NADPH oxidase deficiency results in reduced alveolar macrophage 5-lipoxygenase expression and decreased leukotriene synthesis

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Abstract: Reactive oxygen intermediates (ROI) play an important role in cell signaling in addition to their role in microbial killing. We have shown previously that exogenous ROI regulate activity of the enzyme 5-lipoxygenase (5-LO) in alveolar macrophages (AM). Here, we examined the role of endogenous ROI, specifically generated by NADPH oxidase, in the regulation of leukotriene (LT) synthetic capacity in AM, which from NADPH oxidase knockout (KO) mice, was significantly less than that from wild-type (WT) AM. The decrease in LT synthesis could not be explained by reduced release of the substrate for 5-LO, arachidonic acid. However, the expression of 5-LO was reduced ~50% in AM from NADPH oxidase KO mice compared with WT mice. Reduced 5-LO expression could be reproduced by treating WT AM with ROI scavengers and with selective pharmacologic inhibitors of NADPH oxidase. Furthermore, conditioned media from WT AM augmented 5-LO metabolism in AM from NADPH oxidase KO mice. This decrease in 5-LO expression in NADPH oxidase KO cells was associated with decreased expression of the transcription factors, specificity protein-1 and early growth response-1, both of which are known to regulate 5-LO mRNA expression. These data reveal a previously unrecognized influence of endogenous ROI generated by NADPH oxidase on expression of the key LT biosynthetic protein, 5-LO. In view of the antimicrobial actions of LT, a reduction in LT synthetic capacity by AM from NADPH oxidase KO mice may contribute to the susceptibility of these animals to infection. *J. Leukoc. Biol.* 82: 1585–1591; 2007.

Key Words: *eicosanoid · reactive oxygen intermediate*

INTRODUCTION

Leukotriene (LT) synthesis from arachidonic acid (AA) is initiated by the Ca^{2+}-dependent activation of 5-lipoxygenase (5-LO) to form LTA_4, which is the precursor for formation of the two major groups of LT: LTB_4 and the cysteinyl LT (cysLT), LTC_4, D_4, and E_4 [1]. LT are proinflammatory mediators, which play an important role in disease states such as asthma [2–4] and lung injury [5]. They are also produced in high levels in pneumonia [6], and recently, LT have been implicated as having a role in host defense against microbial infection [7–10]. Specifically, LT boost phagocytosis and killing of microorganisms in a number of phagocytic cell types including alveolar macrophages (AM) [9], blood neutrophils (PMN) [11], and peripheral blood monocytes [12]. Synthesis of LT following cell activation or phagocytosis of microorganisms is accompanied by the production of other mediators including reactive oxygen intermediates (ROI) [13].

ROI, such as superoxide, are formed in macrophages constitutively and especially following phagocytosis or stimulation by various biologically active substances including PMA, LPS, TNF-α, and IL-1β. ROI may be generated from a variety of sources including activation of mitochondrial enzymes [14], endoplasmic reticulum enzymes [15], and peroxisomes enzymes [16]. The enzyme NADPH oxidase is an important source of ROI with a key role in antimicrobial killing [17] and cell signaling. In addition, activation of oxygenase enzymes, e.g., 5-LO and cyclooxygenase, may result in ROI formation [13, 18].

ROI in turn are also known to regulate LT in several ways. First, ROI have a biphasic concentration-dependent effect on 5-LO activity, with low doses necessary for enzyme activity but higher doses causing inactivation [19–21]. It is thought that these effects reflect alterations in redox tone, which influence the nonheme iron in the catalytic site of 5-LO. Second, ROI have also been shown to degrade LT in PMN [22]. This may account for reports that PMN from subjects with chronic granulomatous disease displayed greater levels of LTB_4 and LTC_4 [23]. Despite the enormous impact of respiratory infections on

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morbidity and mortality and the fact that NADPH oxidase and LT participate in antimicrobial defense, little is known about the influence of NADPH oxidase on LT biosynthesis.

In this study, we examined the role of NADPH oxidase in the regulation of 5-LO metabolism in AM, the resident defender of the pulmonary alveolus. We demonstrate for the first time that genetic and pharmacologic disruption of NADPH oxidase results in impaired LT biosynthesis and define relevant mechanisms for this interaction.

MATERIALS AND METHODS

Cell isolation and culture of rat and murine AM

Mice

AM were isolated from C57BL/6 NADPH oxidase wild-type (WT) and knockout (KO; $gp91^{phox\ -/-}$) mice (Jackson Laboratory, Bar Harbor, ME, USA) [24, 25]. After anesthesia with ketamine (80–120 mg/kg) and xylazine (5–10 mg/kg) by i.p. injection, mice were killed by exsanguination. Following exposure of the tracheobronchial tree, harvesting of AM was performed by bronchoalveolar lavage (BAL) using 1 ml aliquots of EDTA-containing phosphate buffer to a total of 5 ml. AM ($0.5–1.0\times10^6$) were obtained per mouse. As assessed by Diff-Quik staining, AM purity of cell monolayers was >98% following a 1-h adherence step. Viability was >95%, as assessed by trypan blue exclusion. Isolated AM were resuspended in LPS-free DMEM (Life Technologies, Grand Island, NY, USA) at 0.5×10^6/ml and plated as follows: 0.2 ml/well in 96-well plates for enzyme immunoassay (EIA) and 5 ml/50 mm culture plate for immunoblot analysis.

Rats

AM were also obtained from 150 g-specific, pathogen-free, female Wistar rats, as described previously [26]. AM ($8–10\times10^6$) were obtained per rat. BAL cells were pelleted by centrifugation at 500 g and were >90% AM when adhered, as determined by Diff-Quik staining.

Coincubation of AM from NADPH oxidase WT and KO mice

AM were harvested from NADPH oxidase WT and KO animals. Using Costar Transwell inserts (Cole-Palmer, Vernon Hills, IL, USA), AM from the WT and KO animals were plated in wells or inserts separately, and then, inserts were placed in wells for overnight incubation. Specifically, AM from KO animals were plated in wells with or without inserts containing AM from WT animals. After the inserts were removed, cells in the wells were subsequently washed and stimulated with or without A23187 or harvested for Western blot analysis.

Quantitation of 5-LO metabolism in intact cells

Following adherence and subsequent incubation for 16 h in DMEM containing 10% FCS (HyClone, Logan UT, USA), the cells were washed three times in media and subsequently incubated with or without Ca^{2+} ionophore A23187 (1 μM, Calbiochem, La Jolla, CA, USA) to stimulate maximal release and metabolism of endogenous AA. Capacity for 5-LO metabolism in intact cells was measured by EIA (Cayman Chemicals, Ann Arbor, MI, USA) determination in cell-free supernatants of the predominant 5-LO products cysLT in murine cells and LTB_4 in rat cells. HPLC analysis was used to quantify A23187-stimulated AA release, following overnight prelabeling of cells with [^{3}H]AA (Du Pont-New England Nuclear, Boston, MA, USA) [27]. Cells were stimulated in the presence of 0.1% BSA (Sigma Chemical Co., St. Louis, MO, USA), which binds AA and prevents its metabolism and reacylation into membrane lipids. HPLC analysis of radiolabeled cell products was performed on supernatants of cells activated with A23187 in the absence of albumin to permit metabolism and thereby confirms the EIA results about LT synthesis and determines effects on other pathways such as cyclooxygenase.

5-LO cell-free activity assay

Freshly isolated rat AM (25×10^6 cells) were suspended immediately in cold homogenization buffer (50 mM Tris, pH 7.8, NaCl 150 nM) containing antiproteases (Roche Complete protease inhibitor cocktail tablet) and disrupted using a Branson sonifier (100% duty cycle, 3×15 s). The resulting homogenates (250 μl per experimental condition) were kept at 4°C, incubated with 10 μM diphenylene iodonium (DPI; or vehicle) for 30 min, and then activated with 1 mM $CaCl_2$, 25 μM ATP, and 50 μM AA for 5 min at 37°C. Incubations were stopped by the addition of 0.5 ml cold (4°C) stop solution (MeOH/MeCN, 50/50, v/v) containing 12.5 ng 19-OH-PGB_2 and PGB_2 as internal standards. The denatured samples were spun to remove the precipitated material, and the supernatants were harvested for the analysis of 5-hydroxy-eicoasatetraenate biosynthesis by HPLC. Briefly, the samples were diluted with acidified water (0.01% acetic acid) to a final volume of 5 ml and then loaded on a C_{18} Sep-Pak cartridge (60 mg, Waters Corp., Milford, MA, USA), which had been preconditioned with 2 ml 63/32/5 (MeOH/MeCN/H_2O; 0.01% acetic acid) and 2 ml acidified water. The C_{18} cartridges were then washed with 2 ml acidified water, and the eicosanoids were eluted with 1 ml 63/32/5. The resulting eluates were then analyzed by HPLC [27].

Immunoblot analysis of cytosolic phospholipase A2 ($cPLA_2$), 5-LO, specificity protein-1 (Sp-1), and early growth response-1 (Egr-1)

The relative cellular expression of two key proteins involved in LT biosynthesis, $cPLA_2$ and 5-LO, was determined by Western blot analysis. Crude lysates were prepared as described [27] and subjected to SDS-PAGE by the method of Laemmli on 10% acrylamide gels. Proteins were transferred overnight to nitrocellulose membranes and probed with rabbit polyclonal antibodies against human leukocyte 5-LO (1:3000 dilution, provided by Dr. Jilly Evans, Merck Frosst, Quebec, Canada [28]); human $cPLA_2$ (1:1000 dilution, provided by Dr. James Clark, Wyeth Research, Cambridge, MA, USA) [8]; Sp-1 (1:500 dilution, eBioscience, San Diego, CA, USA); and Egr-1 (1:500 dilution, R&D Systems, Minneapolis, MN, USA). After washing, blots were incubated for 1 h with HRP-conjugated anti-rabbit IgG (Amersham, Arlington Heights, IL, USA) at a dilution of 1:5000. Membranes were then washed and incubated for 1 min with ECL detection reagents (Amersham) and exposed to film for varying time periods to ensure that densitometric quantitation was performed under conditions in which band density and exposure time were linearly related. Video densitometry was performed using Image [National Institutes of Health (NIH)] software (Scion Corp. Frederick, MD, USA).

Modulation of ROI

Scavenging of ROI was performed by adding tetrakis(4-benzoic acid)porphyrin (TBAP; Calbiochem-Novabiochem, La Jolla, CA, USA), a stable, cell-permeable metalloporphyrin, which catalyzes the dismutation of superoxide and hydrogen peroxide [29]. Relatively selective pharmacologic inhibition of NADPH oxidase was performed by using a 10-μM concentration of the flavoprotein inhibitor DPI overnight [30] (Sigma Chemical Co.).

Data analysis

Where indicated, data were expressed as mean ± SEM. Intergroup differences were analyzed by ANOVA, and statistical significance was assessed by the Scheffe test; $P < 0.05$ was considered significant.

RESULTS

ROI regulate LT synthesis in murine AM

We have shown previously that low concentrations of exogenous ROI stimulate LT synthesis in rat AM [21]. We now wished to assess the role endogenous ROI played in regulation of LT synthesis in AM using a combination of pharmacological and genetic approaches. The ROI scavenger TBAP suppressed cellular 5-LO metabolism (**Fig. 1A**), indicating that endoge-

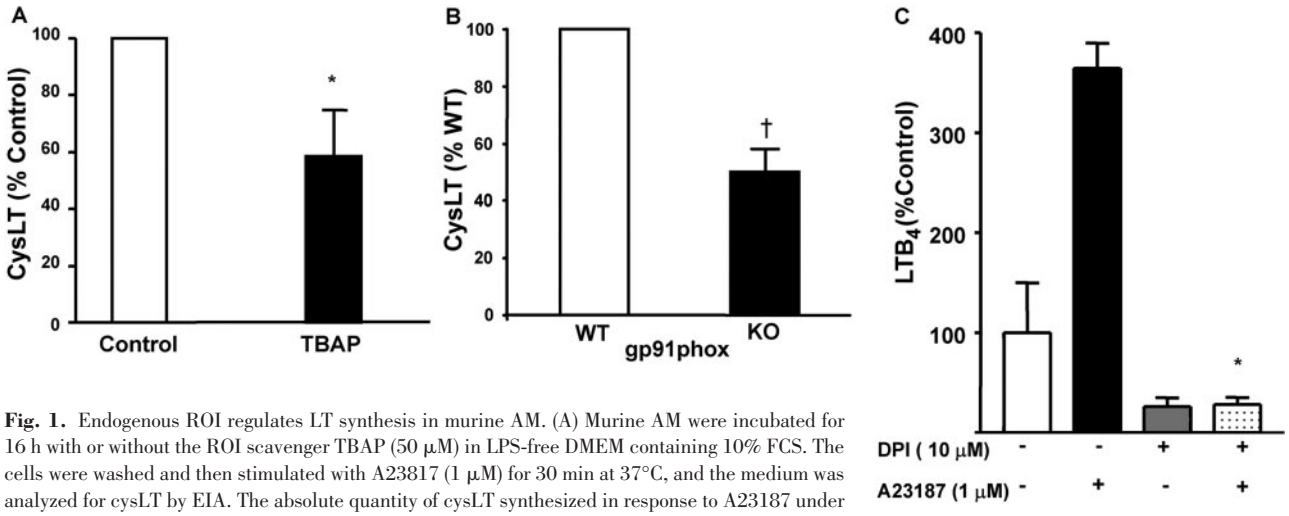

Fig. 1. Endogenous ROI regulates LT synthesis in murine AM. (A) Murine AM were incubated for 16 h with or without the ROI scavenger TBAP (50 μM) in LPS-free DMEM containing 10% FCS. The cells were washed and then stimulated with A23817 (1 μM) for 30 min at 37°C, and the medium was analyzed for cysLT by EIA. The absolute quantity of cysLT synthesized in response to A23187 under control conditions was 4347 ± 1224 pg/ml. Mean data from three experiments are shown; *, $P = 0.05$. (B) Murine AM from NADPH oxidase WT and KO mice were adhered in LPS-free DMEM for 1 h, washed, and incubated with LPS-free DMEM containing 10% FCS. Following washing, the cells were stimulated with A23817 (1 μM) for 30 min at 37°C. The medium was then analyzed for cysLT by EIA. Mean data from three experiments are shown. The absolute level of cysLT produced by AM from NADPH oxidase WT mice was 3572 ± 493.4 pg/ml; †, $P < .001$; $n = 3$. (C) AM from rats were incubated with and without the NADPH oxidase inhibitor DPI (10 μM) for 16 h in LPS-free DMEM containing 10% FCS. The cells were subsequently washed, plated with fresh DMEM, and stimulated with or without A23187 (1 μM) for 30 min. LTB_4 was quantitated in medium by EIA, and data are expressed as percent of control. The absolute level of LTB_4 produced by rat AM was 3675 ± 562.6 pg/ml. Mean data from four experiments are shown; *, $P < 0.001$; $n = 4$.

nous ROI play a role in up-regulating LT synthesis in murine AM, as they do in rat AM.

Reduced LT synthesis in AM from NADPH oxidase KO mice

We next used a genetic approach to determine specifically the role of ROI derived from NADPH oxidase in regulating AM LT synthesis. In response to the potent stimulus A23187, synthesis of CysLT, the major 5-LO product of murine AM, was decreased in cells from NADPH oxidase KO mice compared with AM obtained from WT mice of the same background strain (Fig. 1B). Treatment with a relatively selective NADPH oxidase inhibitor, DPI, also reduced the maximum A23187-stimulated LTB_4 synthesis in rat AM (Fig. 1C). However, when we examined the direct effect of DPI on 5-LO cell-free activity in rat AM whole cell homogenates, we demonstrated no effect (mean 88.2%; $n=2$) compared with control.

Effect of NADPH oxidase on macrophage AA release

Any change in cellular 5-LO metabolism can be dictated by upstream availability of substrate for the enzyme 5-LO. Therefore, we examined the release of AA, the substrate for the 5-LO enzyme. As shown in **Figure 2A**, there was no change in stimulated AA release from [^{3}H]AA-prelabeled cells obtained from NADPH oxidase KO mice compared with AM from WT mice. The mean and SEM for AA release by AM from NADPH oxidase KO mice were 99.0 ± 5.8% ($n=3$; $P=$ns) of that determined for cells from WT mice. The mean release of [^{3}H]AA was 0.81 ± 0.52% of incorporated radioactivity in AM from NADPH oxidase WT mice. In keeping with the unaltered release of AA in AM from NADPH oxidase KO mice, there was also no difference in $cPLA_2$ expression (Fig. 2B). Levels of cyclooxygenase metabolites determined by HPLC analysis were unchanged as expected in NADPH oxidase KO mice compared with WT mice (data not shown), as there was no increase in AA release.

Reduced 5-LO expression in AM from NADPH oxidase KO mice

As shown in **Figure 3A**, the expression of 5-LO, as determined by Western blot analysis, was decreased significantly (by ~50%) in AM from NADPH oxidase KO mice compared with WT controls. Mean densitometry of Western blots from six independent experiments demonstrated 5-LO expression in KO at 51.3 ± 12.5% of the WT level ($P<0.05$; Fig. 3A).

Scavenging ROI reduces 5-LO expression and activity in AM

Having shown reduced 5-LO expression in AM from NADPH oxidase KO mice compared with WT mice, we next sought to determine if this finding could be reproduced pharmacologically in vitro. We therefore examined the effect of treating macrophages with the relatively selective NADPH oxidase inhibitor DPI. Treatment of rat AM with DPI (10 μM) for 16 h led to decreased expression of 5-LO (Fig. 3B), consistent with an effect of NADPH oxidase on 5-LO expression (31.5±2.5% of control levels; $P<0.05$; $n=3$). In addition, rat AM, incubated with and without the ROI scavenger TBAP (50 μM) for 16 h, demonstrated reduced expression of 5-LO in parallel with the decrease in LT synthetic capacity when compared with control cells (Fig. 3C).

Reconstitution of AM LT synthesis from NADPH oxidase KO mice

As the experiments described above indicated that reduced ROI generation was associated with a reduction in cellular

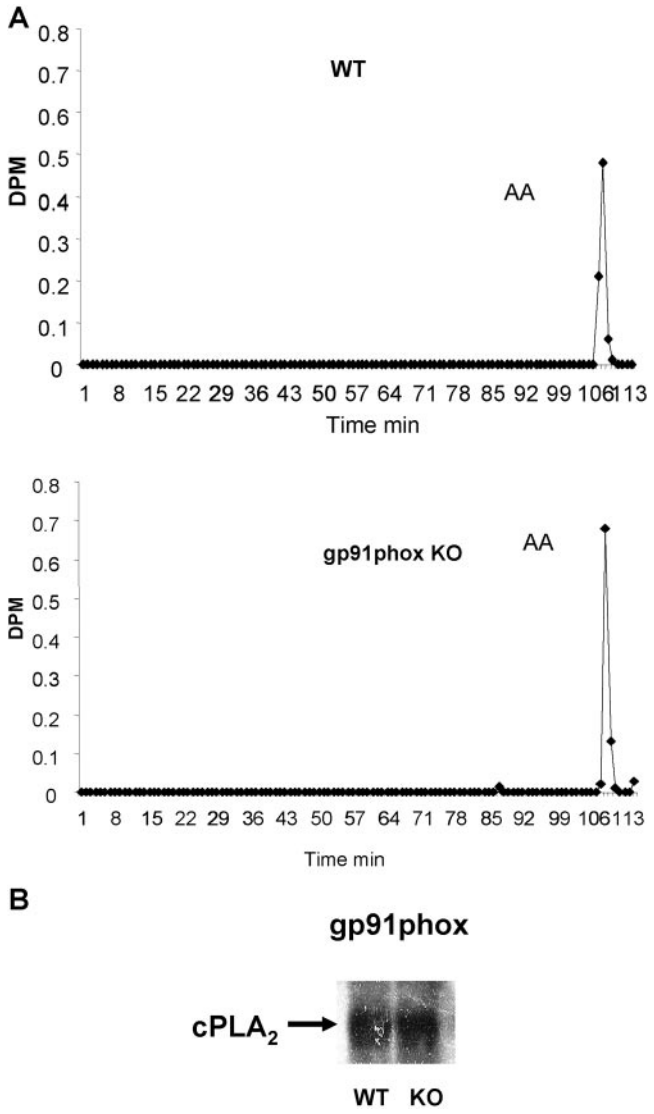

Fig. 2. No change in stimulated AA release from [^{3}H]AA-prelabeled cells obtained from NADPH oxidase KO mice compared with AM from WT mice. (A) Murine AM from NADPH oxidase WT and KO mice were prelabeled with [^{3}H]AA overnight, washed, and stimulated with A23187 (1 μM) for 30 min in the presence of 0.1% BSA. Free [^{3}H]AA in cellular supernatants were separated by HPLC. The peak was identified by coelution with an authentic AA standard and released fatty acids expressed as a percentage of incorporated radioactivity. A representative profile of three separate experiments is shown. (B) A representative Western blot from $n = 3$ total experiments demonstrates the expression of $cPLA_2$ in AM from NADPH oxidase WT and KO mice.

ability to synthesize LT, we wished to determine if adding back conditioned media from AM from NADPH oxidase KO cells restored 5-LO metabolic capacity. AM from NADPH oxidase WT and KO mice were incubated separately and together in a dual chamber culture plate overnight, allowing the conditioned media from each cell type to bathe the other. The cells incubated alone and in combination were then separated and stimulated with A23187 for 30 min. LT generation was increased in the AM from the NADPH oxidase KO mice, which had been exposed to conditioned media from cells from NADPH oxidase WT mice (**Fig. 4A**). Furthermore, conditioned media from AM from NADPH oxidase WT mice also partially restored 5-LO expression in AM from KO mice (Fig. 4B). These observations suggest that mediators elaborated by AM from NADPH WT animals can partially reconstitute LT synthesis in cells from NADPH oxidase null animals.

Reduced expression of Sp-1 and Egr-1 in DPI-treated AM and in AM from NADPH oxidase KO mice

Sp-1 and Egr-1 are zinc finger transcription factors, which regulate transcription of many inflammatory genes, including 5-LO, by binding to the G + C-rich promoter sequence. Sp-1 may activate the murine 5-LO promoter by interacting directly with the basal transcription apparatus or by recruitment of a coactivator [31]. Likewise, Egr-1 is known to activate the human 5-LO promoter [32]. The cysteine residues on zinc finger transcription factors are sensitive to the redox state of the cell [33], and increased expression and activation of Sp-1 and Egr-1 occur following treatment of cells with H_2O_2 [34, 35]. Therefore, we examined the expression of Sp-1 and Egr-1 in AM treated with DPI for 16 h. Sp-1 and Egr-1 expression was decreased markedly in AM from DPI versus untreated AM (**Fig. 5A**). Likewise, expression of Sp-1 and Egr-1 was decreased in AM from NADPH oxidase KO mice compared with WT mice (Fig. 5B).

DISCUSSION

Chronic granulomatous disease encompasses a heterogeneous group of disorders characterized by genetic defects in the ability of phagocytes to generate ROI from molecular oxygen [36, 37]. Specifically, these involve mutations, which result in the loss or functional inactivation of one of the components of the multicomponent NADPH oxidase complex. The most common form of chronic granulomatous disease (accounting for ~70% of cases) arises from mutations in the gene encoding $gp91^{phox}$ [17]. Patients with chronic granulomatous disease typically experience recurrent infections caused by bacterial and fungal pathogens. In descending order of frequency, these infections include pneumonia, suppurative adenitis, osteomyelitis, bacteremia/fungemia, and superficial skin infections. The recent recognition that endogenous LT are also necessary for optimal innate immunity in vivo and phagocytosis and killing in vitro highlights the potential significance of the interactions between ROI and enhanced LT synthesis.

Our study used genetic and pharmacologic approaches to demonstrate for the first time that NADPH oxidase up-regulates LT synthesis in AM. This represents a selective up-regulation of 5-LO metabolism mediated via augmented expression of 5-LO expression but not of $cPLA_2$ with increased AA release. Conditioned media of AM from WT NADPH oxidase mice partially restored LT synthesis and 5-LO expression in cells from NADPH oxidase KO animals. It is likely that other mediators and cytokines including IFN-γ, apart from ROI in the conditioned media, resulted in this increase in LT synthesis and 5-LO expression. The transcription factors Sp-1

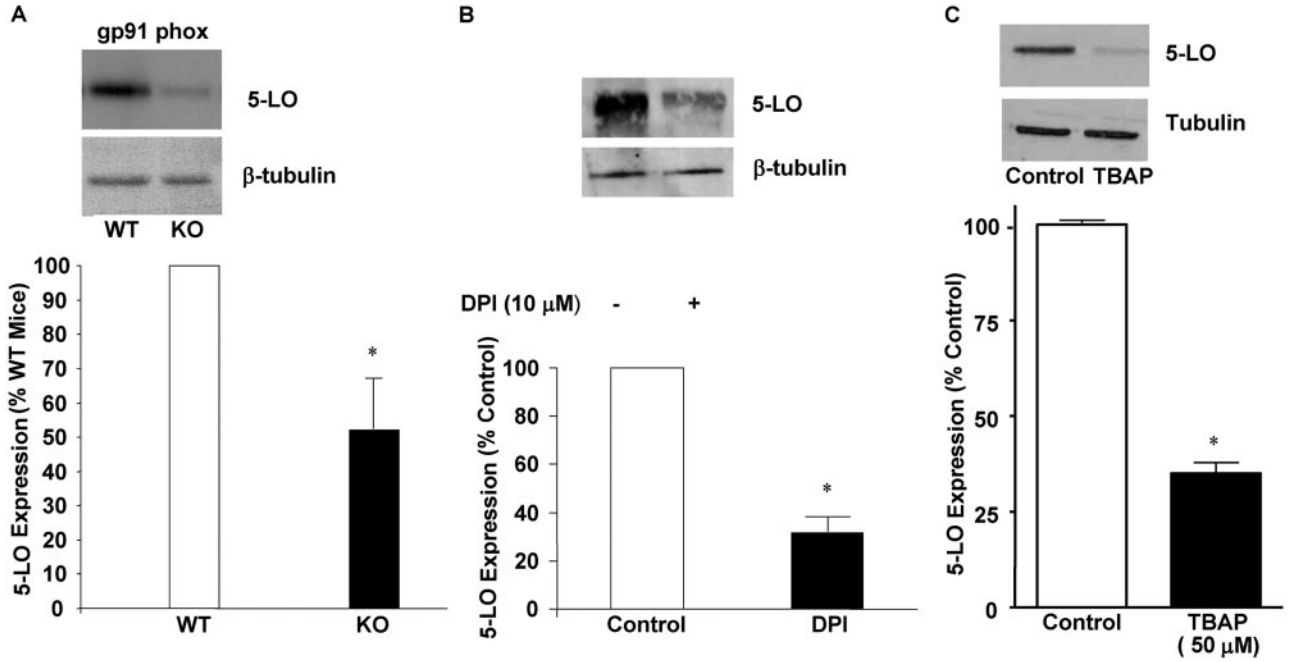

Fig. 3. Reduced 5-LO expression in AM from NADPH oxidase KO mice. (A) AM were harvested from NADPH oxidase WT and KO mice. Equal amounts (20 μg protein) of crude AM lysate from mice from both genotypes were subjected to immunoblot analysis for 5-LO. To confirm equal loading of protein, the blots were also probed for β-tubulin. (Upper panel) A representative autoradiograph of an experiment from a total of six independent experiments. (Lower panel) Relative expression of 5-LO in AM from NADPH oxidase KO mice, as assessed by densitometry and expressed as a percent of values derived from control AM; $n = 6$; *, $P < 0.05$. (B) Rat AM were incubated with or without the selective NADPH oxidase inhibitor DPI at a concentration of 10 μM for 16 h. Equal amounts (20 μg protein) of crude cellular lysate from DPI-treated and control cells were subjected to immunoblot analysis for 5-LO and β-tubulin. (Upper panel) Representative autoradiograph of a Western blot from one of three independent experiments. (Lower panel) Relative expression of 5-LO from DPI-treated AM expressed as a percent of values derived from control AM; $n = 3$; *, $P < 0.05$. (C) AM from rats and mice were incubated with and without the ROI scavenger TBAP (50 μM) for 16 h in LPS-free DMEM containing 10% FCS. Equal amounts (20 μg protein) of crude AM lysate were subjected to immunoblot analysis for 5-LO and β-tubulin. (Upper panel) A representative autoradiograph of a Western blot from one of three independent experiments. (Lower panel) Relative expression of 5-LO from TBAP-treated AM expressed as a percent of values derived from control AM; $n = 3$, *, $P < 0.05$.

and Egr-1 appear to play an important role in the up-regulation of 5-LO expression by NADPH oxidase-generated ROI. Overall, the main observations of this manuscript suggest that the 5-LO metabolism in AM is dependent on ROI synthesis by NADPH oxidase.

Generation of low concentrations of ROI has been shown to up-regulate LT biosynthesis [21]. Our first step was to demonstrate that ROI are important in the regulation of LT synthesis in murine macrophages, similar to their role in rat AM. Scavenging cellular ROI with TBAP resulted in a consistent reduction of LT synthesis in murine AM. These experiments also further emphasized the importance of endogenous ROI generation in cellular 5-LO metabolism. Given the recognition that redox tone influences 5-LO activity, the finding was not surprising. However, the finding that TBAP treatment also reduced 5-LO expression in murine and rat AM was unexpected. Next, we sought to confirm this finding using a genetic model of impaired ROI generation. We used the NADPH oxidase KO model because of the known reduced capacity of cells from these animals to elaborate ROI [13, 24]. Maximal LT synthetic capacity of AM from NADPH oxidase KO mice was reduced compared with WT mice. This reduced capacity was not explained by a difference in AA release, the substrate for the 5-LO enzyme. However, the reduction in LT synthesis was associated with a reduction in 5-LO expression, in parallel with the effects observed for treatment of WT cells with TBAP and DPI. This observation of reduced LT synthesis in AM from NADPH oxidase KO mice contrasts with that reported for PMN from patients with chronic granulomatous disease [22]. This difference may be explained by the lesser capacity of AM than PMN to generate ROI.

The human [38] and murine [31] 5-LO promoter sites have been cloned and characterized in a number of cell types. A number of transcription factors have been shown to interact with the 5-LO promoter, including zinc finger transcriptional proteins such as Sp-1 and Egr-1 [31, 39–41]. ROI are known to regulate these zinc finger sites on the transcription factors [33, 35]. Increased expression and activation of Egr-1 occur following treatment of cells with hydrogen peroxide [34]. Sp-1 is also regulated by redox-related mechanisms [42]. Transfection of macrophages with these transcription factors and/or using Sp-1 and Egr-1 WT and KO mice may help characterize the role of these factors in the regulation of 5-LO expression in future studies.

Little is known about the effect of ROI on the expression of 5-LO. Other investigators examined the effect of hyperoxia on 5-LO expression in the lungs of newborn rat lung tissue [43]. LT synthesis and total lung expression of 5-LO were increased in animals treated with hyperoxia. However, it was unclear if this effect was because of an increased inflammatory response

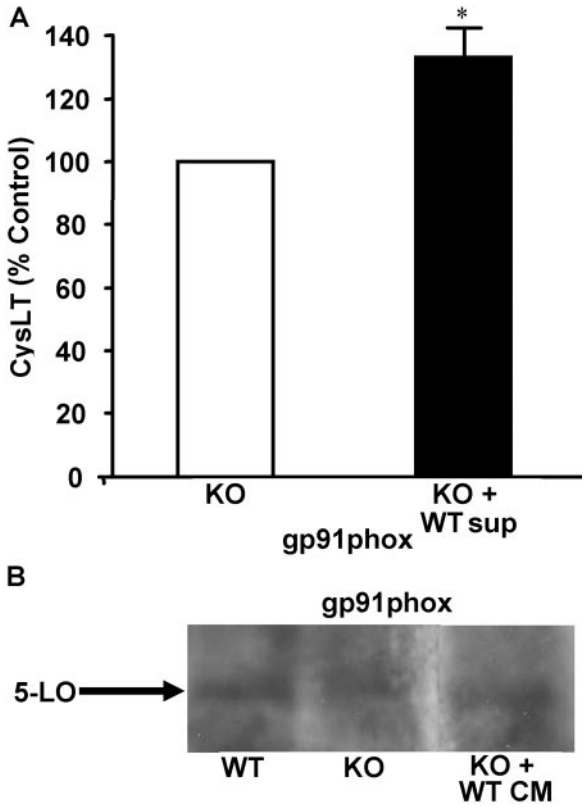

Fig. 4 . Conditioned media (CM) of AM from NADPH WT mice augments cellular LT synthesis in AM from KO animals. (A) Using Costar Transwell inserts, AM from NADPH oxidase KO animals were plated in wells with or without inserts containing AM from WT animals. After 16 h of culture, the inserts were removed, and cells in the wells were subsequently washed and stimulated with A23187 (1 μM) for 30 min. A representative experiment of three performed is shown. (B) Crude lysates were obtained from AM from NADPH oxidase WT and KO mice incubated alone or following coculture and subjected to immunoblot analysis for 5-LO. Shown is a representative autoradiograph of a Western blot from three independent experiments. $n = 3$; $^*P < 0.05$.

with greater leukocyte numbers or if there was increased expression of these key proteins per cell. Selenium deficiency is also associated with altered 5-LO metabolism. In animal models of selenium deficiency and cells incubated under selenium-deficient conditions, increased LT synthesis has been observed [44]. This phenomenon may be explained by an increase in the endogenous hydroperoxide tone caused by a decrease in glutathione (GSH) peroxidase activity and hydroperoxide-GSH peroxidase activity. Indeed, leukocytes from selenium-deficient rats demonstrated a sevenfold increase in LT synthesis. Taken together, these data support our findings that ROI regulate LT biosythesis positively.

In summary, ROI play a significant role in up-regulating 5-LO metabolism in AM. A reduced capacity to generate ROI in NADPH oxidase KO mice is associated with a reduced AM LT synthetic capacity and a decrease in 5-LO expression. In view of the role of LT in host defense, this reduction in AM 5-LO metabolic capacity in NADPH oxidase KO mice may contribute further to the susceptibility of these animals to infection. Thus, this study provides important new insights into the link between LT and ROI—two key components in pulmonary host defense. It remains to be determined whether LT deficiency contributes to the clinical expression of increased infections in patients with chronic granulomatous disease.

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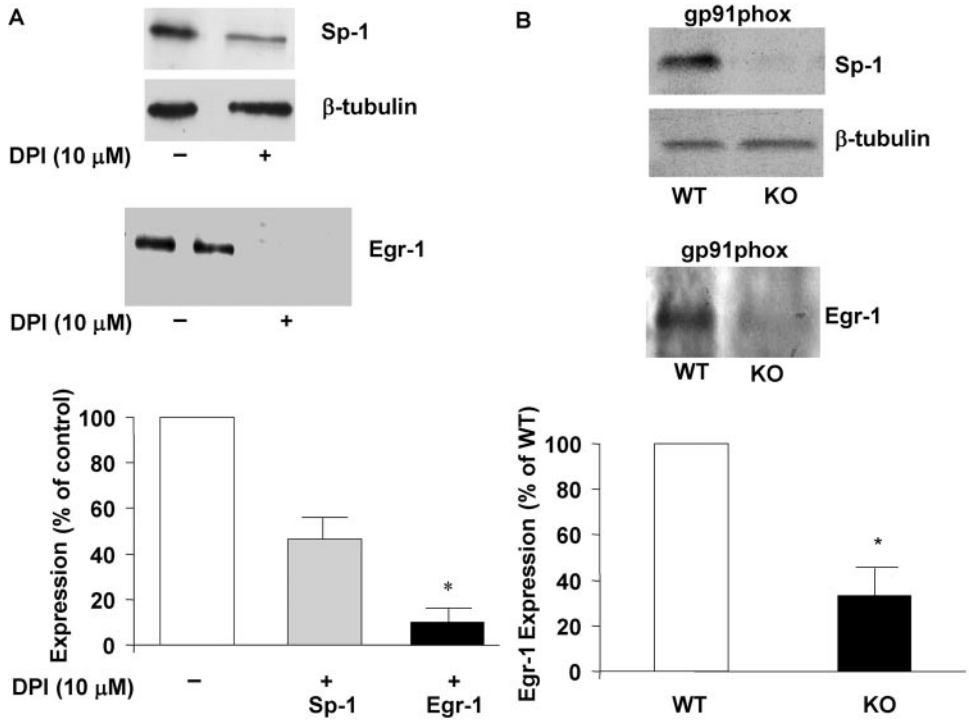

Fig. 5. Reduced expression of Sp-1 and Egr-1 in DPI-treated AM and in NADPH oxidase KO mice. (A) Rat AM were incubated with or without the NADPH oxidase inhibitor DPI at a concentration of 10 μM for 16 h. Crude lysates were obtained for Western blot analysis. Similarly, crude lysates were obtained from AM from NADPH oxidase WT and KO mice. Equal amounts (20 μg protein) of crude cellular lysate from DPI-treated and control cells were subjected to immunoblot analysis for Egr-1, Sp-1, and β-tubulin. (Upper panel) Representative autoradiograph of a Western blot from one (Sp-1) of three independent experiments and one (Egr-1) of six independent experiments. (Lower panel) relative expression of Sp-1 and Egr-1 from DPI-treated AM, as assessed by densitometry and expressed as a percent of values derived from control AM; $n = 3$; *, $P < .05$. (B) AM from NADPH oxidase WT and KO mice were subjected to immunoblot analysis for Sp-1 and Egr-1. (Upper panel) Representative autoradiograph of a Western blot from three independent experiments. (Lower panel) Relative expression of Egr-1 in AM from KO animals compared with WT animals expressed as a percent of values derived from WT AM; $n = 3$; *, $P < 0.05$.

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