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Ym1/2 Promotes Th2 Cytokine Expression by Inhibiting 12/15(S)-Lipoxygenase: Identification of a Novel Pathway for Regulating Allergic Inflammation

Yeping Cai,* Rakesh K. Kumar,† Jiansheng Zhou,* Paul S. Foster,‡ and Dianne C. Webb2*

The Ym1/2 lectin is expressed abundantly in the allergic mouse lung in an IL-13-dependent manner. However, the role of Ym1/2 in the development of allergic airways disease is largely unknown. In this investigation, we show that treatment of mice with anti-Ym1/2 Ab during induction of allergic airways disease attenuated mediastinal lymph node production of IL-5 and IL-13. Ym1/2 was found to be expressed by dendritic cells (DCs) in an IL-13-dependent manner and supplementation of DC/CD4+ T cell cocultures with Ym1/2 enhanced the ability of IL-13−/− DCs to stimulate the secretion of IL-5 and IL-13. Affinity chromatography identified 12(S)-lipoxygenase (12/15-LOX) as a Ym1/2-interacting protein and functional studies suggested that Ym1/2 promoted the ability of DCs to stimulate cytokine production by inhibiting 12/15-LOX-mediated catalysis of 12-hydroxyeicosatetraenoic acid (12(S)-HETE). Treatment of DC/CD4+ T cell cultures with the 12/15-LOX inhibitor baicalein enhanced, whereas 12(S)-HETE inhibited the production of Th2 cytokines. Notably, delivery of 12(S)-HETE to the airways of mice significantly attenuated the development of allergic airways inflammation and the production of IL-5 and IL-13. In summary, our results suggest that production of Ym1/2 in response to IL-13 promotes Th2 cytokine production and allergic airways inflammation by inhibiting the production of 12(S)-HETE by 12/15-LOX. The Journal of Immunology, 2009, 182: 5393–5399.

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2 Address correspondence and reprint requests to Dr. Dianne C. Webb, Division of Molecular Bioscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia 0200. E-mail: dianne.webb@anu.edu.au
3 Abbreviations used in this paper: DC, dendritic cell; BALF, bronchoalveolar lavage fluid; 12(S)-HETE, 12-hydroxyeicosatetraenoic acid; 12/15-LOX, 12/15-lipoxygenase; MLN, mediastinal lymph node; WT, wild type; 15(S)-HETE, 15-hydroxyeicosatetraenoic acid; HpETE, hydroxyperoxyeicosatetraenoic acid; LDL, low-density lipoprotein; PPARγ, peroxisome proliferator-activated nuclear receptor γ.

Notably, since T cells do not bind IL-13 or express an IL-13 receptor (10), the influence of IL-13 on T cell function is likely through an intermediary rather than as a direct effect on T cells. To investigate intermediaries that may function downstream of IL-13, we previously used a protein-profiling approach to demonstrate that the Ym2 chitinase-like lectin was progressively and abundantly up-regulated in the lung during the development of allergic inflammation by a process that was dependent on CD4+ T lymphocytes and signaling by IL-13 and IL-4 (7, 11). Notably, instillation of IL-13 into the lungs of naive mice was sufficient to induce high-level expression of Ym2 (11). The closely related Ym1 isomer (91% identity with Ym2) was constitutively expressed in the mouse lung, but not up-regulated by allergic responses to the same extent as Ym2 (11). Because they can only be distinguished by gene sequence analysis, many studies do not specifically differentiate between these isomers and refer to these proteins collectively as Ym1/2.

The observation that Ym1/2 is abundantly expressed during the development of allergic airways disease has resulted in escalating use of this protein as a convenient marker of allergic airway inflammation and alternatively activated macrophages (12–17). Importantly, human YKL-40, which is highly homologous to mouse Ym1/2, is similarly elevated in asthmatics and is correlated with airway remodeling, reduced forced expiratory volume in 1 s, and greater patient dependence on asthma medications (18). However, although the expression of YKL-40 parallels the severity of asthmatic responses, it is not known whether “recovery of YKL-40 from these patients indicates either a causative or a sentinel role for this molecule in asthma” (18).

Some insight into the function of Ym1/2 has been generated by studies showing that Ym1 was expressed in DCs from the draining lymph nodes of Brugia malayi-infected mice in a Th2-dependent manner (13) and that treatment of DCs with the lipid-lowering drug, simvastatin, stimulated the ability of DCs to promote Th2 biasing in cocultured CD4+ T cells by a mechanism dependent on Ym1 (19). Because these studies suggested that Ym1/2 plays a role in DC function and as we have shown that IL-13 regulates both expression of...
Ym1/2 and the maturation and function of DCs (9), we considered that Ym1/2 could function as an interface between IL-13 and the ability of this cytokine to influence the functional interaction between DCs and CD4⁺ T cells. Therefore, aims of the current study were to 1) characterize the role of Ym1/2 in regulating cytokine production in the lymphoid compartment during allergic airway inflammation, 2) identify the relevant target protein(s) with which Ym1/2 interacts, and 3) define the mechanistic pathway by which Ym1/2 may regulate the functional interplay between DCs and CD4⁺ T cells.

Materials and Methods

Induction of allergic airways disease

Allergic airway inflammation was induced in 6- to 8-week-old BALB/c wild-type (WT) and IL-13⁻/⁻ mice (20) by i.p. sensitization (day 0) with 50 μg of OVA (fraction V; Sigma-Aldrich) mixed with 20 μl of Rehydregel (Reheis) made up to a total volume of 200 μl with 0.9% sterile saline. On days 12, 14, 16, and 19, all mice were challenged with an aerosol of 10 mg/ml OVA in 0.9% saline three times for 30 min per day with 30-min breaks between aerosols as previously described (9). In some experiments, mice were pretreated with 1 mg of purified rabbit polyclonal anti-Ym1/2 Ab (11) or 1 mg of purified rabbit control Ab injected i.p. during the aerosol challenge period on days 12, 15, and 17. In other experiments, 40 μl of 500 ng/ml 12-hydroxyeicosatetraenoic acid (12(S)-HETE; Cayman Chemicals) or vehicle control was delivered to the nares of fluorothane-anesthetized mice 2 h before each OVA challenge. Mice were sacrificed on day 20 and bronchoalveolar lavage fluid (BALF) was obtained by cannulating the trachea and gently flushing the airways with two 1-ml volumes of PBS. Lungs were also dissected from the thoracic cavity for recovery of mediastinal lymph nodes (MLN). All mice were treated according to Australian National University Animal Welfare guidelines (Protocol JMB32/07) and housed in a specific pathogen-free facility.

Measurement of cytokine production by MLN

Cells from MLN were isolated, washed in MLC medium (9) and stimulated with 1 mg/ml OVA in MLC medium for 68 h at 37°C and 5% CO2 in a humidified incubator. T cells were purified from MLN of 15–25 allergic mice per treatment group as described previously (9). Essentially, MLN were incubated at 37°C for 15 min in 2.5 ml of collagenase buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 1.8 mM CaCl₂) containing 300 U/ml collagenase type IV (Worthington Biochem) and 200 U/ml DNase I (Roche Diagnostics). A cell suspension was prepared from the collagenase-digested MLN by gently pushing the tissue through a cell strainer as previously described (9). Cells were washed and incubated with Fc block (FcγII/III; BD Biosciences) and then enriched for expression of CD11c⁺ DCs or CD4⁺ T cells using the MiniMACS magnetic bead system according to the manufacturer’s recommendations (Miltenyi Biotec). The eluate was passed through a second column to enhance purity of the CD11c⁺ or CD4⁺ T cells. For mixed cell cultures, 2 × 10⁴ CD4⁺ T cells and 10⁵ CD4⁺ T cells were plated per well of round-bottom 96-well plates and then incubated for 5 days at 37°C and 5% CO₂. Negative controls consisted of the same numbers of either CD4⁺ T cells or DCs. In some experiments, cocultures were treated during the 5-day incubation with either 16 or 32 ng/ml 12(S)-HETE, 10 μM baicalein (BIOMOL), purified Ym1/2 (2 μg/ml), or appropriate vehicle controls.

C18 extraction of culture supernatants and measurement of 12(S)-HETE and 15(S)-HETE

DC/CD4⁺ T cell culture supernatants were extracted by reverse-phase chromatography using SPE (C18) cartridges (Cayman Chemicals) as described by the manufacturer. Essentially, proteins were precipitated with methanol, which was then evaporated. The dried sample was resuspended in 50 mM acetate buffer (pH 4.0) and applied to an activated C-18 column. After washing with ultra-pure H₂O and then hexane, hydrophobic material was eluted from the column with 1% methanol in ethyl acetate. After evaporating the organic solvent, the HETE-containing sample was reconstituted in enzyme immunoassay buffer and 12(S)-HETE and 15(S)-HETE were assayed using Correlate-enzyme immunoassay 12(S)-HETE or 15(S)-HETE enzyme immunoassay kits per the manufacturer’s instructions (Asay Designs).

Statistical analysis

The significance of differences between experimental groups was analyzed using the Student unpaired t test. Values are reported as the mean ± SEM. Differences in means were considered significant if p < 0.05.

Results

Ym1/2 enhances Th2 cytokine production in allergic mice

To confirm that expression of Ym1/2 was regulated by IL-13 during the development of allergic airways disease, we compared expression of Ym1/2 in the BALF of WT and IL-13⁻/⁻ mice. Whereas Ym1/2 was barely detectable in the lungs of nonallergic WT mice, it was highly expressed in allergic WT mice. In marked
To investigate molecular mechanisms underlying the ability of Ym1/2 to potentiate production of IL-5 and IL-13, we used affinity chromatography to identify proteins that interact with Ym1/2. Purified Ym1/2 (Fig. 2A) was covalently coupled to a solid-phase matrix and incubated with combined cytoplasmic and membrane fractions of detergent-solubilized lung cells from allergic mice. Nonbinding proteins were removed by washing the matrix with PBS (Fig. 2B, lanes 1–4) and Ym1/2-interacting proteins were then dissociated from the matrix with the strong anionic detergent SDS. A protein band of ~70 kDa was identified in the SDS eluate (Fig. 2B, lane 5) and excised from the gel for in-gel digestion with trypsin to generate peptide fragments. MALDI mass spectrometry was used to determine the size of these peptides and to generate a “peptide fingerprint” (Fig. 2C) that enabled protein identification using analysis with the SWISS-PROT and TREMBL databases. Analysis of the molecular masses of the tryptic-generated peptides putatively identified the protein as arachidonate 12-LOX: murine leukocyte type (molecular mass, 75.4 kDa), also known as 12/15(5)-lipoxygenase (12/15-LOX). Western blot confirmed the identity of the Ym1/2-interacting protein as 12/15-LOX (Fig. 2D).

**DCs and CD4+ T cells differentially express Ym1/2 and 12-LOX**

Having identified 12/15-LOX as a Ym1/2-interacting protein, we next investigated whether these proteins formed a functional interaction that mediated the ability of Ym1/2 to influence Th2 cytokine production by MLN cells. RT-PCR showed that both ym1/2 and Alox15 were expressed in MLN from allergic mice (Fig. 3A), suggesting that Ym1/2 could both interact with 12/15-LOX and exert its Th2-promoting effect in a localized manner in the MLN. Analysis of ym1/2 and Alox15 expression revealed a distinct pattern in DCs and CD4+ T cells. In purified DCs, both ym1/2 and Alox15 were expressed in an IL-13-dependent manner (Fig. 3, B and C). In contrast, CD4+ T cells expressed high levels of Alox15, although ym1/2 was barely detectable (Fig. 3, B and C). These findings suggested that a functional interaction between these proteins could occur either in an autocrine fashion in WT DCs or in a paracrine fashion such that Ym1/2 is secreted from DCs to influence the function of 12/15-LOX in CD4+ T cells.
Ym1/2 modulates Th2 cytokine and 12(S)-HETE production

Because treatment of allergic mice with anti-Ym1/2 Ab attenuated Th2 cytokine production and because we had shown that ym1/2 was expressed in DCs derived from MLN of allergic mice, we next investigated whether Ym1/2 potentiates the ability of DCs to stimulate Th2 cytokine production by CD4+ T cells. Because IL-13−/− DCs produced barely detectable levels of ym1/2 (Fig. 3B), we were particularly interested in whether exogenous Ym1/2 could modify the ability of IL-13+/− DCs to stimulate CD4+ T cells. DCs enriched from MLN of allergic WT and IL-13−/− mice typically showed high-level expression of I-A^A MHC class II on 63% of cells and the endocytotic marker CD205 on 19% of cells (Fig. 4A).

DC morphology (data not shown) was consistent with that previously observed for MLN DCs purified using this method (9). Some low levels of contaminating B cells and macrophages were observed, but this was consistently below 5% of viable cells (Fig. 4A). Cocultures of WT DCs with WT CD4+ T cells exhibited significantly increased production of IL-5 (9-fold) and IL-13 (8-fold) compared with CD4+ T cells cultured alone and cytokine production was undetectable in DCs cultured without T cells (data not shown). IL-13−/− DCs were significantly less efficient at stimulating IL-13 production by T cells than WT DCs (Fig. 4C). However, the addition of Ym1/2 to IL-13−/− DCs significantly enhanced their ability to stimulate IL-5 and IL-13 production by cocultured T cells (Fig. 4, B and C). Because our data indicated that Ym1/2 interacted with 12/15-LOX (Fig. 2), we also investigated whether Ym1/2 influenced the function of 12/15-LOX in this culture system. 12/15-LOX inserts molecular oxygen into arachidonic acid, resulting in the formation of 12(S)-HETE and 15(S)-HETE. Notably, mouse leukocyte 12/15-LOX produces three times more 12(S)-HETE than 15(S)-HETE (21). Although 12(S)-HETE was detected in the supernatants of WT DC/WT CD4 cocultures, these levels were amplified in cocultures with IL-13−/− DCs (Fig. 4D). Of particular interest was the observation that exogenous Ym1/2 suppressed 12(S)-HETE production in cocultures with IL-13−/− DCs (Fig. 4D). When cultured in the absence of DCs, WT CD4+ T cells produced high levels of 12(S)-HETE (11.48 ± 0.93 ng/ml), suggesting that the predominant source of 12/15-LOX in this system is the T cell and that coculture with DCs inhibits T cell production of 12(S)-HETE. Because 12/15-LOX can also produce 15(S)-HETE, we determined whether Ym1/2 also influenced production of this eicosanoid. 15(S)-HETE was detected in the supernatants of cocultures of WT or IL-13−/− DCs with WT CD4+ T cells, but 15(S)-HETE was not significantly higher in cultures with IL-13−/− DCs (Fig. 4E). Although exogenous Ym1/2 significantly suppressed production of 15(S)-HETE, it was only reduced by ~30%, whereas 12(S)-HETE was suppressed by ~95%. Collectively, these data show that Ym1/2 can enhance Th2 cytokine production, particularly IL-5, by CD4+ T cells. Additionally,
12(S)-HETE production is amplified in the absence of both DC-derived IL-13 and reduced endogenous Ym1/2 and is potently suppressed by exogenous Ym1/2.

12/15-LOX regulates the function of CD4\(^+\) memory Th2 cells

Because the levels of 12(S)-HETE in DC/CD4\(^+\) T cell cocultures correlated inversely with IL-5 and IL-13 production (Fig. 4), we tested whether exogenous 12(S)-HETE could directly influence the ability of DCs to stimulate CD4\(^+\) memory Th2 cells. Because lower levels of endogenous 12(S)-HETE were produced in cocultures containing WT DC (Fig. 4D), these cells were used to determine the effects of exogenous 12(S)-HETE. We found that increasing the levels of 12(S)-HETE in cultures significantly suppressed production of IL-5 and IL-13 (Fig. 5A). We then used the 12/15 LOX inhibitor baicalein to investigate the impact of 12/15-LOX on cytokine production. For this experiment, we used IL-13\(^{-/-}\) DCs to stimulate WT CD4\(^+\) T cells since these cultures had produced the highest levels of 12(S)-HETE (Fig. 4D) and the effects of the 12/15-LOX inhibitor might be more pronounced under these conditions. In direct contrast to treatment with exogenous 12(S)-HETE, baicalein enhanced the ability of IL-13\(^{-/-}\) DCs to stimulate IL-5 and IL-13 production by WT CD4\(^+\) T cells (Fig. 5B). Although we cannot exclude the possibility that baicalein may influence other lipoxygenase-related enzymes, treatment of cultures with baicalein significantly inhibited 12(S)-HETE production (vehicle, 5.65 ± 0.25; baicalein, 2.35 ± 0.15 ng/ml), but interestingly, not 15(S)-HETE production (vehicle, 3.56 ± 0.26; baicalein, 3.50 ± 0.14 ng/ml). These data demonstrate that 12/15-LOX activity suppresses Th2 cytokine production in the MLN of mice with allergic airways disease.

12(S)-HETE inhibits eosinophilia and Th2 cytokine production in vivo

Because 12(S)-HETE suppressed Th2 cytokine production in vitro, we tested the possibility that 12(S)-HETE may also attenuate Th2 cytokine production and the severity of allergic responses in vivo. When 12(S)-HETE was delivered intranasally to WT mice during Aeroallergen challenge, the resulting blood neutrophilia and eosinophilia, airway (cells in BALF) and peribronchial eosinophilia (Figs. 6, A–D), and production of IL-5 and IL-13 by MLN (Fig. 6E) were significantly attenuated when compared with vehicle controls.
control-treated mice. Collectively, these data identify 12(S)-HETE as a novel and potent inhibitor of allergy-driven eosinophilia and production of the Th2 cytokines IL-5 and IL-13 in vivo.

Discussion

In this study, we have identified Ym1/2 as an inhibitor of a previously undisclosed 12/15-LOX/12(S)-HETE-dependent regulatory pathway that attenuates both the ability of DCs to stimulate Th2 cytokine production by CD4⁺ T cells and the development of allergic airways inflammation.

First, our data established that Ym1/2, which occurs in the allergic state in response to IL-13, potentiates Th2 cytokine production by MLN cells. To understand the molecular mechanisms underlying this observation, we used an affinity chromatography-based approach, which showed that Ym1/2 interacted with 12/15-LOX, the ortholog of human 15-LOX1 (22). Because inhibiting Ym1/2 in vivo reduced Th2 cytokine production by MLN, we also investigated whether the interaction between Ym1/2 and 12/15-LOX regulated the function of MLN-derived DCs and CD4⁺ T cells, which are key players in initiating and maintaining Th2 cytokine production. Coexpression studies showed that Ym1/2 and Alox15 transcripts were generated in WT DCs in an IL-13-dependent manner, whereas WT CD4⁺ T cells expressed Alox15, but not ym1/2 transcripts.

Murine 12/15-LOX generates a variety of products including 12(S)- and 15(S)-hydroperoxyeicosatetraenoic acid (HpETE) from arachidonic acid. These HpETE derivatives are readily oxidized in vivo by cytosolic glutathione-dependent peroxidases to the more stable 12(S)- and 15(S)-HETE derivatives (23), which are important components in biochemical pathways that have been linked to a number of disease processes (22). However, because the production of 12(S)-HETE by 12/15-LOX is three times more efficient than its production of 15(S)-HETE (21) and because it appears that 15(S)-HETE is also generated from non-12/15-LOX sources in the allergic mouse lung (Fig. E1 in Ref. 24), for the current study, we elected to focus specifically on the role of 12(S)-HETE in regulating Th2 immune responses. A culture model was established using DCs and CD4⁺ T cells purified from the MLN of allergic mice and, to mirror the effects seen with Ab-mediated neutralization of Ym1/2 in vivo, purified Ym1/2 was added to cocultures of these cells. Ym1/2 significantly potentiated the ability of IL-13⁻⁻ DCs to stimulate IL-5 production, in particular, in cocultured T cells, although IL-13 production was also enhanced. Interestingly, the concentration of 12(S)-HETE was significantly higher in cocultures with IL-13⁻⁻ DCs than those with WT DCs, suggesting an inverse relationship between Ym1/2 expression and 12(S)-HETE production. Furthermore, addition of purified Ym1/2 to cocultures with IL-13⁻⁻ DCs significantly suppressed the production of 12(S)-HETE. When cultured without DCs, CD4⁺ T cells produced higher levels of 12(S)-HETE than when these cells were cultured in the presence of either WT or IL-13⁻⁻ DCs, suggesting that the interaction between CD4⁺ T cells and DCs suppressed production of T cell-derived 12(S)-HETE and that this could be mediated by the Ym1/2 secreted by DCs.

Since Ym1/2 and 12/15-LOX are coexpressed in WT DCs, it is possible that these proteins interact within these cells. However, the observation that cocultures containing IL-13⁻⁻ DCs, which express negligible levels of 12/15-LOX and CD4⁺ T cells, produce high levels of 12(S)-HETE suggests that the 12(S)-HETE is generated predominantly by the T cell. This notion is compounded by our observation that CD4⁺ T cells cultured in the absence of DCs produce even higher levels of 12(S)-HETE than when cultured with DCs. Because Ym1/2 is not expressed by the CD4⁺ T cell, it seems likely that Ym1/2 secreted by DCs interacts with 12/15-LOX in or at the surface of the T cell. The mechanism by which secreted Ym1/2 inhibits 12/15-LOX, a predominantly cytoplasmic protein at first seems incongruous. However, 12/15-LOX has been extensively studied for its ability to oxidize low-density lipoproteins (LDLs), a critical step in the formation of atherosclerotic plaques. Although 12/15-LOX predominantly localizes to the cytoplasm, treatment of macrophages with LDLs induced a rapid translocation of 12/15-LOX to the plasma membrane (25). Additionally, 12/15-LOX expressed in J774A cells was capable of stereo-specific oxygenation of LDLs in the extracellular medium, suggesting that the enzyme directly interacts with extracellular LDL particles that make contact with the cell membrane (26). 12/15-LOX also translocates to the cell membrane of emerging filopodia when macrophages are incubated with apoptotic cells (27). Considering these data, we think it feasible that on appropriate activation of CD4 T cells, 12/15-LOX translocates to the plasma membrane, allowing its accessibility to DC-derived Ym1/2.

In addition to its production of 12(S)-HETE, 12/15-LOX is known to also produce 15(S)-HETE, but does so less efficiently to generate a 12(S)-HETE:15(S)-HETE ratio of 3:1 (21). However, our data showed that in DC/CD4⁺ T cell cultures, 15(S)-HETE was produced more efficiently in relation to 12(S)-HETE than previously observed for 12/15-LOX. Additionally, 15(S)-HETE production was not inhibited by the 12/15-LOX inhibitor baicalein and Ym1/2, which we showed interacted with and inhibited 12/15-LOX, was less efficient at inhibiting 15(S)-HETE than 12(S)-HETE production. Considering that 15(S)-HETE production persists in 12/15-LOX⁻⁻ mice (Fig. E1 in Ref. 24), these data suggest that 15(S)-HETE is also partially produced from non-12/15-LOX sources in our culture systems.

The observation that Ym1/2 potentiated Th2 cytokine production and concomitantly suppressed the production of 12(S)-HETE indicated that 12/15-LOX activity might directly modulate Th2 cytokine production. Indeed, when applied to DC/CD4⁺ T cell cocultures, exogenous 12(S)-HETE attenuated Th2 cytokine production and, conversely, treatment with the 12/15-LOX inhibitor baicalein stimulated Th2 cytokine production. We then extended this observation to determine whether 12(S)-HETE influenced the development of allergic airways disease in vivo. Compared with vehicle control, intranasal delivery of 12(S)-HETE during aeroallergen challenge potentiated the severity of blood, lung, and airway eosinophilia and, consistent with our observations that 12(S)-HETE influenced T cell function in vitro, significantly inhibited Th2 cytokine production.

To date, it is unclear how 12(S)-HETE regulates Th2 cytokine production. However, a candidate pathway may be through regulation of the peroxisome proliferator-activated nuclear receptor (PPARγ). 12/15-LOX is known to interfere with T cell proliferation by generating lipid metabolites, including 12(S)-HETE, that can function as ligands for PPARγ (28–30). Notably, treatment of mice with the endogenous PPARγ ligand 15-deoxy-Δ12,14-PGJ₂ or with synthetic ligand significantly reduces lung inflammation, mucus production, and remodeling following induction of allergic airways disease (31, 32). Although further studies are required, the levels of 12(S)-HETE produced in the allergic lung would make it more likely endogenous ligand of PPARγ than 15-deoxy-Δ12,14-PGJ₂, which some have suggested is produced at too low a level to be considered a physiological ligand for PPARγ (33).

In summary, we have provided evidence for a mechanism by which Ym1/2 produced by DCs down-regulates the activity of 12/15-LOX and the resulting production of 12(S)-HETE, leading to potentiation of the production of IL-5 and IL-13 by CD4⁺ T cells. Although recent studies have suggested that products of the 12/
15-LOX biosynthetic pathway may contribute to allergic airway inflammation (24, 34), these studies are confounded by the use of 12/15-LOX−/− mice in which the deficiency in 12/15-LOX stimulates a default pathway that channels arachidonic acid through the 5-LOX pathway with subsequent up-regulation of proinflammatory cysteinyl leukotrienes (24, 35). Additionally, it is likely that 12/15-LOX−/− mice are deficient in a complex mixture of lipid mediators that mediate both pro- and anti-inflammatory pathways. In contrast, by applying 12(5-HEPE) directly to the mouse airway, we have defined a previously undisclosed pathway for regulating Th2 cytokine production and identified 12(S)-HEPE as a novel inhibitor of allergic inflammation. We propose that a pathophysiological function of the IL-13-dependent Ym1/2 lectin is attenuation of the inhibitory effects of 12/15-LOX and thus potentiation of allergic inflammation. However, further studies are required to determine whether Ym1/2 modulates the biosynthesis of molecules other than 12(S)-HEPE by the 12/15-LOX pathway. Interestingly, the human Ym1/2 homolog YKL-40, which has been linked to disease severity in asthmas (36), has recently been ascribed a proinflammatory role in chronic obstructive pulmonary disease (37). Whether YKL-40 can similarly potentiate aspects of allergic inflammation in asthmatics awaits further investigation.

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Disclosures

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References