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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**
From the Department of Medicine, Allergy-Immunology Division, Northwestern University Feinberg School of Medicine, Chicago, Ill

Abstract

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.

Keywords

Asthma; allergy; inflammation; lipoxygenase; IL-13; IgA; B cells; airway; lung

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

Reprint requests: Douglas A. Kuperman, PhD, Division of Allergy-Immunology, 240 E, Huron, Room M-317, Chicago, IL., d-kuperman@northwestern.edu.

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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.

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 Vovalbumin in 200μL 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 transcripts6 by using the Taqman method of real-time PCR and Iμ–Cα postswitch IgA transcripts11 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 (Fig 1, *A*). Furthermore, 12/15-$LO^{-/-}$ mice were almost completely protected from the induction of specific IgE antibodies in serum after 2 and 4 airway exposures (Fig 1, *B*). Likewise, 12/15-$LO^{-/-}$ mice were at least 4-fold protected from the induction of specific IgG1 antibodies (Fig 1, *C*). Specific IgG2a antibodies were not detected after 2 airway exposures; however, 12/15-$LO^{-/-}$ mice were approximately 3-fold protected compared with wild-type responses after 4 airway exposures (Fig 1, *D*). After 4 airway exposures, 12/15-$LO^{-/-}$ mice were almost completely protected from allergen-induced increases in expression of T_H2 cytokines (IL-4 and IL-13) and a T_H1 cytokine (IFN-γ), as well as induction of transcripts encoding Muc5ac and Gob-5 that are associated with allergen-induced mucus metaplasia (Fig 1, *E*).[10] Compared with wild-type mice, there was an equivalent increase in alveolar macrophage numbers, but significantly lower numbers of eosinophils were observed in BALF from 12/15-$LO^{-/-}$ mice (see Table E1 in this article's Online Repository at www.jacionline.org).

To study the influence of 12/15-LO on systemic sensitization induced by intraperitoneal injection of allergen, we used the protocol shown in Fig 2. Under this protocol, there were no differences between wild-type and 12/15-$LO^{-/-}$ mice for ovalbumin-specific IgE antibodies in serum 7 days after either the first or second intraperitoneal injections (Fig 2, *A*). The development of ovalbumin-specific IgG1 antibodies in sera was not detected 7 days after the first intraperitoneal injection, and there were no differences in the specific IgG1 levels 7 days after the second intraperitoneal injection (Fig 2, *B*). Some mice that were sensitized by means of intraperitoneal injection also received a brief period of allergen exposures to the airways. This protocol also results in the development of STAT6-dependent experimental asthma.[9] Ovalbumin-specific IgG2a responses only developed after the brief airway exposures to allergen. In this case 12/15-$LO^{-/-}$ mice were approximately 3-fold protected compared with wild-type mice (Fig 2, *C*). There were no differences observed for the induction of transcripts encoding IL-4, IL-13, and IFN-γ cytokines (Fig 2, *D*) and Muc5ac and Gob-5 (data not shown), and no differences were observed for the allergen-induced profile of cells in BALF (see Table E2 in this article's Online Repository at www.jacionline.org).

12/15-LO$^{-/-}$ mice were only protected from allergic sensitization and airways inflammation when the airways route of allergen exposure was used. This suggested a lung-restricted protective influence of 12/15-LO deficiency on systemic sensitization. One possibility was that 12/15-LO deficiency enhanced a first-line physical or immune barrier function in the lung. The levels of serum proteins in BALF were evaluated in wild-type and 12/15- LO$^{-/-}$ mice at baseline and in response to LPS treatment, which induces neutrophilic inflammation, dilation of epithelial tight junctions, and influx of serum proteins into the lung, to determine whether 12/15-LO regulated the physical permeability of the lung.[17] Mice were treated with PBS (control animals) or LPS directly to the airways, and then BSA was injected into the venous circulation as an exogenous serum protein tracer. The next day, no differences between wild-type and 12/15-LO$^{-/-}$ mice were detected for BSA (Fig 3, *A*), mouse serum albumin (Fig 3, *B*), and IgG (Fig 3, *C*) in BALF at baseline and in response to LPS treatments. Because baseline BSAwas not altered, decreased lung permeability was unlikely to explain the decreased susceptibility of 12/15-LO$^{-/-}$ mice to the development of antibody responses induced by airways-specific allergen exposures. However, IgA levels were approximately 4-fold increased at baseline in BALF from 12/15-LO$^{-/-}$ mice (Fig 3, *D*), suggesting a previously unrecognized role for 12/15-LO in the regulation of lung mucosal immunity.

In the process of active epithelial transport of IgA, the pIgR (also membrane SC) is cleaved, resulting in release of bound SC incorporated into SIgA. The levels of SC and IgA in BALF and IgA in serum were compared between in-house C57Bl/6 wild-type and 12/15-LO$^{-/-}$ mice and genetically diverse age- and sex-matched inbred mouse strains (AKR/J, C57Bl/6, BALB/c, and 129/SvJ) that had recently arrived at our facility to determine whether increased IgA levels in BALF of 12/15-LO$^{-/-}$ mice might be derived from epithelial transport and to determine the possible contributions of environmental and genetic influences. There were at least 3.5-fold greater levels of SC detected in BALF from 12/15-LO$^{-/-}$ mice compared with wild-type mice by means of direct ELISA (Fig 4, *A*), and this result was confirmed by means of Western immunoblotting (data not shown). No differences in SC were observed between in-house C57Bl/6 wild-type mice and recently purchased C57Bl/6 wild-type mice, suggesting that the environment of the mouse facility did not contribute significantly to the results. The increase of SC and IgA levels in BALF caused by 12/15-LO deficiency was large compared with the baseline variability between strains (Fig 4, *A* and *B*), and this result renders unlikely a significant effect of genetic variance. The levels of IgA in serum were not increased in 12/15-LO$^{-/-}$ mice (Fig 4, *C*), further suggesting that the increased IgA levels detected in BALF from these mice was of mucosal origin. The levels of IgA in serum were significantly greater in the 129/SvJ strain than in any other strain tested. However, this did not translate to increased SC and IgA levels in BALF from the same mice, suggesting that genetic polymorphisms can regulate systemic IgA production independently of mucosal IgA production. Baicalein inhibits 12/15-LO activity,[18] and treatment of wild-type mice with Baicalein increased IgA levels in BALF (Fig 4, *D*) but not in serum (data not shown). These results suggest that the 12/15-LO enzyme exerts an inhibitory effect on SIgA in the airways.

If the increased SIgA levels in BALF from 12/15-LO$^{-/-}$ mice reflected increased local production of IgA, then IgA levels in the lung tissue of 12/15-LO$^{-/-}$ mice should be increased compared with that seen in wild-type mice. To test this, blood was cleared from mouse lungs, and the whole lungs were repeatedly lavaged (5 times) to remove IgA from the lumen. IgA levels were measured in each washing and in the supernatants of homogenized right lung tissue by means of ELISA. After 5 washes, IgA levels in BALF from 12/15-LO$^{-/-}$ mice were dramatically reduced (Fig 5, *A*). However, the lung tissue of 12/15-LO$^{-/-}$ mice still retained much more IgA than that of wild-type mice (Fig 5, *B*), which is consistent with increased IgA production in the lungs of 12/15-LO$^{-/-}$ mice. The pIgR can also transport polymeric IgM, resulting in secretory IgM. However, the levels of IgM in BALF and lung tissue were unaltered in 12/15-LO–deficient mice (data not shown).

The expression of cell-surface markers on dispersed lung cells was analyzed by means of flow cytometry to evaluate whether increased local IgA production in 12/15-LO–deficient mice was associated with increased mucosal B-cell activation (Table I). Compared with wild-type mice, the percentage of live cells that expressed the B cell–specific cell-surface marker CD19 trended lower in the lungs of 12/15-LO$^{-/-}$ mice. This might be explained by loss of CD19 expression as mouse B cells become terminally differentiated plasma cells.[19] However, a greater percentage of CD19^{+} cells expressed the early activation markers CD69 (very early activation antigen) and CD25 (low affinity IL-2 Receptor α chain) in dispersed lung cells from 12/15-LO$^{-/-}$ mice.

The levels of IgA-specific postswitch transcripts in blood-free lung tissue were detected by means of end-product PCR to evaluate further the effect of 12/15-LO deficiency on mucosal B-cell activation (Fig 6, *A*). After 30 cycles of PCR with 1.0 μg input whole-lung cDNA from a wild-type mouse, a faint band was detected, and no bands were noted in subsequent 4-fold dilutions of input cDNA. In comparison, 1.0 μg of whole-lung cDNA from a 12/15-LO$^{-/-}$ mouse generated a very dense band. A band was easily visualized at 0.25 μg input cDNA, and a faint band was still detected at 0.06 μg input cDNA. The 15-kd J chain is produced by plasma cells, and it links IgA monomers into polymers, which is critical for the transport of polymeric IgA by pIgR.[4] Significantly greater levels of transcripts encoding J chain were detected in the lungs of 12/15-LO$^{-/-}$ mice compared with that detected in the lungs of wild-type mice (Fig 6, *B*). APRIL and BAFF are cytokines involved in mucosal B-cell activation. APRIL and BAFF bind to their coreceptors, BCMA and TACI. BAFF can also bind to BAFF-R.[20] There were increased transcript levels of TACI (Fig 6, *C*) and BCMA (Fig 6, *D*) receptors in the lungs of 12/15-LO$^{-/-}$ mice. However, no differences in expression of IL-5, IL-10, IL-6, TGF-β1, BAFF, BAFF-R, and APRIL were detected (data not shown). These results suggested that increased mucosal B-cell activation resulted in increased local production of IgA and J chain, which likely contributed to increased SIgA levels in 12/15-LO$^{-/-}$ mice. However, an increased capacity for airway epithelial cells to transport IgA might have also contributed to increased SIgA levels because the levels of transcripts encoding pIgR were approximately 4-fold greater in the lungs of 12/15-LO$^{-/-}$ mice compared with those of wild-type mice (Fig 6, *E*). The levels of transcripts encoding the epithelial-specific product Clara cell secretory protein were not altered (Fig 6, *F*), suggesting that 12/15-LO deficiency did not generally increase epithelial gene expression.

The levels of 12(S)-HETE and 15(S)-HETE and the combined levels of CysLTs, including LTC_4, LTD_4, and LTE_4, were measured in BALF by means of ELISA to evaluate the effects of 12/15-LO deficiency on AA metabolism in the lung (see Fig E1 in this article's Online Repository at www.jacionline.org). Compared with those of wild-type mice, 12(S)- and 15(S)-HETE levels were reduced and CysLT levels were increased in BALF of 12/15-LO$^{-/-}$ mice. Levels of LTB_4, the combined levels of PGE_1 and PGE_2, and the levels of 13(S)-HODE were not altered (data not shown).

DISCUSSION

Systemic antibody responses and allergic airways inflammation occurring as a consequence of systemic and mucosal allergen exposures were evaluated in wild-type and 12/15-LO$^{-/-}$ mice. In the mucosal allergen exposure model, 12/15-LO$^{-/-}$ mice demonstrated markedly diminished IgE antibody responses and less eosinophilic airways inflammation. In the systemic allergen exposure model, 12/15-LO had no obvious role in systemic sensitization and eosinophilic inflammation. This suggested a lung-restricted influence of 12/15-LO on allergic sensitization and, consequently, the downstream development of allergic airways inflammation.

There was evidence of increased mucosal-specific production of SIgA in the airways of 12/15-LO$^{-/-}$ mice. This might explain why 12/15-LO$^{-/-}$ mice were protected from allergic sensitization and airways inflammation in the airways exposure model but not in the systemic exposure model. For example, SIgA blocks allergen penetration of mucosal surfaces[3] and induces inhibitory effects on dendritic cell maturation[21] and antigen presentation.[22] A previous report demonstrated that delivery of allergen-specific IgA to the airways of mice leads to protection from airways hyperreactivity, eosinophilia, and secondary increases in allergen-specific IgE induced by airways exposure to allergen in mice that were previously exposed to allergen by means of intraperitoneal injection.[23] On the other hand, SIgA has been observed to activate human eosinophils.[24] Based on the present results, increased 15-LO-1 expression and activity in the airways of subjects with asthma[7] might account for the suppression of SIgA seen in BALF from subjects with asthma.[5] Suppression of SIgA could be beneficial by lessening eosinophil activation. However, it might also leave individuals with asthma relatively more susceptible to the development of IgE and inflammatory responses that occur as a result of aeroallergen exposures.

As expected, reductions of the 12/15-LO metabolites 12(S)- and 15(S)-HETE occurred as a consequence of 12/15-LO deficiency. Although a large decrease of 12(S)-HETE was observed, significant levels of 15(S)-HETE remained in the lungs, indicating that 15(S)-HETE can be produced independently of 12/15-LO. The interaction of AA with mouse Alox8 results in the formation of 15(S)-HETE,[25] and therefore Alox8 (the homolog of human 15-LO-2) might be an important source of 15(S)-HETE in the lungs.We observed increased CysLT levels in lungs of 12/15-LO$^{-/-}$ mice, as previously reported in 12/15-LO–deficient peritoneal macrophages.[26] This might reflect the effects of substrate diversion, inhibitory effects of 12/15-LO products on 5-LO activity, or both. However, 12/15-LO$^{-/-}$ mice had similar responses to wild-type mice in the systemic model and were protected in the mucosal model, and therefore it appears that the increased CysLT levels did not exert a profound proinflammatory effect on the end points evaluated.

Eicosanoids potently regulate immune cells through the actions of specific cell-surface receptors.[27] However, to the best of our knowledge, only PGE_2 is known to directly promote antibody production,[28] and no changes in the combined levels of PGE_1 and PGE_2 were detected. The SC inhibits the ability of phospholipase A_2 to mediate the release of AA from cell membranes.[29] Therefore suppression of AA release, mediated by increased SC in 12/15-LO$^{-/-}$ mice, might have contributed to protection from allergic sensitization and airways inflammation in these mice. However, if this were a dominant effect of SC in this model, then one would have expected reductions of CysLT levels in 12/15-LO$^{-/-}$ mice rather than the increases that were observed. The precise molecular pathway by which changes in AA metabolites link 12/15-LO deficiency to increased SIgA levels remains largely undefined.

T_H1-dependent experimental allergic encephalomyelitis is enhanced in 12/15-LO$^{-/-}$ mice,30 suggesting that 12/15-LO has a protective role in the central nervous system. 12/15-LO–deficient mice have an expansion of myeloid cells but not lymphoid cells (including T and B cells) in the spleen.[31] However, this did not alter baseline levels of IgA, IgG, IgM, and IgE in serum (data not shown). An embryo implantation defect was described in the uterus of 12/15-LO–deficient female mice.32 Theses observations and the present study suggest an important role for 12/15-LO in immune responses and regulation of mucosal surfaces.

In conclusion, induction of 15-LO-1[7] might contribute to the development of allergic sensitization and elicitation of airways inflammation caused by aeroallergen exposures in patients with asthma, potentially through suppression of the first-line mucosal immune defense mediator SIgA.[5]

Clinical implications: Inhibition of 15-LO-1 might boost SIgA levels in the airways and improve the condition of patients with asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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

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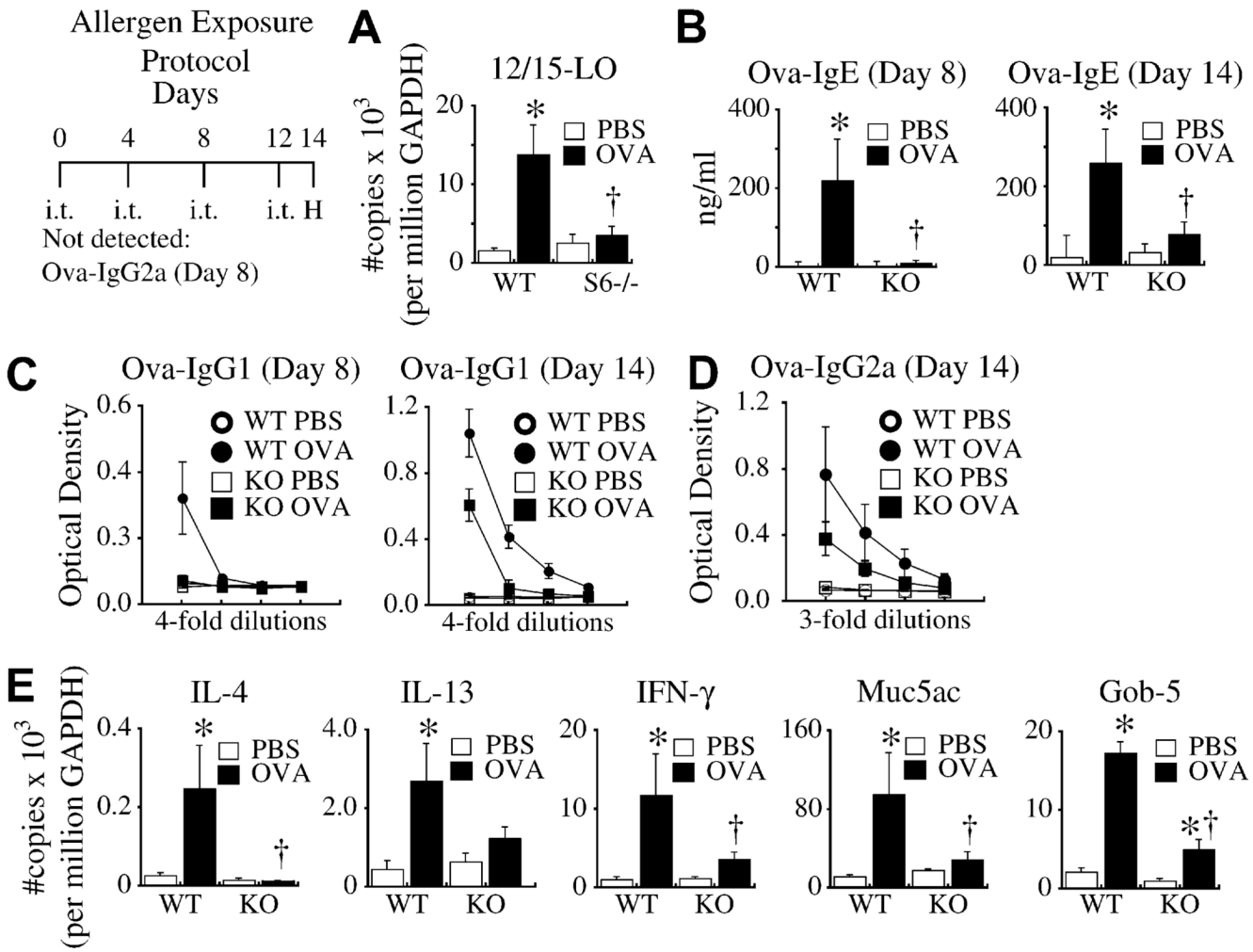

FIG 1.

12/15-LO deficiency protects mice from allergic sensitization induced by airways exposure to allergen. Levels of transcripts encoding 12/15-LO in the lungs of wild-type (*WT*) and STAT6$^{-/-}$ (S6−/−) mice are shown **(A)**. Levels of ovalbumin *(Ova)*–specific IgE **(B)**, ovalbumin-specific IgG1 **(C)**, and ovalbumin-specific IgG2a **(D)** in serum and transcripts encoding IL-4, IL-13, IFN-γ, Muc5ac, and Gob-5 **(E)** in lungs of wild-type (WT) and 12/15-LO$^{-/-}$ *(KO)* mice are also shown. **P* < .05 versus PBS. †P < .05 versus ovalbumin.

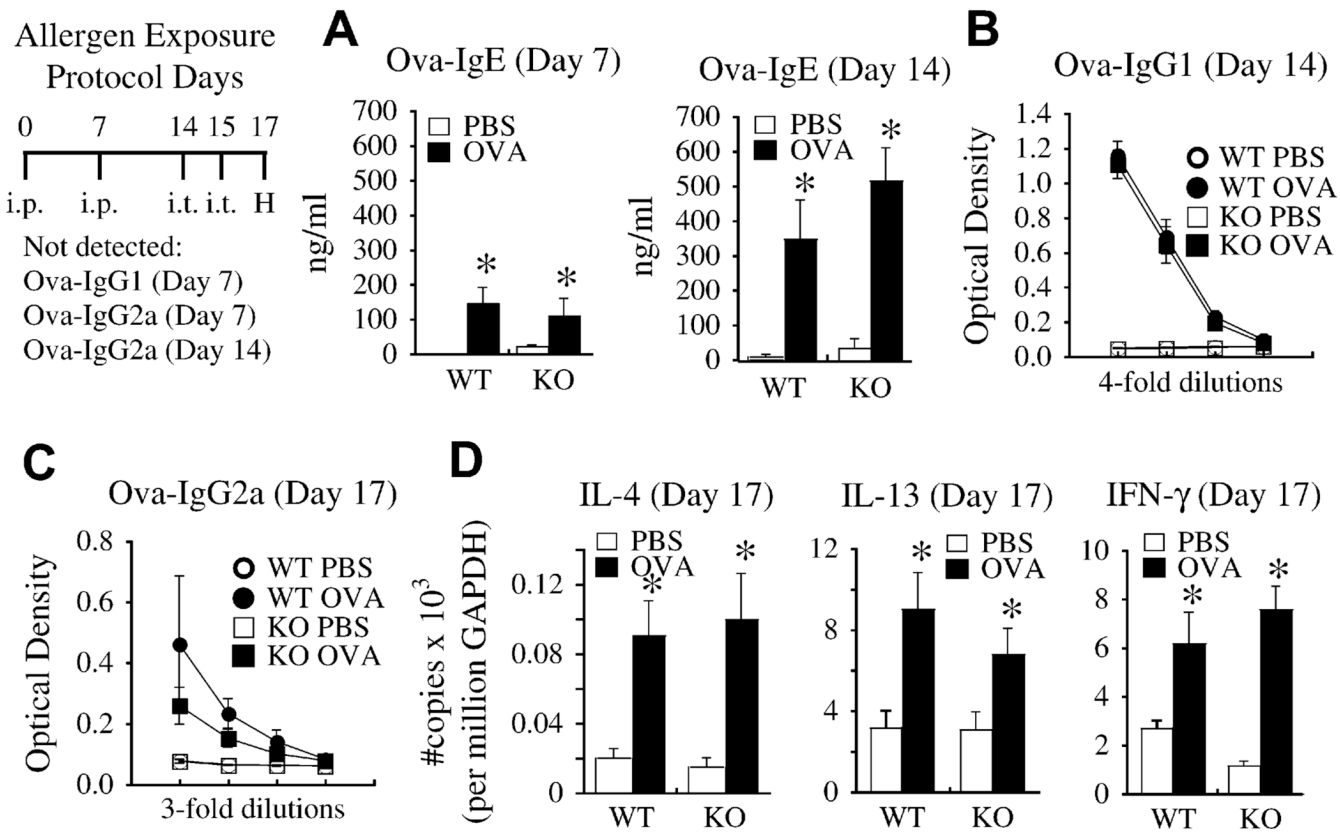

FIG 2.

12/15-LO deficiency does not protect mice from allergic sensitization induced by intraperitoneal exposure to allergen. Levels of ovalbumin *(Ova)*–specific IgE **(A)**, ovalbumin-specific IgG1 **(B)**, and ovalbumin-specific IgG2a **(C)** in serum and transcripts encoding IL-4, IL-13, and IFN-γ (D) in the lungs of wild-type *(WT)* and 12/15-LO$^{-/-}$ (KO) mice are shown. **P* < .05 versus PBS. †P < .05 versus ovalbumin.

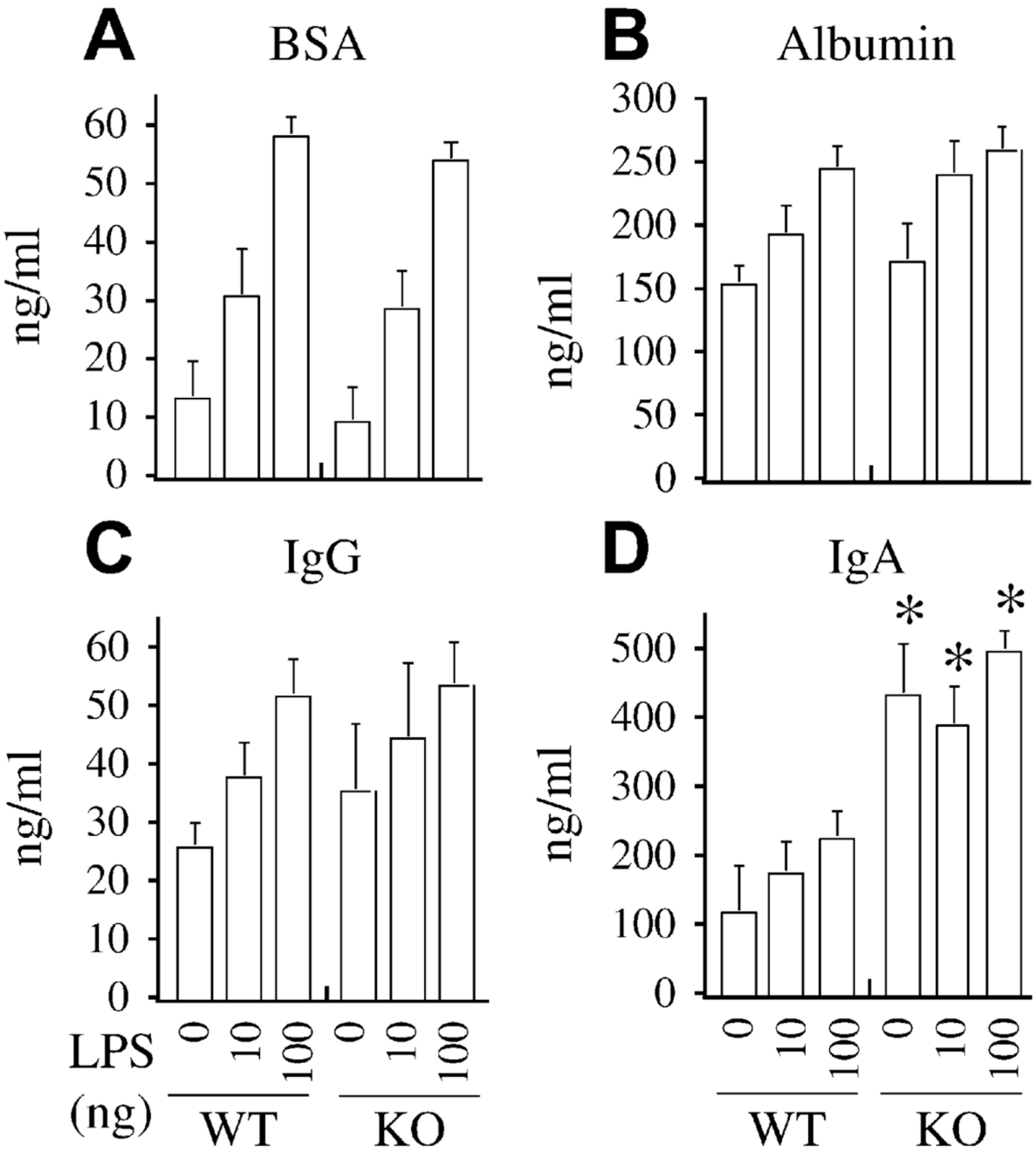

FIG 3.

12/15-LO deficiency increases lung IgA levels but does not alter lung permeability. Levels of BSA **(A)**, albumin **(B)**, IgG **(C)**, and IgA **(D)** in BALF are shown. *WT*, Wild-type mice; *KO*, 12/15-LO$^{-/-}$ mice. *$P < .001$ versus wild-type mice.

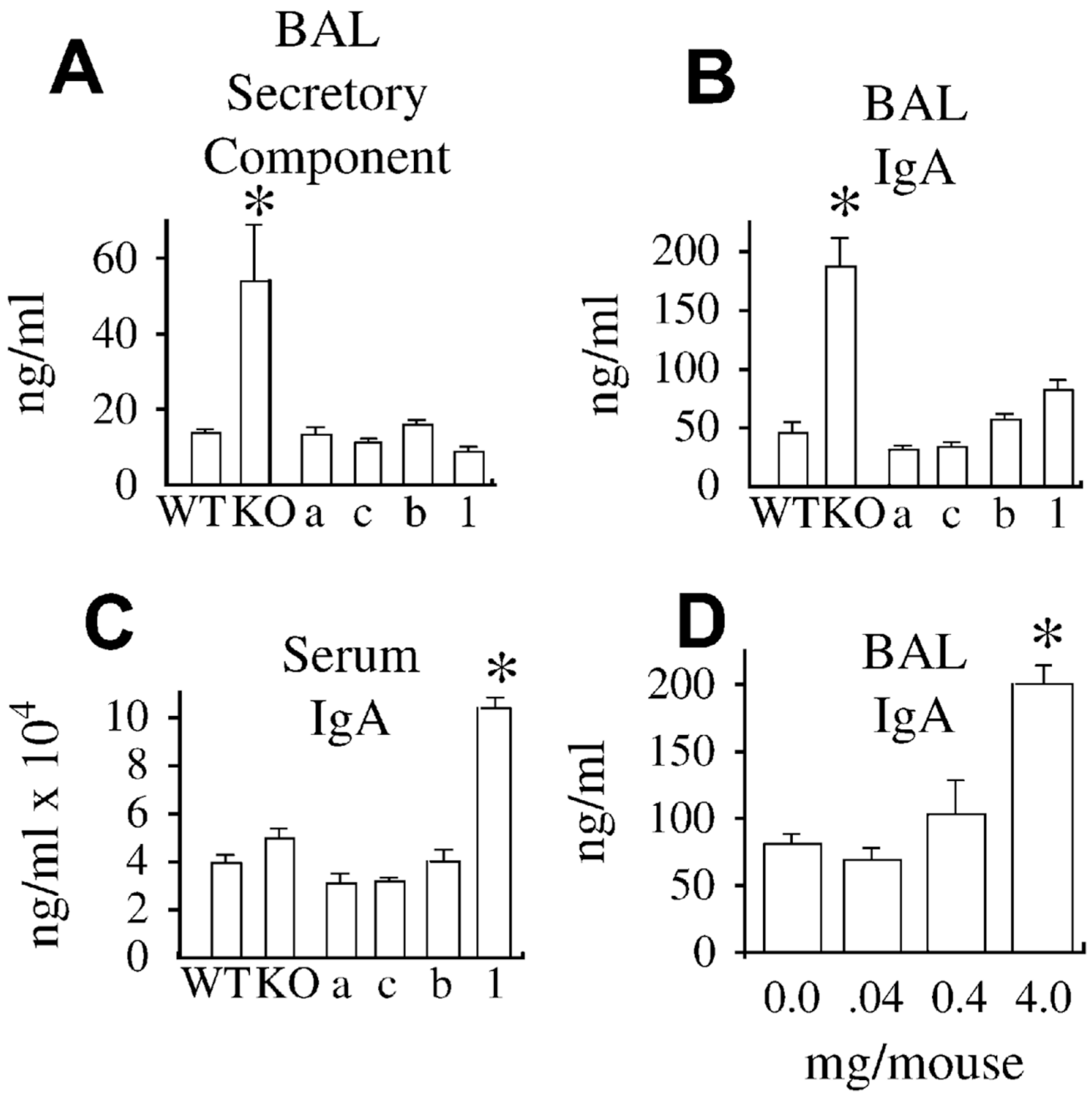

FIG 4.

12/15-LO deficiency and 12/15-LO inhibition increases SIgA levels. Levels of SC **(A)** and IgA **(B)** in BALF and IgA in serum **(C)** from wild-type *(WT)*, 12/15-LO$^{-/-}$ *(KO)*, and AKR/J *(a)*, C57Bl/6 *(c)*, BALB/C *(b)*, and 129/J *(1)* mice are shown. Levels of IgA in BALF from wild-type mice treated with a pharmacologic inhibitor of 12/15-LO are also shown **(D)**. *$P < .05$ versus all other groups.

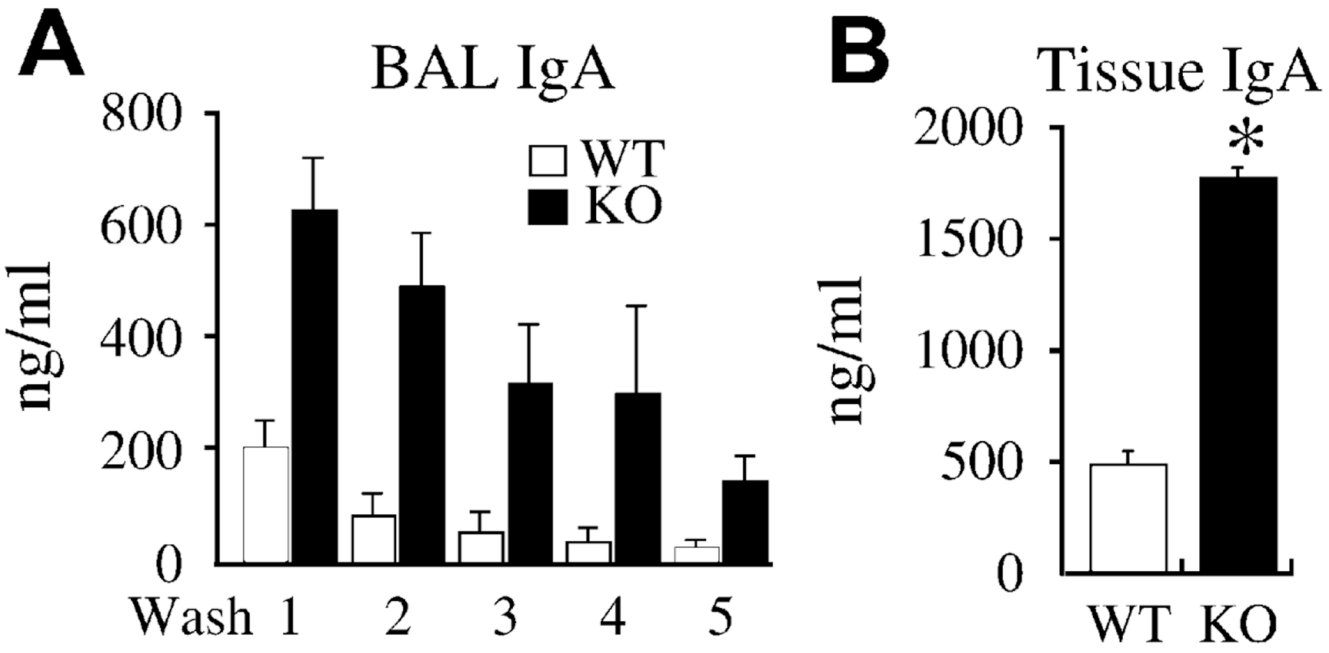

FIG 5.

12/15-LO deficiency increases mucosal-specific production of IgA. Levels of IgA in 5 consecutive bronchoalveolar lavage washings **(A)** and IgA in supernatants from repeatedly washed blood-free homogenized lungs (B) are shown. **P* < 0.001 versus wild-type mice.

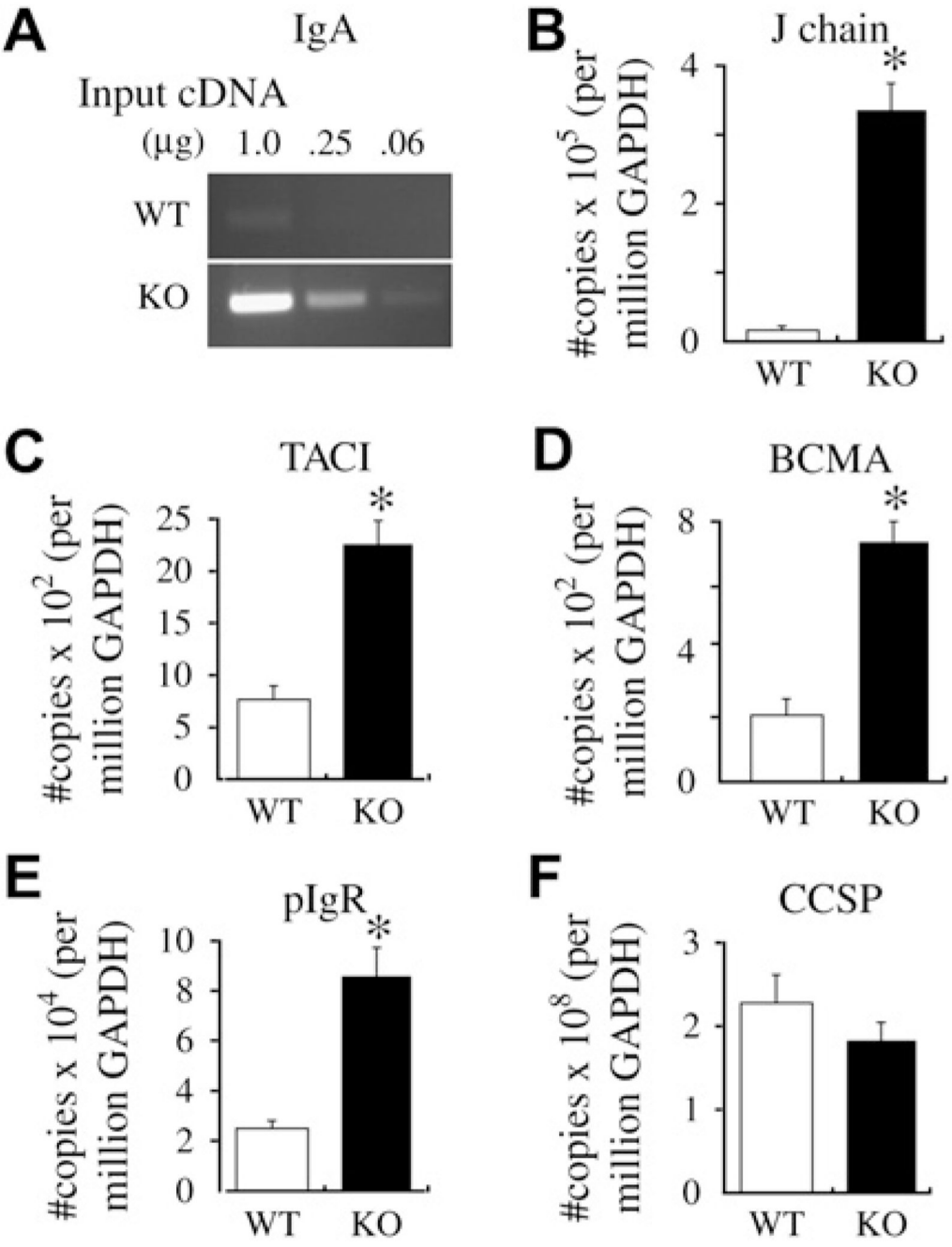

FIG 6.

12/15-LO deficiency increases expression of transcripts encoding the mucosal IgA system. **A**, Representative (n 5 6) levels of IgA transcripts detected in lung tissue from wild-type (*WT*; *upper row*) and 12/15-LO$^{-/-}$ (*KO*; *lower row*) mice. Levels of transcripts encoding J chain **(B)**, TACI **(C)**, BCMA **(D)**, pIgR **(E)**, and Clara cell secretory protein (*CCSP*; **F**) in lung tissue are also shown. **P* < .001 versus wild-type mice.

TABLE I

Cell-surface activation markers

	$CD19^+$ (% of live cells)	**$CD69^+CD25^-$ (% of $CD19^+$ cells)**	**$CD69^-CD25^+$ (% of $CD19^+$ cells)**	**$CD69^+CD25^+$ (% of $CD19^+$ cells)**
WT	29.16 ± 4.60	0.64 ± 0.14	2.00 ± 0.35	3.48 ± 1.10
KO	19.17 ± 5.20	1.38 ± 0.25*	2.63 ± 0.33	7.10 ± 2.36

Values are presented as means ± SEs.

CD19, B cell–specific surface marker; *CD69*, very early activation antigen; *CD25*, low-affinity IL-2 receptor α chain; *WT*, wild-type mice; *KO*, 12/15-$LO^{-/-}$ mice.

*$P < .05$, wild-type versus 12/15-$LO^{-/-}$ (knockout).