, phycoerythrin–anti-CD69, and phycoerythrin-Cy7-anti-CD25 antibodies (mouse B-lymphocyte activation kit; BD PharMingen). 4′-6-Diamidino-2-phenylindole, dihydrochloride staining was used to gate on living cells. Fifty thousand events per sample were collected and analyzed with FACSDiva Software (BD Biosciences).

Transcripts

Published primer sequences were used to detect IL-4, IL-13, IFN-γ, Muc5ac, and Gob-5 transcripts[10] and 12/15-LO transcripts[6] by using the Taqman method of real-time PCR and Iμ–Cα postswitch IgA transcripts[11] by means of end-product PCR. The Taqman method was performed for pIgR (forward primer, 5′-CCACAGAACGCAACAGCAGTAC; reverse primer, 5′-TGGGAGTAGAATTTGCACGGATA; probe, 5′-FAM-AGGA GAGACCTTCACCGTTTCCTG-BHQ-1). The Cyber Green method of real-time PCR was used to detect joining polypeptide (J chain) transcripts.[12] Preverified Taqman assays were used to detect a proliferation-inducing ligand (APRIL), B-cell activating factor from the TNF superfamily (BAFF), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA), BAFF receptor (BAFF-R), IL-6, IL-10, TGF-β1, IL-5, and Clara cell secretory protein (Applied Biosystems, Foster City, Calif). Copy numbers were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[6]

AA metabolites

BALF was analyzed for 12(S)-HETE, 15(S)-HETE, 13(S)-hydroxyoctadecadienoic acid (HODE), leukotriene (LT) B_4, the combined levels of prostaglandin (PG) E_1 and PGE_2, and the combined levels of the cysteinyl leukotrienes (CysLTs) LTC_4, LTD_4, and LTE_4 by using competitive ELISAs (Assay Designs, Inc, Ann Arbor, Mich).

Statistical analysis

ANOVA and Tukey-Kramer posttests were used in the case of 4 experimental groups, or the Student *t* test was used in the case of 2 experimental groups. Means and SEs are shown. *P* values of less than .05 were considered statistically significant. Unless stated otherwise, at least 6 mice per group were evaluated.

RESULTS

To test the influence of 12/15-LO on systemic sensitization occurring as a consequence of mucosal exposures to allergen, we used the protocol shown in Fig 1. This type of repeated airways exposure to allergen induces experimental asthma in mice that is dependent on the IL-4– and IL-13–activated signaling molecule STAT6.[13] 15-LO-1 is an IL-4– and IL-13–induced product of airway epithelial cells,[14] monocytes,[15] and dendritic cells.[16] The STAT6 pathway is important in this study, as suggested by the observation that STAT6-deficient mice did not demonstrate an allergen-induced expression of 12/15-LO transcripts in the lung