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Control of experimental pulmonary tuberculosis depends more on immunostimulatory leukotrienes than on the absence of immunosuppressive prostaglandins

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Abstract

Prostaglandins (PGs) and leukotrienes (LTs) are produced in *Mycobacterium tuberculosis* (Mtb)-infected lungs and have immune suppressive and protective effects, respectively. Considering that both of these mediators are produced during mycobacterial infection, we investigated the specific and relative biological importance of each in regulating host response in experimental tuberculosis. Administration of celecoxib, which was found to reduce lung levels of PGE₂ and increase LTB₄, enhanced the 60-day survival of Mtb-infected mice in 14%. However, administration of MK886, which reduced levels of LTB₄ but did not enhance PGE₂, reduced 60-day survival from 86% to 43% in Mtb-infected mice, and increased lung bacterial burden. MK 886 plus celecoxib reduced survival to a lesser extent than MK 886 alone. MK 886- and MK-886 plus celecoxib-treated animals exhibited reduced levels of the protective interleukin-12 and gamma-interferon. Our findings indicate that in this model, the protective effect of LTs dominates over the suppressive effect of PGs.

Keywords

Mycobacterium tuberculosis; prostaglandins; leukotrienes; cytokines; immunomodulation

1. Introduction

The facultative intracellular acid-fast bacillus *Mycobacterium tuberculosis* (Mtb) is a major human pathogen which infects a third of the world's population and is responsible for ~2 million deaths each year [1]. Murine models of tuberculosis demonstrate that outcome of disease is determined by the nature (i.e., Th1 or Th2 cells) and magnitude of the T cell and cytokine response. Th1-mediated immunity plays a crucial role in host defense against Mtb. Cytokines such as IL-12, IFN- γ , and TNF are essential for protection against this pathogen

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in the mouse model [2–5], while *in vitro* reduced IL-10 production is associated with resistant human disease [6].

Besides cytokines, eicosanoid lipid mediators derived from phospholipase-released arachidonic acid (AA) have been shown to greatly influence host defense against Mtb. They include prostaglandins (PGs) and leukotrienes (LTs), which are involved in numerous homeostatic biological functions and inflammation [7]. Whereas both leukocytes and structural cells synthesize prostanoids, LT synthesis is confined primarily to cells of myeloid origin, by the action of cyclooxygenase (COX) isozymes and 5-lipoxygenase (5-LO), respectively [8, 9]. In particular, cyclooxygenase (COX)-derived prostaglandin E₂ (PGE₂) was shown to be highly expressed at the lungs during the late phase of tuberculosis and its inhibition resulted in the control of infection [10]. PGE₂ inhibits a variety of antimicrobial functions, including phagocytosis [11], bacterial killing [12] and the production of nitrite and Th1 cytokines, such as IL-1 and TNF- α by macrophages [13] and IL-2 and IFN- γ by lymphocytes [14]. Moreover, PGE₂ inhibits IL-12 production by antigen-presenting cells stimulated with LPS, but increases IL-10 production [15, 16]. On the other hand, a study from our group has shown that *in vivo* reduction of endogenous LT synthesis increases susceptibility to mycobacterial infection [17]. This is consistent with increasing recognition of LTs and particularly leukotriene B₄ (LTB₄) as an important mediator in host defense against different pulmonary infectious agents [18–21].

Since PGE₂ and LTB₄ are both produced *in vivo* during infection by Mtb and seem to have opposite effects on antimicrobial defense mechanisms, it is of interest to understand the relative importance of each in regulating the immune response. To address this question, we evaluated survival, bacterial clearance, and cytokine production in Mtb-infected BALB/c mice whose synthesis of PGE₂ and LTB₄ were separately or concomitantly inhibited by treatment with the COX-2 inhibitor celecoxib and/or the 5-LO activation protein (FLAP) inhibitor MK-886, respectively.

2. Material and methods

2.1. Animals

Male 5–8-week-old BALB/c mice were obtained from the animal facilities of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo. All experiments were approved and conducted in accordance with guidelines of the Animal Care Committee of the University. Infected animals were kept in biohazard facilities and housed in cages within a laminar flow safety enclosure under standard conditions.

2.2. Growth of *M. tuberculosis*

M. tuberculosis H37Rv strain (American Type Culture Collection, Rockville, MD, USA) was grown in 7H9 Middlebrook broth for 7 days. The culture was harvested by centrifugation, and the cell pellet was resuspended in sterile phosphate-buffered saline (PBS) and agitated vigorously. Viability of *M. tuberculosis* suspension was tested initially with fluorescein diacetate and ethidium bromide (Sigma, St. Louis, MO, USA) [22]. After 21 days in culture, the viability was again checked by plating 10-fold serial dilutions in Lowenstein-Jensen solid medium (Difco, Detroit, MI, USA) and by counting the colony-forming units (CFU).

2.3. Infection of mice with viable *M. tuberculosis* and treatment with celecoxib and MK 886

Mice were anesthetized with tribromoethanol 2.5% and restrained on a small board. An anterior midline incision was made for tracheal exposure. A 30-gauge needle attached to a tuberculin syringe was inserted into the trachea and intratracheal (i.t.) dispersion was used to

introduce either 100 μ l of phosphate-buffered saline (PBS) or 10^5 viable CFU of *M. tuberculosis* H37Rv in 100 μ l of PBS into the lungs. Animals were divided into five groups. In group 1, animals received i.t. injections of PBS and were given water orally (0.5 ml) for 60 days. In group 2, animals were subjected to i.t. infection with *M. tuberculosis* and water was given 1 h before infection and then every 24 h for 60 days. In group 3, animals were orally treated with celecoxib (5 mg/kg/0.5ml) 1 h prior to infection i.t. with *M. tuberculosis* and again every 24 h for 60 days. In group 4, animals were orally treated with MK-886 p.o. (5 mg/kg/0.5 ml) 1 h prior to infection i.t. with *M. tuberculosis* and again every 24 h for 60 days. In group 5, animals were orally treated with both celecoxib (5 mg/kg/0.5 ml) [23] and MK-886 (5 mg/kg/0.5 ml) 1 h prior to infection i.t. with *M. tuberculosis* and again every 24 h for 60 days. A dose of 5 mg/kg/0.5 ml MK-886 has previously been shown to inhibit the synthesis of leukotrienes in models of pulmonary histoplasmosis and tuberculosis [17, 20].

2.4. Bronchoalveolar lavage fluid (BALF)

On days 30 and 60 following infection, animals were euthanized with sodium pentobarbitone. The chest cavity was carefully opened and the trachea was exposed and catheterized. The catheter was tied in place and sterile PBS was infused in 3×1 -ml aliquots. Lavage fluid was recovered and placed on ice. Total cell counts were immediately performed in a Neubauer Chamber. Differential counts were obtained using Rosenfeld-stained cytospin preparations [24]. For NO measurements, total cells obtained on day 30 post infection from untreated or celecoxib, MK-886 and celecoxib plus MK-886-treated infected mice were plated in 24-well plates for 2 h at 37°C. Non-adherent and adherent cells were collected for determination of neutrophil and alveolar macrophage (AM) counts, respectively. Neutrophil and AM purities of ~ 85 and 95%, respectively, were observed by differential counts using Panotic-staining.

2.5. Quantification of bacterial growth

The number of live bacteria recovered from the lungs was determined as colony-forming units (CFU) by plating 10-fold serial dilutions of homogenized tissue on Middlebrook 7H11 agar (Difco) and counting colonies after 21 days at 37° C [25], and data were expressed as $\log_{10}$ of CFU.

2.6. Measurement of PGE₂ and LTB₄

The lungs were removed on day 30 post-infection for PGE₂ and LTB₄ measurement. Tissue was homogenized (Mixer Homogenizer, Labortechnik, Germany) in 2 ml of RPMI 1640, centrifuged at 1500 X *g* filtered, sterilized, and stored at -70°C until assayed. Specific enzyme immunoassay (Amersham Pharmacia Biotech) was used to quantify the PGE₂ and LTB₄ in the samples [26]. Supernatant dilutions were incubated with conjugated eicosanoid-acetylcholinesterase and with specific antiserum in 96-well plates pre-coated with anti-rabbit immunoglobulin G antibodies. Following overnight incubation at 4°C, plates were washed and enzyme substrate (Ellman's reagent) was added for 60 to 120 min at 25°C. The optical density of samples was determined at 450 nm in a microplate reader, and concentrations of eicosanoids were calculated based on a standard curve.

2.7. Quantitation of cytokines

On days 30 and 60 post infection, lung homogenates from mice infected or uninfected with *M. tuberculosis* and treated with celecoxib, MK-886 or both were obtained. Tissue was homogenized and stored as described above. Commercially available ELISAs were used to measure IL-6, IL-10, IL-12, and IFN- γ (PharMingen, San Diego, CA), as well as TNF- α and IL-1 (R&D Systems, Minneapolis, MN). Sensitivities were > 10 pg/ml.

2.8. Quantitation of nitric oxide (NO)

NO production was assessed by measuring the amount of nitrite in lung homogenates and in supernatants of neutrophils and AMs from BALF of infected mice, all obtained as described above. Neutrophils and AMs were plated at a density of 2×10^5 cell/well in RPMI medium/10% SBF. Cells were stimulated or not with LPS (10 μ g/well) for 4 h at 37°C. After that, cell supernatants were obtained for future NO quantitation. NO production was assessed using the Greiss reagent [27]. Values were determined using a standard curve with serial dilutions of NaNO₂ (Sigma, St. Louis, MO).

2.9. Statistical analysis

Each experiment ($n = 5-8$ mice per group) was repeated at least once. Statistical analyses were performed with ANOVA followed by the Bonferroni test using InStat3.0 (GraphPad Software, San Diego, CA). Differences were considered significant if $P < 0.05$.

3. Results

3.1. PGE₂ and LTB₄ levels in lung of *M. tuberculosis*-infected mice

On the basis of previous work demonstrating the kinetics of PGE₂ and LTB₄ production in lung lipid extracts from *M. tuberculosis*-infected mice [10, 17], we chose day 30 post-infection to determine the effect of treatment with celecoxib, MK-886 or both on lung synthesis of PGE₂ and LTB₄. Fig. 1 shows a 13-fold increase in PGE₂ levels in lung of *M. tuberculosis*-infected mice in comparison with uninfected control mice. As expected, the treatment of infected mice with celecoxib inhibited PGE₂ by ~74%. On the other hand, neither treatment with MK-886 nor MK-886 plus celecoxib altered significantly the PGE₂ synthesis in lung of infected mice.

Compared to control mice, the infection induced ~8-fold increment of LTB₄ in the lungs. While celecoxib treatment did not affect the levels of LTB₄ in *M. tuberculosis*-infected lungs, MK-886 alone or MK-886 plus celecoxib significantly inhibited the LTB₄ production in lung of infected mice by ~61 and 49 %, respectively (Fig. 1).

3.2. Survival of *M. tuberculosis*-infected mice and CFU recovery from lungs

The suppressive effect of PGs and the protective effect of LTs in experimental pulmonary tuberculosis have been reported previously [10, 17]. We now sought to investigate the relative and combinatorial effects of these endogenously produced eicosanoids on host survival and mycobacterial growth. As expected, the administration of celecoxib increased 60-day survival from 86% to 100% in Mtb-infected mice and significantly decreased the CFU number in the lungs at days 30 and 60 post-infection (Fig. 2A and B), while the treatment of infected mice with the 5-LO pathway inhibitor MK-886 increased both 60-day mortality from 14% to ~55% and lung bacterial burden by ~1.3 log₁₀. Treating infected mice with MK-886 plus celecoxib increased mortality from 14% to 35% and lung CFU by 1.9 and 1.6 log₁₀ on days 30 and 60, respectively (Fig. 2A and B).

3.3. Effect of PG and LT inhibition on lung leukocyte recruitment during *M. tuberculosis* infection

We have previously shown that the *in vivo* pharmacological abrogation of LTs in *M. tuberculosis*-infected mice did not alter BALF cell counts [17]. We verified here that treatment of infected mice with MK-886 alone did not affect neutrophil migration to the lungs as judged by the BALF cell counts (Fig. 3A). We next evaluated the impact of both PG and LT inhibitors on cell numbers in the lungs at day 30 and 60 post-infection. Treatment of infected mice with celecoxib alone or MK-886 plus celecoxib partially

inhibited neutrophil count numbers by ~ 22 and 33 % respectively at 30 days and 48 and 40 % respectively, at 60 days postinfection. However, the neutrophil numbers were still higher than those seen in uninfected control mice. In addition, no significant changes in mononuclear cell counts were observed in infected mice treated with celecoxib, MK-886 or both together (Fig. 3B).

3.4. Effect of PG and LT inhibition on cytokines and NO in the lungs of *M. tuberculosis*-infected mice

We next determined whether the treatment with PG and LT inhibitors altered production of mediators involved in the inflammatory and immune response to *M. tuberculosis*. As seen in Fig. 4, there was a differential modulation of cytokines by PG and LT inhibitors during infection. Compared to infected untreated mice, there were no changes in the IL-1 by lungs of infected mice treated with celecoxib, MK-886 or both at 30 days postinfection. At this time point, MK-886 enhanced while MK-886 plus celecoxib reduced IL-6 synthesis by 27% and 24%, respectively. On day 60, the treatment of infected mice with celecoxib inhibited IL-1 and IL-6 production by 86% and 60.5% in comparison with infected untreated mice, whereas MK-886 enhanced 33% and 43%, respectively (Fig. 4A and B). Compared to Mtb-infected untreated mice, LT inhibition by MK-886 treatment did not affect TNF- α production at 30 and 60 days and PG inhibition by celecoxib reduced TNF- α production by ~51% in lungs of infected mice on day 60 post-infection. When both eicosanoids were inhibited, reduction of ~28% in TNF- α levels were observed in lungs of infected mice on day 30 (Fig. 4C). At 30 days post-infection, treatment of infected mice with MK-886 and MK-886 plus celecoxib inhibited IL-12 production by 36% and 47% respectively. At 60 days post-infection, the inhibition was 43.5% and 55 % respectively. In contrast, at 60 days post-infection, treatment of infected mice with celecoxib increased ($P > 0.05$) the IL-12 production in lungs (Fig. 4D). At 30 days post-infection, the treatment of infected mice with MK-886 or celecoxib plus MK-886 inhibited IFN- γ production by 48.6% and 29%, respectively, while only celecoxib treatment increased IFN- γ levels by 52.3%, in comparison with infected untreated mice (Fig. 4E). Compared with lungs of infected untreated mice, the treatment with celecoxib was able to selectively inhibit IL-10 production by 50% at 30 days and by 52% at 60 days post-infection. On day 30, MK-886 or MK-886 plus celecoxib treatments did not affect IL-10 production by lungs of infected mice (Fig. 4F). Also, treatment with MK-886 or celecoxib plus MK-886 resulted in ~ 51% and 50.1% reduction of NO production, respectively, assessed on day 30 (Fig. 4G).

3.5. AMs, but not neutrophils, are the target for PG and LT modulation during host defense to Mtb

Neutrophils and mononuclear cells are recruited to the lung during Mtb infection (Fig. 3) and play an important role in mediating both innate and immune responses to this infection. To determine the relative importance of each of these cell types as targets for the modulation of mycobacterial host defense by eicosanoids, we isolated both cell types on day 30 post-infection and evaluated their capacity for NO production. Both types of plated cells were stimulated with LPS (10 μ g/well) or medium alone for 4 h, and the supernatants were used for NO₂⁻ measurement. The synthesis of NO by neutrophils from infected-mice was not statistically different from that of celecoxib, MK-886, or celecoxib + MK-886-treated-infected mice (Fig. 5A). AMs obtained from infected mice treated with celecoxib exhibited slightly greater NO synthesis ($P > 0.05$), while those from both MK-886- and celecoxib + MK-886- treated infected mice exhibited a decreased ability to produce NO when compared to untreated Mtb-infected AMs ($P < 0.05$; $P < 0.01$) (Fig. 5B).

4. Discussion

Work from our laboratory has indicated that LTs play an important role in the host immune response to pulmonary TB [17]. Moreover, it was previously shown that pharmacological suppression of PGs contributed to the enhanced clearance of bacilli in lungs of *M. tuberculosis*-infected mice [10]. Although these previous studies were useful for characterizing the potential immunomodulatory effects of each of these classes of eicosanoids individually in TB infection, the relative importance of each eicosanoid when both are present has not been evaluated. This study demonstrates that PGs and LTs have opposing effects in regulating the protective Th1 responses against mycobacterial infection *in vivo*. Also, the net immunosuppressive response seen in infected mice caused by celecoxib plus MK-886 in our model suggests that the effects of LTs dominate over PGs one.

After cellular activation, released AA is oxygenated by 5-LO to yield LTs, lipoxins and 5-HETE, or by COX isozymes to yield PGs, prostacyclins and thromboxanes. Since both metabolic pathways utilize a common substrate, the potential for interaction between them exists when inhibitors of each are provided. Several studies have suggested that the blockade of COX-1 and COX-2 pathways by administration of non-steroidal anti-inflammatory drugs enhances metabolism via the 5-LO pathway [28]. Interestingly, in our study, inhibition of COX-2 pathway shifted AA metabolism towards 5-LO activation but the inverse was not observed, indicating that (i) these pathways do not proceed in a parallel way but communicate in a dynamic manner and (ii) the protective effect of COX-2 blockade in host defense against Mtb may be due to 5-LO activation and increased leukotriene production. In fact, infected mice treated with celecoxib exhibited a 60-day survival of 100% in Mtb-infected mice and decreased CFU in lungs that could be suggested by an indirect increase of LT-enhanced alveolar macrophage functions.

When both pathways were simultaneously blocked, we found inhibition of LTB₄ production but PGE₂ production was unaltered, which can explain the predominance of LT effects on the biological features observed during the infection. Although not addressed in this study, one possible explanation by the fact that MK-886 prevented the PGE₂ inhibition induced by celecoxib could be that in presence of COX-2 inhibition by celecoxib, perhaps MK-886, by inhibiting 5-LO metabolism, preferentially shunts AA to the COX-1 metabolism. The effect of dual blocking was similar to the obtained with LT-specific inhibition, where CFU recovery in lungs of infected mice was augmented in comparison to untreated mice, as we previously have shown [17], suggesting that, when both mediators are present in Mtb-challenged lungs, inhibition of LT synthesis seems to be more important than PGE₂ presence. It is notable that neutrophil recruitment was attenuated compared to untreated infected mice when both COX-2 and 5-LO pathways were inhibited, whereas it was not when only the 5-LO pathway was inhibited. In the absence of LTs, PGs inhibition is not significantly able to change the biological features of infection. In fact, Rangel-Moreno and co-workers [10] have reported that pharmacological suppression of PGE₂ synthesis during the chronic phase of disease contributed to the decreased recovery of bacilli in lungs of *M. tuberculosis*-infected mice. The PGE₂ effect on TB pathogenesis likely involves its well-known effect in down-modulating the Th1 cytokines. Increased production of PGE₂ by macrophages caused suppression of IL-12 production by these cells and IFN- γ by T lymphocytes, resulting in development of Th2 response [29]. Also, it was demonstrated that the incubation of dendritic cells with exogenous PGE₂ induced IL-10 production and the treatment with a selective inhibitor of COX-2 blocked this production [16]. In agreement with these results, we observed an increased production of IL-12 and IFN- γ and inhibition of IL-10 after treatment with celecoxib during tuberculosis infection. Based on our data, we

now suggest that the protective host defense seen during absence of the immunosuppressive PGs during TB infection may be, at least in part, associated with LT presence.

Simultaneous inhibition of COX-2 and 5-LO has been shown to be useful to ameliorate inflammation by reducing pro-inflammatory and chemotactic mediators [30]. In order to verify whether blocking both pathways would result in additive effects on the inflammatory response, cellular recruitment and pro-inflammatory cytokines were evaluated. The production of inflammatory cytokines TNF- α , IL-1, and IL-6 by cells obtained from *M. tuberculosis*-infected mice and treated with both inhibitors was similar to that from cells of *M. tuberculosis*-infected mice treated with MK-886 only. The same was observed for cytokines IL-12, IFN- γ and IL-10. Thus, the blockade of both pathways has similar biological consequences as blocking the LT pathway alone, namely reduced cytokines which would be expected to activate alveolar macrophages following cytokine inhibition preventing the optimal clearance of bacilli.

During infection with intracellular pathogens, the production of IL-12 is essential for T lymphocyte differentiation to the protective Th1 pattern [31]. The role of IL-12 in the protection against *M. tuberculosis* was demonstrated using IL-12 knockout mice [32] as well as transgenic overexpression of IL-12 [33]. In murine tuberculosis, IL-12 is a critical cytokine that generates a protective immune response through induction of IFN- γ by Th1 lymphocytes. This cytokine is able to activate macrophages to release toxic substances for *M. tuberculosis*, such as reactive intermediates of oxygen and nitrogen [5]. In this context, the dual 5-LO and COX-2 blockade significantly reduced NO synthesis by lung cells to a degree similar to that observed with isolated 5-LO blockade. On the other hand, IL-10 directly inhibits macrophage activation, negatively modulating the intensity of cellular immune response [34].

In summary, our results showed that during infection with *M. tuberculosis*, celecoxib reduced PGE₂ and increased LTB₄, and also resulted in increases in IL-12 and IFN- γ and decreases in IL-10. MK-886 and MK-886 plus celecoxib reduced LTB₄ but did not influence PGE₂, and led to reduced formation of IL-12, IFN- γ , NO and of the IFN- γ /IL-10 ratio. PGs and LTs exerted opposing effects on regulation of protective Th1 responses against mycobacterial infection *in vivo*. Finally, to define the cell types affected by administration of COX-2 or 5-LO inhibitors, lung infiltrating cells from BALF were assessed for NO generation. Production of NO by neutrophils from infected mice was not affected by *in vivo* treatment with MK-886, celecoxib, or MK-886 plus celecoxib. However, NO production by AMs from infected mice was decreased following *in vivo* treatment with either MK-886 or celecoxib plus MK-886. Thus, although our data show that the treatment of infected mice with celecoxib alone or MK-886 plus celecoxib partially inhibited neutrophil accumulation in BALF and did not alter mononuclear cell accumulation, it is possible that the treatments affect the activation status of AMs, rather than their numbers. Neutrophils have a slightly greater capacity to synthesize LTs compared to macrophages but both respond similarly to LTB₄ through B leukotriene receptor 1/2 (BLT1/BLT2) [35]. It has been reported that LTB₄ enhances Fc γ R-mediated phagocytosis in AMs by decreasing BLT1-triggered cAMP [36]. Also, IgG-opsonized targets decrease intracellular cAMP levels in AMs via BLT1 signaling; this was not observed in peritoneal macrophages or neutrophils [37]. Our data show that AMs, but not neutrophils, are the target for the critical role played by LTB₄ enhancement of host defense against Mtb. Importantly, our *in vivo* findings employing dual inhibition of PGs and LTs suggest that the immunostimulant effects of LTB₄ dominate over the immunosuppressive actions of PGE₂.

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Fig. 1.

Lung PGE₂ and LTB₄ production in *M. tuberculosis*-infected mice and their inhibition by celecoxib and/or MK 886. Enzyme immunoassay quantification of PGE₂ and LTB₄ concentrations in lungs from mice submitted to either i.t. PBS injection and daily p.o. doses of water or i.t. infection with *M. tuberculosis* (Mtb) and daily p.o. doses of water, celecoxib (5 mg/kg/0.5 ml) or MK 886 (5 mg/kg/0.5 ml). Lungs were removed on day 30 and eicosanoid concentrations in lung homogenate supernatants are expressed as pg/ml. Data are presented as mean $\pm$ SEM from one experiment representative of two independent experiments (n = 6 mice per group). * PBS + H₂O or Mtb + H₂O vs indicated groups. $P < 0.05$.

Fig. 2.

Opposing effects of PGs and LTs on mouse survival and mycobacterial clearance. **(A)** Survival of mice after i.t. PBS injection and daily p.o. administration of water, after i.t. infection with *M. tuberculosis* (Mtb) and daily p.o. administration of water, celecoxib (5 mg/kg/0.5 ml) and/or MK 886 (5 mg/kg/0.5 ml). Mice were monitored for 60 days (n = 7 mice per group). **(B)** Bacterial load in lungs. Tissue samples were harvested at 30 and 60 days following Mtb-infection with daily administration of water, celecoxib and/ or MK 886. Data are expressed as mean $\pm$ SEM from two independent experiments (n = 9 mice per group). * Mtb + H₂O vs Mtb + celecoxib and/or MK. $P < 0.05$.

Fig. 3. Effect of celecoxib and celecoxib plus MK 886 on alveolar leukocyte numbers during *M. tuberculosis* infection. BALF cells were obtained from mice after i.t. injection of PBS and daily p.o. administration of water; i.t. infection with *M. tuberculosis* (Mtb) and daily p.o. administration of water, celecoxib (5 mg/kg/0.5 ml) or MK 886 (5 mg/kg/0.5 ml). **(A)** Neutrophils. **(B)** Mononuclear cells. Cells were enumerated and identified after Rosenfeld staining. Data are presented as mean $\pm$ SEM from two independent experiments ($n = 7-9$ mice per group). * PBS + H₂O or Mtb + H₂O vs indicated groups. $P < 0.05$.

Fig. 4. Administration of celecoxib and/or MK 886 modulates levels of cytokines and antimicrobial mediator in lung tissue of infected mice. Levels of IL-1 (A), IL-6 (B), TNF-α (C), IL-12 (D), IFN-γ (E), IL-10 (F) and NO (G) were determined in supernatants of homogenized lung at the indicated periods by ELISA. Data are expressed as mean ± SEM from two independent experiments (n = 6–8 mice per group). * PBS + H₂O or Mtb + H₂O vs indicated groups. P < 0.05.

Fig. 5. Alveolar macrophages (AMs), but not neutrophils, are involved in the modulation of *M. tuberculosis* host defense by 5-LO and COX-2 inhibitors. Neutrophils and AMs were collected from BALF on day 30 post infection and plated at the density of 2×10^5 cells/well. Both cell types were stimulated or not with LPS (10μg/well) for 4 h at 37°C. Thereafter, neutrophil (A) and AM (B) supernatants were obtained for NO quantitation. Data are expressed as mean $\pm$ SEM from one experiment (n = 5–6). *Mtb unstimulated vs Mtb stimulated with LPS $P < 0.05$; #Mtb + H₂O vs Mtb + MK $P < 0.01$; ## Mtb + H₂O vs Mtb + MK + celecoxib $P < 0.001$