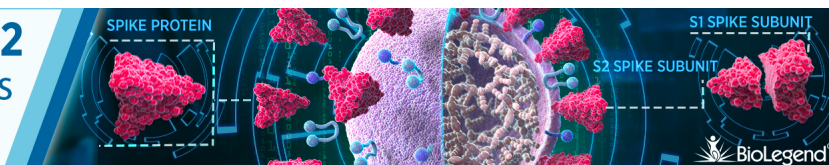


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J Immunol 2005; 175:6878-6884; ;
doi: 10.4049/jimmunol.175.10.6878
<http://www.jimmunol.org/content/175/10/6878>

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The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Contrasting Effects of Cyclooxygenase-1 (COX-1) and COX-2 Deficiency on the Host Response to Influenza A Viral Infection¹

Michelle A. Carey,* J. Alyce Bradbury,* John M. Seubert,* Robert Langenbach,[‡] Darryl C. Zeldin,* and Dori R. Germolec^{2†}

Influenza is a significant cause of morbidity and mortality worldwide despite extensive research and vaccine availability. The cyclooxygenase (COX) pathway is important in modulating immune responses and is also a major target of nonsteroidal anti-inflammatory drugs (NSAIDs) and the newer COX-2 inhibitors. The purpose of the present study was to examine the effect of deficiency of COX-1 or COX-2 on the host response to influenza. We used an influenza A viral infection model in wild type (WT), COX-1^{-/-}, and COX-2^{-/-} mice. Infection induced less severe illness in COX-2^{-/-} mice in comparison to WT and COX-1^{-/-} mice as evidenced by body weight and body temperature changes. Mortality was significantly reduced in COX-2^{-/-} mice. COX-1^{-/-} mice had enhanced inflammation and earlier appearance of proinflammatory cytokines in the BAL fluid, whereas the inflammatory and cytokine responses were blunted in COX-2^{-/-} mice. However, lung viral titers were markedly elevated in COX-2^{-/-} mice relative to WT and COX-1^{-/-} mice on day 4 of infection. Levels of PGE₂ were reduced in COX-1^{-/-} airways whereas cysteinyl leukotrienes were elevated in COX-2^{-/-} airways following infection. Thus, deficiency of COX-1 and COX-2 leads to contrasting effects in the host response to influenza infection, and these differences are associated with altered production of prostaglandins and leukotrienes following infection. COX-1 deficiency is detrimental whereas COX-2 deficiency is beneficial to the host during influenza viral infection. *The Journal of Immunology*, 2005, 175: 6878–6884.

Influenza virus continues to be a major cause of morbidity and mortality worldwide despite the availability of vaccines and antiviral agents. This virus is responsible for an average of 140,000 hospitalizations and 36,000 deaths annually in the United States alone, and the problem is increasing due to the aging population and the susceptibility of the elderly (1). The emergence of highly pathogenic avian influenza viruses has raised international concern that we are on the verge of a global pandemic (2). Influenza is therefore a virus of enormous public health importance. Certain evidence suggests that the host response to the virus rather than the cytopathic effects of the virus itself is responsible for the morbidity and mortality associated with influenza infection and other viral infections. In contrast to measles, smallpox, and poliomyelitis, influenza is caused by viruses that undergo continuous antigenic change and that possess an animal reservoir. Thus, eradication of the disease will be difficult to ever achieve (3). Greater understanding of the endogenous regulatory pathways that modulate the host response to influenza viral infection is prudent and may yield insight into new prophylactic and therapeutic strategies.

Eicosanoids are lipid mediators derived from arachidonic acid that play critical roles in the host response to infection (4–6). The

cyclooxygenase (COX)³ enzymes, which catalyze the first step in the biosynthesis of prostaglandins from arachidonic acid, have specifically been implicated as being important in host responses to infection (5, 7, 8), and the ability of COX products to modulate inflammation and immune responses is well documented (9–14). In humans, prostanoid production can be altered genetically via polymorphisms in the COX enzymes (15–17). In addition, COX enzymes are major targets of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, and naproxen (18) and the more recently introduced selective COX-2 inhibitors such as celecoxib and rofecoxib. Importantly, NSAIDs are used clinically for fever control and alleviation of symptoms during influenza viral infection.

No studies to date have addressed the roles of COX-1 and COX-2 in influenza viral infection. Because the host response is critical in determining morbidity and mortality following influenza infection and the COX enzymes have important effects in inflammation and host response to other infectious agents, a better understanding of the effects of COX enzymes in the host response to influenza is highly desirable. Thus, the purpose of this study was to examine the effect that deficiency of either COX-1 or COX-2 has on the host response to influenza viral infection and to begin to understand the mechanisms involved.

Materials and Methods

Mice

All animal studies were conducted in accordance with principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at the National Institute of Environmental Health Sciences (NIEHS). Female, pathogen-free, 3–5 mo old COX-1^{-/-}, COX-2^{-/-} and

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Received for publication April 14, 2005. Accepted for publication September 1, 2005.

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¹ This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (Research Triangle Park, NC).

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³ Abbreviations used in this paper: COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; WT, wild type; BAL, bronchoalveolar; TCID₅₀, tissue culture ID₅₀; LT, leukotriene.

wild type (WT) mice were of a hybrid C57BL/6J x 129/Ola genetic background (19, 20) intercrossed for >30 generations and bred at Taconic Farms. This age range was used because of the time necessary to breed sufficient numbers of mice and to genotype the animals and also so that results are comparable with other studies with these mice. Mice were genotyped by PCR using DNA isolated from tail pieces as previously described (21). They were housed under identical conditions and fed NIH 31 rodent chow (Agway) ad libitum.

Viral infection model

Before infection, mice were weighed and rectal temperatures were recorded electronically (Thermalert TH-5; Physitemp). A frozen aliquot of influenza A/Hong Kong/8/68 (H3N2) was used to prepare dilutions in HBSS containing 200 PFU in 50 μ l. The virus was a generous gift from Dr. R. Luebke (U.S. Environmental Protection Agency, Research Triangle Park, NC). Mice were lightly anesthetized with isofluorane and infected by intranasal instillation of 25 μ l/nostril. Control mice of each genotype, sham infected with HBSS, were included throughout the study to exclude any possible effects of vehicle on endpoints examined (data not shown). Body weight and temperature were recorded daily during the course of infection. Mice were euthanized at 1, 4, and 6 days postinfection except in mortality studies where mice were observed for 12 days and mortality was recorded on a daily basis.

Bronchoalveolar lavage (BAL)

Mice were anesthetized by i.p. injection of sodium pentobarbital (80 mg/kg). Lungs were lavaged with two 1-ml aliquots of HBSS that were subsequently combined. Approximately 90% of the total instilled volume was consistently recovered. The BAL fluid was placed on ice and centrifuged at $360 \times g$ for 10 min at 4°C. Aliquots of BAL fluid for cytokine analyses were stored at -80°C. Cells were resuspended in 1 ml of HBSS and counted using a Coulter counter (Z1 model; Coulter Electronics). Slides of BAL fluid cells were prepared (Cytospin 3; Shandon), stained with Wright-Giemsa (Fisher Scientific), and differentiated using conventional morphological criteria in a blinded fashion.

Quantitation of IL-1 β , IL-6, TNF- α , IFN- α , and IFN- γ in BAL fluid

Levels of IL-1 β , IL-6, TNF- α , IFN- α , and IFN- γ in BAL fluid were measured with the commercially available ELISA kits from R&D Systems according to the manufacturer's instructions.

Pulmonary virus quantitation

On days 1, 4, and 6 of infection, lungs were homogenized in ice-cold HBSS (10% w/v). The homogenates were centrifuged at $1000 \times g$ for 30 min to remove cell debris and the supernatants were stored at -80°C until assay. To determine the tissue culture ID₅₀ (TCID₅₀) of virus in the lungs, confluent monolayers of Madin-Darby canine kidney cells on 96-well microtiter plates were infected with one-half log₁₀ dilutions of lung homogenates. After 3–4 days of incubation at 37°C, the wells were observed for cytopathic effect. The wells with cytopathic effect were counted, and the TCID₅₀ was calculated according to the Reed-Muench method (22).

BAL fluid eicosanoid quantitation

PGE₂ levels were determined by a radioimmunoassay and leukotriene (LT)C₄/LTD₄/LTE₄ levels by an enzyme immunoassay, both supplied by Amersham Biosciences and used according to the manufacturer's instructions. LTB₄ levels were determined by an enzyme immunoassay from Cayman Chemical according to the manufacturer's instructions.

Statistical analyses

Results are expressed as means \pm SEM. Groups were compared by ANOVA followed by multiple comparison of means with Newman-Keuls multiple comparison test. Survival was analyzed using the χ^2 test with Fisher's exact method. All statistics were performed using GraphPad Prism (version 4) statistical software (GraphPad Software). Values of $p < 0.05$ were considered significant.

Results

Clinical signs of infection

Influenza infection produced a biphasic temperature response in WT animals consisting of an initial hyperthermic response on day 1 postinfection and a progressive hypothermic response from day

2 of infection onwards (Fig. 1A). Similarly, COX-1^{-/-} mice exhibited a biphasic response although the hyperthermic response was greater on day 1 (+1.23°C vs +0.58°C, $p < 0.05$), and there was a slightly sharper decline in body temperature on days 4 (-3.1°C vs -2.7°C, $p < 0.05$) and 5 (-3.5°C vs -2.8°C, $p < 0.05$) compared with WT mice. In contrast to WT and COX-1^{-/-} mice, there was no hyperthermic response in COX-2^{-/-} mice on day 1 postinfection, rather they exhibited a slight drop in body temperature. On day 2, COX-2^{-/-} mice were less hypothermic than WT and COX-1^{-/-} mice (-0.8 vs -1.5 and -1.9°C, respectively, $p < 0.05$). Consistent with the lack of hyperthermic response and blunted hypothermic response in COX-2^{-/-} animals, they lost significantly less weight relative to WT and COX-1^{-/-} animals on days 2–6 postinfection ($p < 0.05$) (Fig. 1B). These results indicate that influenza infection induced more severe illness in COX-1^{-/-} mice and less severe illness in the COX-2^{-/-} mice compared with WT mice.

Reduced mortality in COX-2^{-/-} mice following influenza infection

To determine whether the milder clinical manifestations of disease in the COX-2^{-/-} mice affected survival, we monitored daily mortality following infection. By day 12 of infection, 87.5% of WT

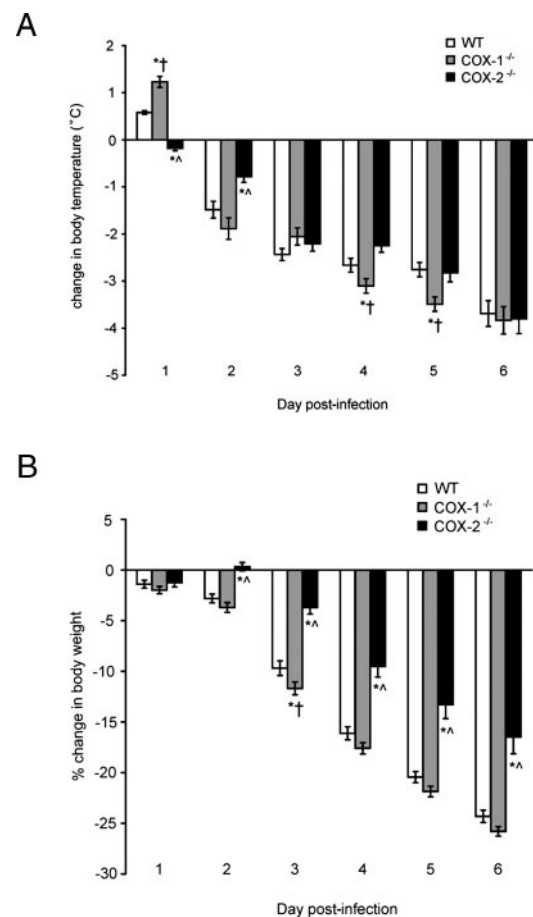


FIGURE 1. Time course of body temperature (A) and body weight (B) changes following infection with influenza virus in WT, COX-1^{-/-}, and COX-2^{-/-} mice. Before infection, mice were weighed and rectal temperatures were recorded electronically. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2). Body weight and temperature were recorded daily; $n = 24$ –30 mice per group. Data represent the means \pm SEM; *, $p < 0.05$ vs WT; ^, $p < 0.05$ vs COX-1^{-/-}; †, $p < 0.05$ vs COX-2^{-/-}.

mice and 96.7% of COX-1^{-/-} succumbed to the illness. In contrast, there were significantly more survivors in the COX-2^{-/-} group where only 66.7% succumbed to infection ($p < 0.05$) (Fig. 2). These results suggest that deficiency of COX-2 leads to enhanced survival following influenza A viral infection.

Contrasting inflammatory response to influenza infection in COX-1^{-/-} and COX-2^{-/-} mice

Pulmonary recruitment and accumulation of inflammatory cells is considered an important aspect of the innate host response to respiratory viral infection. On day 1 postinfection, BAL fluid contained mainly macrophages with a small number of infiltrating neutrophils and lymphocytes in all three groups. However, there were reduced numbers of macrophages in BAL fluid from influenza infected COX-2^{-/-} mice relative to WT ($p < 0.05$) (Fig. 3A). Peak cellular infiltration of the airways occurred on day 4 of infection with COX-1^{-/-} mice having elevated total cells, specifically neutrophils, relative to WT ($p < 0.05$) (Fig. 3B). In contrast, total cells, neutrophils, and macrophages were markedly lower in the BAL fluid of COX-2^{-/-} mice ($p < 0.05$) (Fig. 3B). By day 6, there were no differences in total BAL fluid cells between WT and COX-1^{-/-} mice; however, total BAL fluid cells in COX-2^{-/-} mice were still significantly reduced compared with WT and COX-1^{-/-} levels ($p < 0.05$) (Fig. 3C). These results suggest that COX-1 and COX-2 have significant but opposite effects on the modulation of inflammatory cell recruitment to the influenza-infected airway.

Effects of COX-1 and COX-2 deficiency on the airway cytokine response to influenza infection

In addition to cell recruitment, release of cytokines is another important aspect of the innate host response to pulmonary influenza viral infection. We measured various proinflammatory and antiviral cytokines in the BAL fluid on days 1, 4, and 6 postinfection. The kinetics of release of the proinflammatory cytokines TNF- α and IL-1 β were faster in COX-1^{-/-} mice with peak levels detected on day 4 in COX-1^{-/-} mice and day 6 in WT mice. In contrast, on day 6, TNF- α and IL-1 β levels in the COX-2^{-/-} mice were blunted compared with WT and COX-1^{-/-} levels ($p < 0.05$) (Figs. 4, A and B). IL-6 levels in WT BAL fluid peaked on day 6, but levels in COX-1^{-/-} and COX-2^{-/-} BAL fluid were markedly attenuated relative to WT ($p < 0.05$) (Fig. 4C). There were no differences between the genotypes with respect to BAL fluid IFN- α levels at all time points examined (results not shown). BAL fluid IFN- γ levels peaked on day 6 of infection in WT mice. COX-1^{-/-} mice had comparative levels, whereas levels in the COX-2^{-/-} mice were significantly reduced ($p < 0.05$) (Fig. 4D). These results suggest that COX-1 negatively regulates and COX-2 positively regulates the release and kinetics of expression of specific

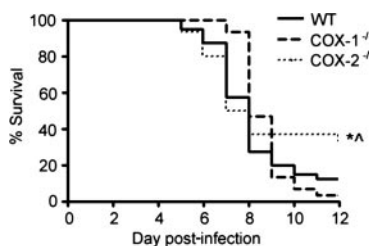


FIGURE 2. Mortality following influenza infection. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2) and were observed for 12 days. Mortality was recorded daily; $n = 30$ – 40 mice per group. *, $p < 0.05$ vs WT; ^, $p < 0.05$ vs COX-1^{-/-}.

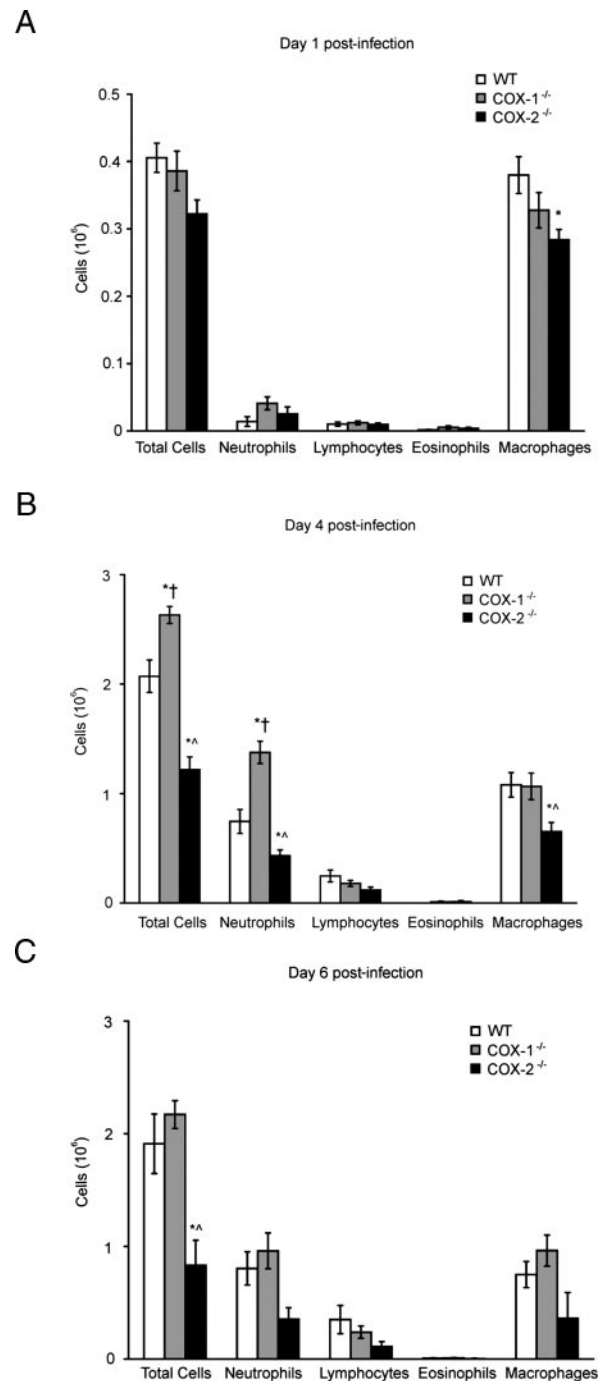


FIGURE 3. BAL fluid cellularity on days 1 (A), 4 (B), and 6 (C) of influenza infection in WT, COX-1^{-/-}, and COX-2^{-/-} mice. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2). BAL was performed on days 1, 4, and 6 following infection, and BAL fluid cells were differentiated. $n = 6$ – 10 mice per genotype except on day 6 where $n = 3$ for COX-2^{-/-} mice. *, $p < 0.05$ vs WT; ^, $p < 0.05$ vs COX-1^{-/-}; †, $p < 0.05$ vs COX-2^{-/-}.

cytokines in the influenza-infected airway with the exception being IL-6, which appears to be positively regulated by both COX-1 and COX-2.

Lung viral titers

Viral titers in WT and COX-2^{-/-} mice peaked on day 4, whereas in COX-1^{-/-} mice, there was no significant difference in viral

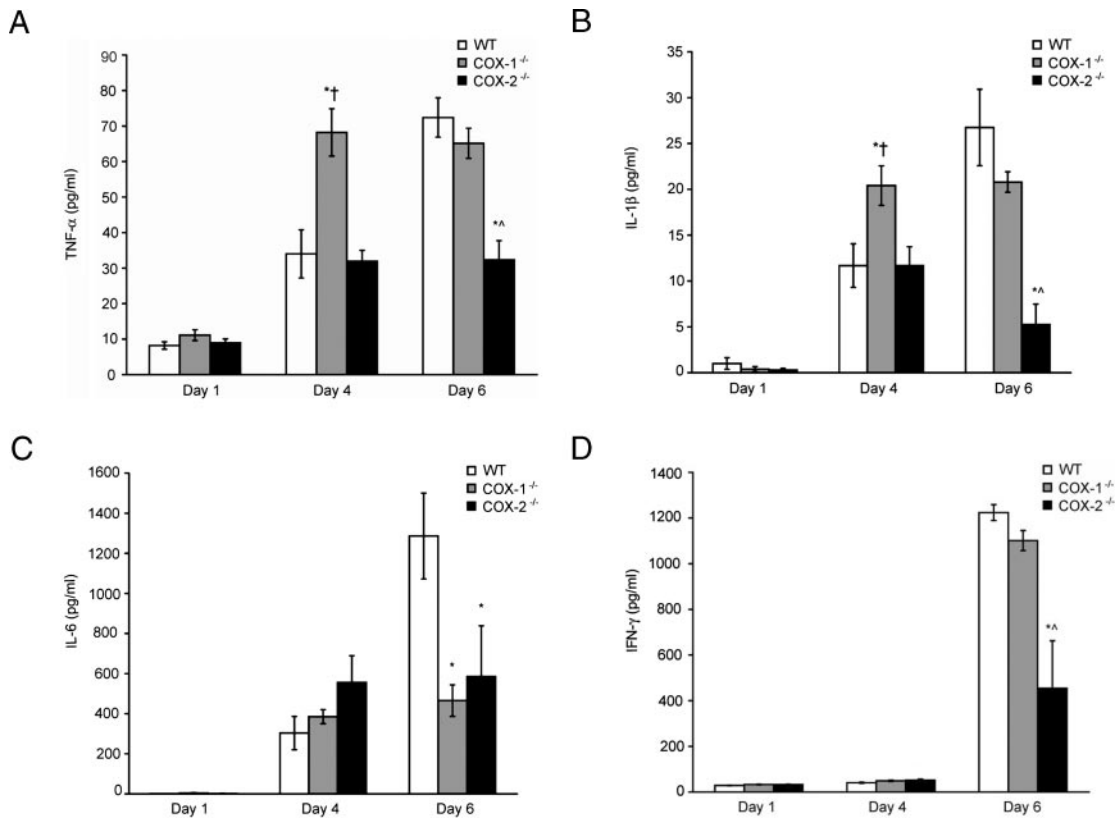


FIGURE 4. Kinetics of appearance of TNF- α (A), IL-1 β (B), IL-6 (C), and IFN- γ (D) in BAL fluid in WT, COX-1^{-/-}, and COX-2^{-/-} mice on days 1, 4, and 6 of infection. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2). BAL was performed on days 1, 4, and 6 following infection and BAL fluid cytokines were quantified by ELISA. $n = 7-10$ mice per group on days 1 and 4 and $n = 4-7$ on day 6. *, $p < 0.05$ vs WT; ^, $p < 0.05$ vs COX-1^{-/-}; †, $p < 0.05$ vs COX-2^{-/-}.

titers on any of the days examined (Fig. 5). On day 1 of infection, viral titers were elevated in the lungs of COX-2^{-/-} mice relative to WT and COX-1^{-/-} mice, but this increase was not statistically significant. However, by day 4 of infection there was an approximate 6-fold increase in viral titers in the lungs of COX-2^{-/-} mice relative to WT ($p < 0.05$) (Fig. 5). By day 6, there was a marked reduction in viral titers in COX-2^{-/-} mice. These data suggest that COX-2 is important for the early control of influenza virus repli-

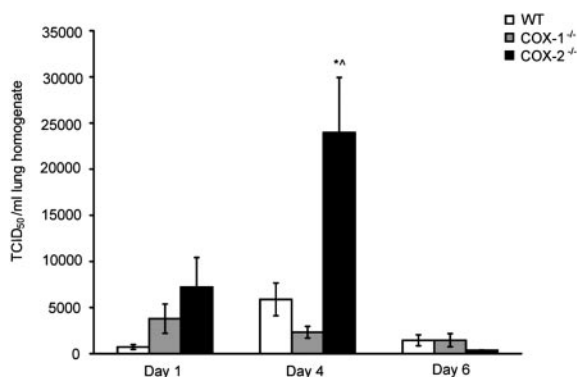


FIGURE 5. Lung viral titers. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2). On days 1, 4, and 6 of infection, lungs were homogenized and the TCID₅₀ of virus in the lungs was determined using Madin-Darby canine kidney cells; $n = 5-9$ mice per group. *, $p < 0.05$ vs WT; ^, $p < 0.05$ vs COX-1^{-/-}.

cation in the lung but not required for efficient clearance of virus from the lung.

Altered BAL fluid eicosanoid profiles following influenza infection in COX-1^{-/-} and COX-2^{-/-} mice

To determine whether there was altered arachidonic acid metabolism following influenza infection in the COX-1^{-/-} and COX-2^{-/-} mice, we measured levels of PGE₂, LTB₄ and cysteinyl LTs in BAL fluid. On days 1, 4, and 6 of infection, there were no differences between WT and COX-2^{-/-} mice in BAL fluid PGE₂ levels. In contrast, BAL fluid PGE₂ was markedly lower in COX-1^{-/-} airways than in WT airways on days 1 and 4, and COX-2^{-/-} airways on days 1, 4, and 6 (Fig. 6A). On day 1 of infection cysteinyl LT levels were higher in BAL fluid from COX-2^{-/-} mice relative to WT and COX-1^{-/-} mice, but the increase was not statistically significant. However, by day 4 of infection, cysteinyl LT levels were significantly elevated in COX-2^{-/-} airways relative to WT and COX-1^{-/-} airways. By day 6 of infection, there were no differences in levels between the genotypes (Fig. 6B). There were no significant differences between the groups in BAL fluid levels of LTB₄ following influenza infection on days 1, 4, or 6 (data not shown). These results demonstrate that following influenza infection, there is altered arachidonic acid metabolism in COX-1^{-/-} and COX-2^{-/-} mice. The reduced PGE₂ synthesis in COX-1^{-/-} mice and enhanced cysteinyl LT production in COX-2^{-/-} mice could contribute, in part, to the observed differences in host response to influenza viral infection.

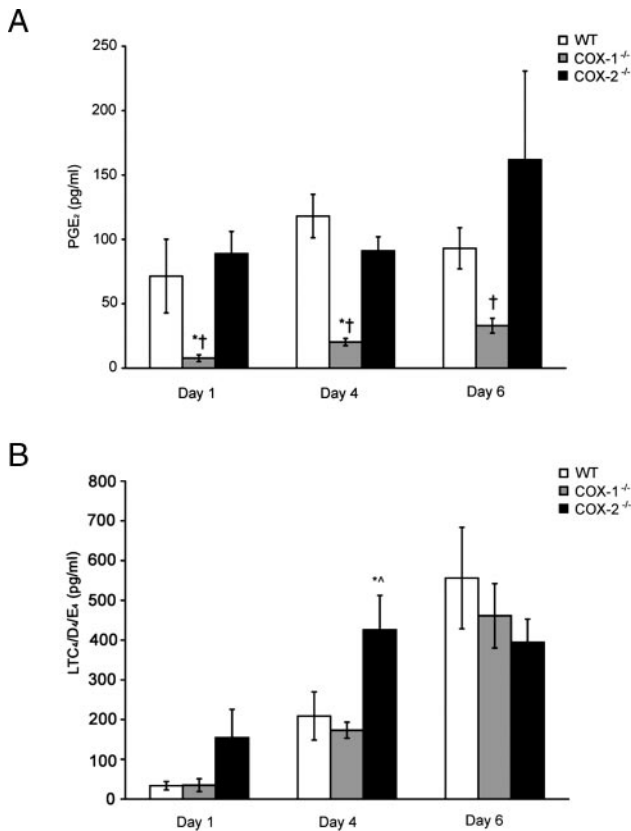


FIGURE 6. BAL fluid PGE₂ (A) and cysteinyl LTs (B) levels. Mice were infected intranasally with 200 PFU of influenza A/Hong Kong/8/68 (H3N2). BAL was performed on days 1, 4, and 6 following infection and BAL fluid PGE₂ and cysteinyl LTs were quantified by immunoassay; $n = 4$ –10 mice per group. *, $p < 0.05$ vs WT; †, $p < 0.05$ vs COX-1^{-/-}.

Discussion

Influenza virus is a significant public health problem and is responsible for more morbidity and mortality than any other infectious agent in history (23). Few other diseases inflict such an economic burden with large costs associated with healthcare and productivity losses due to absenteeism. The host inflammatory response is a critical determinant of morbidity and mortality following infection (24). The involvement of the COX pathway in inflammatory processes and modulation of immune responses is well established. In addition, the COX pathway has been implicated in modulating the host response to infection (5, 7, 8) and is a major target for the widely prescribed NSAIDs and the newer COX-2 inhibitors such as celecoxib and rofecoxib. Importantly, no studies to date have examined the relative effects of COX-1 and COX-2 in the host response to influenza viral infection. In the present study, we define central but opposite effects of COX-1 and COX-2 enzymes in the host response to this infection.

Hypothermia and weight loss are well documented features of viral influenza infection in the mouse (25–30). In the present study, we observed some differences in the early temperature response to influenza infection in the COX-1^{-/-} and COX-2^{-/-} mice. COX-1^{-/-} mice had a higher hyperthermic response on day 1 and a slightly steeper decline in body temperature on days 4 and 5 relative to WT mice. In contrast, COX-2^{-/-} mice failed to manifest the hyperthermic response on day 1 and development of the hypothermic response was blunted on day 2 of infection. By day 3, there were no temperature differences between the genotypes. The reduced weight loss and blunted temperature response following

influenza infection are consistent with a blunting of the acute phase response in the COX-2^{-/-} mice resulting in reduced mortality.

The mechanisms regulating the clinical signs of illness in influenza infection, such as hypothermia, weight loss, and death are not fully understood. The host responds to infection by release of a variety of chemokines and proinflammatory cytokines. Macrophages release predominantly proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α (31). TNF- α , ILs, and IFN- γ have been shown to induce or modulate hypothermia. The hyperthermic response to influenza virus is attenuated in IL-1 β -deficient (32) and IL-6-deficient mice (33). In addition to modulating hypothermia, proinflammatory cytokines have also been proposed to mediate sickness behaviors such as anorexia, weight loss, and wasting (34, 35). In the present study, we observed that the kinetics of cytokine release and the magnitude of responses were different between the genotypes. TNF- α , IFN- γ , and IL-1 β were all reduced in the BAL fluid from the COX-2^{-/-} mice consistent with the blunted temperature response, reduced weight loss, and enhanced survival. TNF- α and IL-1 β levels peaked earlier in the COX-1^{-/-} mice relative to WT, which is consistent with the enhanced temperature response in these mice on day 1 of infection. In addition to modulating some of the clinical signs, IFN- γ is an important antiviral cytokine. We observed that IFN- γ levels peaked on day 6 of infection in WT and COX-1^{-/-} mice; however, levels in COX-2^{-/-} mice were markedly blunted. This reduction in IFN- γ is consistent with the milder clinical signs and markedly elevated viral titers found in the lungs of COX-2^{-/-} mice. Therefore, one potential mechanism for the differential effects of COX-1 and COX-2 on the host response to and mortality following influenza infection is via modulation of these proinflammatory and antiviral cytokines.

It is well known that PGs can affect cytokine production during an inflammatory response (36–38). PGE₂ has been shown to be an important lipid mediator that dramatically limits TNF- α production (39). COX-1^{-/-} mice have lower levels of BAL fluid PGE₂ at baseline and following allergen challenge (40). In the present study, we also observed markedly reduced levels of BAL fluid PGE₂ in COX-1^{-/-} mice following influenza infection. The reduction in PGE₂ in COX-1^{-/-} mice may have removed the “brake” on TNF- α production and be a potential mechanism for the enhanced TNF- α release in the COX-1^{-/-} mice. Disruption of COX-1 leads to “shunting” of arachidonic acid down the lipoxygenase pathway following allergen challenge as evidenced by enhanced levels of BAL fluid LTB₄ and cysteinyl LTs (40). LTs have been implicated in inflammatory processes and are also emerging as important components of the innate immune response (41). Therefore, altered production of LTs due to enhanced availability of the substrate arachidonic acid could play a role in the differential host responses to influenza infection in COX-1^{-/-} and COX-2^{-/-} mice. Interestingly, following influenza infection, we observed no “shunting” in the COX-1^{-/-} mice but significantly elevated levels of the cysteinyl LTs in the airways of COX-2^{-/-} mice. The cysteinyl LTs are generally associated with an allergic state and in the promotion of a Th2 response (42–44). Enhanced levels of cysteinyl LTs in the influenza infected COX-2^{-/-} mice may be antagonizing the development of a strong Th1 response. Indeed, COX-2^{-/-} mice have markedly lower levels of the prototypic Th1 cytokine IFN- γ in BAL fluid after infection. Alternatively, the enhanced levels of cysteinyl LTs may be a reflection of the enhanced viral titers in the lungs of the COX-2^{-/-} mice as respiratory viral infection is associated with elevated LT levels (41, 45).

It has been previously shown that deficiency or inhibition of COX-1 and COX-2 can modulate the recruitment of inflammatory cells into the lung. In an allergen model, deficiency of either COX-1 or COX-2 leads to an enhanced inflammatory response in

the airways (14, 40). In contrast, in the present study, we observed that deficiency of COX-1 leads to increased cellular influx, whereas deficiency of COX-2 attenuates the recruitment of inflammatory cells. Thus, the role of COX-1 and COX-2 in the lung response to a stimulus depends on the specific stimulus involved. These findings highlight the importance of investigating the distinct roles of these two enzymes in various disease states and models.

The recruitment of leukocytes to the site of infection is critical for clearance of the pathogen; however, if the inflammatory response is left unchecked, it can cause extensive damage to surrounding tissues. Neutrophils play an important role in the clearance of virus from the infected lungs. Neutrophils also play an important role in the pathophysiology of influenza as they cause narrowing of terminal bronchi and bronchioli (29), and they are a significant source of tissue injury during the innate immune response (46). Consequently, a fine balance exists between the ability of the host immune system to clear the virus and the damage that this immune response causes to the delicate architecture of the lung. In this study, we observed two extremes in the inflammatory response by the host and the consequences of those extremes. The COX-1^{-/-} mice displayed an exaggerated inflammatory response to influenza virus infection, whereas the response in COX-2^{-/-} mice was attenuated. The degree of inflammation correlated with indicators of morbidity and mortality in the COX-2^{-/-} mice. The enhanced inflammatory response in the COX-1^{-/-} mice was associated with higher levels of the proinflammatory cytokines in the lung. The reduced inflammatory response in the COX-2^{-/-} mice was associated with reduced cytokines, milder clinical signs, and enhanced survival. Indeed, the COX-2^{-/-} mice had enhanced survival despite markedly elevated viral titers. These findings support the notion that morbidity and mortality from influenza viral infection has more to do with the host's response to the virus than to the cytopathic effects of the virus itself. Similar results were obtained by Dawson et al. (24) who observed enhanced survival of CCR2^{-/-} mice associated with reduced lung inflammation despite elevated viral titers. In contrast, they observed enhanced mortality in the CCR5^{-/-} mice that was associated with severe lung inflammation. Similarly, following infection with lymphocytic choriomeningitis virus, mice with a vigorous immune response clear the virus but succumb to the immunopathological sequelae induced by the infection, whereas mice with an attenuated immune response survive despite having very high titers of the virus (47).

NSAIDs are used clinically for fever control and alleviation of symptoms during influenza viral infection. If we extrapolate our results to a clinical setting, treatment with NSAIDs such as ibuprofen or aspirin, which have predominantly COX-1 inhibiting activity (18), may produce more severe inflammation whereas the use of COX-2 inhibitors such as celecoxib or rofecoxib during influenza infection may improve the clinical course. In summary, our findings suggest that COX-1 and COX-2 have critical but contrasting effects on the host immune response to influenza viral infection, possibly mediated via altered production of PGs and LTs following infection. Deficiency of COX-1 results in an enhanced inflammatory response and earlier release of proinflammatory cytokines. In contrast, deficiency of COX-2 results in reduced inflammation and proinflammatory cytokine release, reduced morbidity, and, despite higher viral titers, enhanced survival.

Acknowledgments

We gratefully acknowledge Drs. Farhad Imani and Steven Kleeberger for helpful comments during the preparation of this manuscript.

Disclosures

The authors have no financial conflict of interest.

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