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Lpa2 Is a Negative Regulator of Both Dendritic Cell Activation and Murine Models of Allergic Lung Inflammation

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Negative regulation of innate immune responses is essential to prevent excess inflammation and tissue injury and promote homeostasis. Lysophosphatidic acid (LPA) is a pleiotropic lipid that regulates cell growth, migration, and activation (1). Extracellular LPA is generated primarily by hydrolysis of lysophosphatidylcholine by the enzyme autotaxin (or lysophospholipase D) (2, 3). A major advance in the field came from the molecular cloning of specific LPA receptors (4–6). There are at least five established LPA receptors, three of which belong to the Edg family, lpa2 (edg4) is expressed by dendritic cells (DC) and other innate immune cells. In this article, we show that DC from lpa2−/− mice are hyperactive compared with their wild-type counterparts and are less susceptible to inhibition by different LPA species. In transient-transfection assays, we found that lpa2 overexpression inhibits NF-κB–driven gene transcription. Using an adoptive-transfer approach, we found that allergen-pulsed lpa2−/− DC induced substantially more lung inflammation than did wild-type DC after inhaled allergen challenge. Finally, lpa2−/− mice develop greater allergen-driven lung inflammation than do their wild-type counterparts in models of allergic asthma involving both systemic and mucosal sensitization. Taken together, these findings identify LPA acting via lpa2 as a novel negative regulatory pathway that inhibits DC activation and allergic airway inflammation. The Journal of Immunology, 2012, 188: 000–000.

Originally described as an intermediate in intracellular lipid biosynthesis, lysophosphatidic acid (LPA; or monoacyl-sn-glycero-3-phosphate) is now recognized as a pluripotent extracellular mediator that regulates cell growth, migration, and activation (1). Extracellular LPA is generated primarily by hydrolysis of lysophosphatidylcholine by the enzyme autotaxin (or lysophospholipase D) (2, 3). A major advance in the field came from the molecular cloning of specific LPA receptors (4–6). There are at least five established LPA receptors, three of which belong to the Edg family: LPA1 (Edg2), LPA2 (Edg4), and LPA3 (Edg7) (7). Signal transduction via these classical LPA receptors leads to activation of MAPKs, PI3K, and Rho kinases, which affect cell activation, survival, and migration (reviewed in Ref. 8). Activation of Gai and PI3K/Akt is emerging as particularly important for LPA-directed cell migration (9–13).

LPA has physiologic roles in wound repair and development (14–20) and emerging roles in disease states, including cancer, atherosclerosis, lung fibrosis, and asthma (21–31). LPA may play a broader role in regulating innate and adaptive immune responses (32). For example, constitutive expression of autotaxin in high endothelial venules contributes to lymphocyte homing to secondary lymphoid organs, presumably by inducing local LPA production and T cell emigration from the vasculature (33). Although LPA has proinflammatory effects (28), it can also inhibit inflammation in some contexts. For example, i.v.-injected LPA protected mice from LPS-induced peritonitis (34), and LPA attenuated cytokine secretion in human monocyte-derived dendritic cells (DC) (9, 35). However, the inhibitory mechanisms and receptor(s) by which LPA attenuates immune responses remain poorly defined.

In this article, we report that LPA2 (edg4) negatively regulates DC activation and allergic airway inflammation using different mouse models of asthma. Compared with wild-type controls, lpa2−/− DC exhibit a hyperactive phenotype both in vitro in DC/T cell coculture, as well as following adoptive transfer into the mouse airway. Although LPA inhibits activation of wild-type DC in response to different pattern recognition receptor ligands, lpa2−/− DC are resistant to LPA-dependent inhibition. In transfection studies, we show that expression of LPA2 inhibits LPS-induced NF-κB activation. Finally, we studied allergic airway inflammation using OVA as a model allergen and protocols known to induce DC activation by either systemic immunization (OVA plus alum i.p.) or mucosal immunization (inhaled OVA plus low-dose LPS); lpa2−/− mice developed greater allergen-driven lung inflammation than did their wild-type counterparts. Taken together, these studies uncover a novel anti-inflammatory role for lpa2 and identify a new pathway involved in the suppression of innate immune responses.

Materials and Methods

Mice

Wild-type mice on the C57BL/6 background were from The Jackson Laboratory. LPA2-deficient (lpa2−/−) mice were derived from frozen gene-
targeted embryos provided by Deltagen (San Mateo, CA), in collaboration with GlaxoSmithKline (GSK), and backcrossed for more than six generations onto the C57BL/6 background. Wild-type and gene-targeted mice were maintained at the University of Rochester, and age- and gender-matched littermate controls were used in all experiments. C57BL/6.PL OT-II TCR transgenic mice recognizing OVA peptide OVA323-339 in the context of I\(^{\text{b}}\) were a gift of Dr. David Topham (University of Rochester). Mouse protocols were reviewed by the University of Rochester Committee on Animal Resources and the GSK Institutional Animal Care and Use Committee and were conducted in accordance with institutional guidelines and the GSK Policy on the Care, Welfare, and Treatment of Laboratory Animals.

Bone marrow-derived DC

DC were derived from bone marrow precursors using modifications of previously published protocols (36). Briefly, wild-type and lpa\(^{2/-}\) mice were euthanized and prepared aseptically to remove femurs and tibias for bone marrow harvest. On day 0, bone marrow cells were seeded at a density of 1 \(\times\) 10\(^{5}\) cells/ml in RPMI 1640 media supplemented with 10% heat-inactivated FBS (lot #103057; Tissue Culture Biologicals, Los Alamitos, CA), 1 M HEPES, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 20 mM 2-ME, and 50 mg/ml gentamicin using sixwell plates (media and additives from Life Technologies, Carlsbad, CA), supplemented with 25 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ). The media were changed and supplemented with the same concentration of GM-CSF together with 10 ng/ml IL-4 (PeproTech) on days 2, 5, and 7. Bone marrow-derived DC (BM-DC) grown using this protocol typically express CD11c, high levels of MHC class II (Ia/Je) and CD11b, and low levels of CD80 and plasmacytoid dendritic cell Ag 1 (Ab panels available upon request). On day 8, DC were harvested and cocultured with allogeneic T cells at DC/T cell ratios of 1:1, 1:5, 1:25, and 1:125, and 1:625. Cell proliferation was analyzed 72 h following coculture using a BrdU Cell Proliferation ELISA (Roche). Cell supernatants were analyzed by ELISA (IL-6 detection limit: 1.6 pg/ml, TNF-\(\alpha\) detection limit: 1.8 pg/ml, vascular endothelial growth factor [VEGF] detection limit: 3 pg/ml; Quantikine Kits, R&D Systems) or multiplex cytokine/chemokine bead array (IL-1\(\alpha\), IL-\(\beta\), IL-2, IL-4, IL-5, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, TNF-\(\alpha\), eotaxin, G-CSF, GM-CSF, IFN-\(\gamma\), MCP-1, MIP-1\(\alpha\), MIP-1\(\beta\), RANTES, and KC; Bio-Plex Pro Mouse Cytokine 23-plex Assay; BioRad, Hercules, CA), according to the manufacturers’ instructions.

Transfection of HEK293T cells with LPA2

HEK293T cells stably transfected with TLR4 and MD2 (a kind gift of Dr. Jung Dong Li, University of Rochester Medical Center, Rochester, NY) were maintained in DMEM (Life Technologies Invitrogen, Carlsbad, CA) containing heat-inactivated 10% FBS (Tissue Culture Biologicals, Los Alamitos, CA) supplemented with 1% nonessential amino acids, 1 mM sodium pyruvate, 0.5 mg/ml penicillin, and 0.1 mg/ml gentamicin. HEK293T cells were seeded at 10\(^7\) cells/ml in 100 mm tissue culture dishes and maintained with 2 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ) and 10% heat-inactivated FBS (lot #103057; Tissue Culture Biologicals, Los Alamitos, CA). Different species of LPA, including 16:0 (1-palmitoyl-2-hydroxy-sn-glycero-3-phospho), 18:1 (1-octadecanoyl-2-hydroxy-sn-glycero-3-phosphate), and 20:4 (1-arachidonoyl-2-hydroxy-sn-glycero-3-phosphate); all from Avanti Polar Lipid, Alabaster, AL) were stored in methanol:H\(_2\)O. Working solutions were prepared fresh by evaporating methanol over N\(_2\) gas and dissolving the residue in PBS containing 1% fatty acid free BSA. Ki16425 was kindly provided by Dr. Andrew Tager (Harvard University, Cambridge, MA), and wortmannin was purchased from Calbiochem.

DC adoptive transfer

Wild-type and lpa\(^{2/-}\) BM-DC were generated, as described above. On day 8, DC were plated at 8 \(\times\) 10\(^4\) cells/condition and pulsed with 400 pg/ml Grade V OVA (Sigma-Aldrich) or saline control. On day 9, 1 \(\times\) 10\(^5\) OVA and control wild-type and lpa\(^{2/-}\) DC were intratracheally instilled by oropharyngeal aspiration (50 \(\mu\)l) into wild-type recipients. The recipients were then aerosol challenged with Grade V OVA inhalation on days 19–22 (1%, 30 min) and sacrificed for analysis of airway inflammation.

OVA sensitization and challenge

Female wild-type and lpa\(^{2/-}\) littermates were used at 6–8 wk of age. We used systemic and mucosal protocols to sensitise and challenge mice using OVA as a model allergen. In the systemic protocol, mice were immunized by i.p. injection of Grade II OVA (20 and 100 \(\mu\)g) plus alum (1.3 and 6.5 mg; Thermo Scientific) on days 0 and 14, respectively, followed by aerosol challenge with Grade V OVA inhalation (1%, 30 min) on days 24, 26, and 28. Some mice were sacrificed on day 10 for analysis of OVA–specific serum IgE levels using a commercially available ELISA (MD Biosciences). To immunize mice via the airway, separate groups of mice were exposed to Grade V OVA (100 \(\mu\)g, Sigma-Aldrich), purified by Endotoxin Removing Columns (Thermo Scientific), either alone or together with low-dose LPS (100 ng; Sigma-Aldrich) by oropharyngeal aspiration (50 \(\mu\)l), followed by aerosol challenge with Grade V OVA inhalation on days 14–16 (1%, 30 min). Bronchoalveolar lavage (BAL) was collected for analysis of cell counts and differentials using standard techniques, as previously reported (37). BAL supernatants were analysed for expression of IL-13 and VEGF using commercially available ELISA kits (eBioscience). Some lungs were inflated with 4% paraformaldehyde and embedded in paraffin, and 8-mm sections were stained with H&E. Lung sections were scored by blinded observers using a semiquantitative scoring system, which takes into account extent and severity of inflammation, on a 0–4 scale.

Bone marrow chimeras

Female wild-type and lpa\(^{2/-}\) littermates were exposed to 1000 rad delivered via a [\(^{137}\)Cs] source using whole-body radiation without shielding. The radiation was delivered in a split dose (500 rad) 4 h apart. Immediately following the second round of radiation, the mice were infused with 1 \(\times\) 10\(^6\) bone marrow cells from wild-type and lpa\(^{2/-}\) donor mice via the tail vein. To permit complete chimerism, we allowed 6 wk of reconstitution before sensitizing and challenging wild-type and lpa\(^{2/-}\) bone marrow chimeras using the mucosal immunization protocol described above.

Results

BM-DC from lpa\(^{2/-}\) mice are hyperactive and not susceptible to inhibition by exogenous LPA

Using primers specific for the classical Edg family of LPA receptors, we confirmed that BM-DC constitutively express lpa1, lpa2, and lpa3, as previously reported for human monocyte-
derived DC (Fig. 1A) (35). The yield of cells and cell surface expression of MHC class II and costimulatory molecules, including CD40, CD80, and CD86, were similar when comparing DC derived from wild-type and lpa2<sup>−/−</sup> littermates (data not shown). However, when comparing the ability of DC to stimulate allogeneic naive CD4<sup>+</sup> T cells in coculture assays, we found that lpa2<sup>−/−</sup> DC induced significantly more T cell proliferation than did their wild-type counterparts, especially at lower DC/T ratios (black bars, Fig. 1B). In coculture supernatants, we detected almost twice as much IL-13 secretion from T cells stimulated with lpa2<sup>−/−</sup> DC compared with their wild-type counterparts (Fig. 1C). In contrast, DC obtained from lpa1 gene-targeted mice behaved similarly to wild-type mice in these assays (gray bars, Fig. 1). Pretreating DC with the LPA1/3 antagonist Ki16425 had no effect on the ability of DC to induce T cell proliferation or activation (38), whereas the PI3K inhibitor wortmannin (0.1–10 μM) inhibited the ability of both wild-type and lpa2<sup>−/−</sup> DC to stimulate T cells equally well (data not shown, Supplemental Fig. 1). These data indicate that, in the absence of lpa2, DC acquire a hyperactive phenotype and suggest that LPA2 is an inhibitory receptor that attenuates DC activation.

We explored this possibility further using two approaches. First, we studied the secretion of inflammatory and immunoregulatory cytokines by wild-type and lpa2<sup>−/−</sup> DC in response to LPS, which is known to activate DC in a TLR4/MyD88-dependent manner. We first confirmed that the expression of TLR4 was not affected by lpa2 deficiency (data not shown). Compared with their wild-type counterparts, we found that when stimulated in the presence of 10% serum, lpa2<sup>−/−</sup> DC secreted significantly more VEGF than did their wild-type counterparts, whereas secretion of other cytokines, including IL-6, IL-12p70, and TNF-α, was similar between genotypes (data not shown, Supplemental Fig. 1). One explanation for this finding is that LPA present in serum-containing tissue culture medium is sufficient to suppress LPS-dependent DC activation in an lpa2-dependent manner. To test this possibility, we next stimulated wild-type and lpa2<sup>−/−</sup> DC under reduced serum conditions with LPS (1 μM), alone or together with 16:0, 18:1, or 20:4 LPA (1–10 μM). If lpa2 normally inhibits DC activation, then wild-type DC should be more susceptible to LPS-dependent inhibition than lpa2<sup>−/−</sup> DC. Fig. 2 shows that exogenous LPA inhibited LPS-driven IL-6 secretion from wild-type (open bars) but not lpa2<sup>−/−</sup> DC (closed bars); the data are expressed as relative inhibition compared with cells stimulated with LPS alone, and the dotted line indicates no inhibition. Table I shows that, under reduced serum conditions, LPS-stimulated lpa2<sup>−/−</sup> DC secreted significantly less IL-10 and more TNF-α than did their wild-type counterparts, whereas IL-12p70 secretion was comparable between genotypes. Furthermore, three exogenous LPA species partially suppressed LPS-driven TNF-α secretion in wild-type DC, an effect that was lost using lpa2<sup>−/−</sup> DC (Table I). Interestingly, both saturated and unsaturated LPA species inhibited LPS-driven IL-6 and TNF-α secretion, which contrasts with the preferential ability of unsaturated LPA species to induce DC migration in vitro (39) (see Discussion).

**Expression of lpa2 inhibits LPS-dependent NF-κB activation**

Signal transduction via the TLR4 receptor complex is known to induce cytokine secretion in an NF-κB–dependent manner. To test the possibility that lpa2 interferes with NF-κB–dependent gene expression, we used HEK293T cells stably expressing TLR4 and MD2, which do not express LPA2 at baseline (data not shown). We first confirmed that, after cotransfection with a full-length expression vector, LPA2 is expressed in these cells and localizes to the cell membrane (data not shown, Supplemental Fig. 3). As expected, LPS induced transcriptional activation of an NF-κB–driven reporter construct in cells cotransfected with an empty expression vector (Fig. 3). In contrast, LPS-dependent NF-κB activation was significantly attenuated in LPA2-expressing cells. Levels of secreted IL-6 were at or below detection limits in these experiments (data not shown). Treatment with exogenous 16:0 LPA alone, or in combination with LPS, did not result in additional inhibition of reporter gene activity (data not shown). Interestingly, transient transfection of an LPA1 expression vector also attenuated LPS-dependent NF-κB activation in HEK293T cells expressing TLR4/MD2 (N. Meednu, unpublished observations): the mechanisms and consequences of this effect are being pursued in a separate study. Taken together, these data support the idea that endogenous serum LPA inhibits LPS-induced NF-κB–dependent gene expression, at least in part, in an lpa2-dependent manner.

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**FIGURE 1.** lpa2-deficient DC are hyperactive in vitro: T cell stimulation. (A) Expression of LPA1–3 by BM-DC using RT-PCR. Wild-type (white bars), lpa1-deficient (gray bars), and lpa2-deficient DC (black bars) on the C57BL/6 background were incubated with allogeneic (BALB/c) naive CD4<sup>+</sup> T cells at the indicated DC:T ratios for 72 h, followed by analysis of T cell proliferation (B) and IL-13 production (C). Results are mean ± SEM (n = 5–6 mice). *p < 0.05.

**FIGURE 2.** lpa2-deficient DC are refractory to inhibition by different LPA species. Wild-type and lpa2-deficient DC were stimulated under reduced serum conditions with LPS alone or together with increasing concentrations of the indicated LPA species (1–10 μM) for 48 h, followed by analysis of IL-6 secretion by ELISA. Results are expressed relative to LPS-induced IL-6 production in the absence of LPA and are the mean ± SEM of n = 3 mice/group. *p < 0.05.
PTX augments the ability of wild-type, but not lpa2<sup>-/-</sup>, DC to stimulate T cell proliferation

Many of the effects of LPA2 are mediated by coupling with G proteins, which stimulate T cell proliferation. PTX augments the ability of wild-type, but not lpa2<sup>-/-</sup> DC to induce T cell proliferation in a dose-dependent manner. Similarly to Fig. 1, we found that untreated lpa2-deficient DC induced more proliferation in responding T cells than did their wild-type counterparts (white bars; p < 0.05 versus wild-type); in contrast to wild-type cells, preincubation with PTX had no significant effect on the T cell-stimulating capacity of lpa2-deficient DC. Because no exogenous LPA was supplied in this experiment, these data further support the idea that LPA present in serum transduces a tonic inhibitory signal that dampens DC activation via LPA2.

Lpa2-deficient DC are hyperactive and proallergic in vivo

Using in vitro assays, we found that lpa2-deficient DC induced more T cell proliferation and IL-12p70 production than did their wild-type counterparts, suggesting that they may promote Th2-driven allergic immune responses in vivo (40, 41). To test this possibility, we used an adoptive-transfer model in which wild-type mice received allergen-pulsed wild-type or lpa2<sup>-/-</sup> DC by intratracheal administration, followed by aerosol allergen challenge using OVA as a model allergen (modified from Refs. 42–44). Mice receiving control, saline-pulsed DC followed by OVA aerosol challenge developed little or no lung inflammation, as determined by BAL cell counts (>95% macrophages), whereas significant lung eosinophilia developed in mice that received OVA-pulsed wild-type DC followed by OVA aerosol challenge (Fig. 6B, data not shown). Interestingly, adoptive transfer of lpa2-deficient, OVA-pulsed DC resulted in substantially more lung inflammation than did transfer of wild-type DC following OVA challenge (Fig. 5). Taken together with the results shown in Figs. 1–4, these data indicate that lpa2-deficient DC are hyperactive in both in vitro and in vivo assays.

Greater allergen-driven airway inflammation in lpa2<sup>-/-</sup> mice compared with controls

Finally, we compared wild-type mice with lpa2-deficient mice in two models of allergic airway inflammation known to involve DC activation. First, we used the well-established model of systemic immunization using i.p. injection of OVA plus alum. Second, we used a mucosal immunization approach and sensitized mice with inhaled endotoxin-free OVA using low-dose LPS (100 ng) as adjuvant (45). Using both systemic- and mucosal-immunization approaches, we found that lpa2-deficient mice developed greater allergic sensitization, airway inflammation, and airway hyperresponsiveness than did their wild-type counterparts (Figs. 6, 7). In the OVA-plus-alum model, lpa2-deficient mice developed more airway eosinophilia and higher BAL IL-13 and VEGF levels at 48 and 72 h following allergen challenge (Fig. 6A–C, data not shown), indicative of greater Th2-driven allergic airway inflammation. To determine whether lpa2 deficiency resulted in augmented allergen sensitization, we sacrificed a separate group of mice 10 d after OVA-plus-alum immunization and found that serum OVA-specific IgE levels were almost twice as high in lpa2-deficient mice compared with wild-type controls (Fig. 6D).

Mucosal immunization protocols result in less severe airway inflammation than does systemic immunization following recall
FIGURE 5. Ipa2-deficient DC are hyperactive and proallergic in vivo. DC from wild-type or Ipa2−/− mice were pulsed with 400 μg/ml OVA for 24 h, and adoptively transferred intratracheally into wild-type recipients. Control mice received unpulsed DC from wild-type mice. Ten days later, mice were challenged for four consecutive days with 1% OVA aerosol and sacrificed 24 h later for analysis of total BAL cell counts (A) and BAL cell differentials using cytospin (B). Results are the mean ± SEM of three independent experiments. *p < 0.05, recipients of wild-type versus Ipa2-deficient DC.

Allergen challenge, but they are a more physiological route of allergen encounter. Using the approach described by Eisenbarth et al. (45), with low-dose LPS as inhaled adjuvant, we found that Ipa2-deficient mice developed a greater influx of inflammatory cells into BAL fluids than did their wild-type counterparts 48 h after allergen challenge (Fig. 7A). Interestingly, although the percentages of eosinophils and neutrophils were similar between the groups (eosinophils: 22 ± 2% versus 17 ± 2% and neutrophils: 8 ± 2% versus 11 ± 3%), wild-type versus Ipa2 knockout, respectively, mean ± SEM of n = 9–11, airway hyperreactivity measured in sedated and paralyzed mice was significantly greater in Ipa2-deficient mice compared with wild-type controls (Fig. 7B). We used reciprocal bone marrow chimeras to investigate the requirement for LPA2 in hematopoietic and nonhematopoietic cells in suppressing allergic lung inflammation following mucosal immunization. We found that wild-type mice reconstituted with Ipa2−/− bone marrow developed significantly greater airway inflammation than did those reconstituted with wild-type bone marrow (Fig. 7C). Furthermore, Ipa2-deficient recipients of either wild-type or Ipa2-deficient bone marrow reacted similarly to wild-type recipients of wild-type bone marrow. These data indicate that Ipa2 expression by a radiosensitive bone marrow-derived cell(s) normally restrains allergic lung inflammation.

**Discussion**

Using complementary approaches, we uncovered a novel role for Ipa2 (Edg4) in suppressing DC activation and allergic immune responses. DC from Ipa2-deficient mice were hyperactive using in vitro assays compared with their wild-type counterparts and induced greater allergic airway inflammation after adoptive transfer in vivo. Wild-type (but not LPA2-deficient) DC were susceptible to inhibition by different exogenous LPA species, and PTX enhanced the ability of wild-type DC to induce T cell proliferation, an effect not seen using LPA2-deficient DC. Collectively, these data support a model in which LPA, acting via LPA2 coupled to cAMP, acts to tonically inhibit DC activation. Thus, in addition to regulating cell recruitment and survival, our data establish a novel role for LPA as a negative regulator of innate immunity.
Negative regulation of innate immune responses is important to prevent excess inflammation and tissue injury (46). Negative regulatory mechanisms have been identified that suppress activation of innate immune cells and the dysfunction of which may be associated with disease states (47). Because LPA is constitutively present in serum and BAL fluids (48, 49), one possibility is that the extravasation of LPA-containing serum into tissues that occurs during inflammation may be sensed as an anti-inflammatory or proresolusion signal (50). In support of this notion, LPA promotes epithelial barrier function, a key step in the restoration of normal tissue integrity following inflammatory insults (51). A corollary of this hypothesis is that dysfunction of the LPA/Lpa2 axis may contribute to persistent inflammation in chronic disease states. Taken together, these findings suggest that lysolipids may play a broader role in dampening immune responses than previously suspected.

Our data support a model in which LPA2 coupling to Gαi suppresses NF-κB–dependent DC activation. Precedence for the idea that PTX can augment DC activation is provided by the work of Ausiello et al. (52), and our data firmly implicate a role for LPA2 in this regard. The C-terminal tail of LPA2 contains unique sequences that support macromolecular complex formation (53), and it is attractive to speculate that this complex negatively regulates TLR4-dependent activation of NF-κB. Future studies will be needed to explore this and other mechanistic possibilities.

We found that heterozygous Lpa2+/− mice were partially protected from lung inflammation following challenge with Schistosoma egg Ag. The reasons for this apparent discrepancy are not immediately obvious, but they may relate to the nature of the Ags used, immunization protocols, or genetic backgrounds. Using bone marrow chimeras, we uncovered a key role for Lpa2 expression by radiosensitive hematopoietic cells in suppressing allergic airway inflammation. Our results using adoptive-transfer experiments firmly implicate DC in this regard and are supported by the observation that OVA–specific IgE responses are enhanced in the absence of LPA2.

LPA is constitutively present in epithelial lining fluids of the human lung and is significantly enriched during the late-phase response following segmental allergen challenge (49). Based on our findings and previously published research, we can construct a working model in which LPA has both pro- and anti-inflammatory effects in asthma. Proinflammatory effects can result from the ability of LPA to promote cell recruitment or activation (9, 27–29), especially in response to submaximal stimuli (55). LPA can also augment airway hyperresponsiveness by direct effects on smooth muscle cells (30, 31). However, because LPA also restores epithelial barrier function (51), enhances IL-13Rα2 expression (56), inhibits epithelial RANTES production (57), and attenuates DC activation (this report), it has the potential to dampen airway inflammation. The observation that LPA is constitutively present in epithelial lining fluids supports its potential role in maintaining lung homeostasis. One intriguing possibility is that LPA contributes to airway remodeling in long-standing asthma. By promoting fibroblast recruitment (25) and smooth muscle mitogenesis (58), local generation of LPA during cycles of airway injury and repair could lead to airway fibrosis and smooth muscle hypertrophy. More research is needed to understand the mechanisms and timing of LPA generation in the airway, as well as the roles of different LPA receptors and target cells in airway inflammation and remodeling.

Taken together, our results establish a novel role for Lpa2 as an inhibitory receptor of a key innate immune cell type and uncover a new pathway involved in tonic dampening of allergic immune responses. Future studies investigating the function of the inhibitory LPA/Lpa2 axis in infectious diseases should prove worthwhile. We also speculate that LPA2-specific agonists may have anti-inflammatory properties and therapeutic efficacy in allergic and inflammatory diseases.

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Disclosures

The authors have no financial conflicts of interest.

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