Published in final edited form as:

Virus Res. 2016 March 2; 214: 26–32. doi:10.1016/j.virusres.2016.01.007.

Prostaglandin E₂ Production during Neonatal Respiratory Infection with Mouse Adenovirus Type 1

Megan C. Procario^{a,1}, Mary K. McCarthy^{b,2}, Rachael E. Levine^{a,3}, Caitlyn T. Molloy^a, and Jason B. Weinberg^{a,b}

^aDepartment of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan

^bDepartment of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan

Abstract

Neonatal mice are more susceptible than adults to mouse adenovirus type 1 (MAV-1) respiratory infection. In adult mice, MAV-1 respiratory infection induces production of prostaglandin E₂ (PGE₂), a lipid mediator that exerts suppressive effects on a variety of host immune functions. We tested the hypothesis that exaggerated PGE₂ production in neonatal mice contributes to increased susceptibility to MAV-1. PGE₂ concentrations were lower in lungs of uninfected neonatal mice than in adults. PGE₂ production was induced by both MAV-1 and a nonspecific stimulus to a greater degree in neonatal mice than in adults, but only in adults was PGE₂ induced in a virus-specific manner. Lung viral loads were equivalent in PGE₂-deficient neonatal mice and wild type controls, as was virus-induced expression of IFN- γ , IL-17A, and CCL5 in the lungs. PGE₂ deficiency had minimal effect on production of virus-specific IgG or establishment of protective immunity in neonatal mice. Collectively, our data indicate that lung PGE₂ production is exaggerated early in life, but this effect does not mediate increased susceptibility to MAV-1 infection.

Keywords

adenovirus; viral pathogenesis; neonatal infection; prostaglandin E₂

1. Introduction

Infants are at an increased risk of infection, in part because of differences between neonatal and adult immune function. Many such differences have been described. For instance, neonates have a relative inability to mount T helper type 1 (Th1) responses (Adkins et al.,

Corresponding author: Jason B. Weinberg, University of Michigan, 7510A Medical Science Research Building I, 1150 West Medical Center Drive, Ann Arbor, Michigan, 48109, jbw@umich.edu; Phone: (734) 764-6265; Fax (734) 936-7083.

¹Current Affiliation: Program in Biomedical Sciences, University of Michigan, Ann Arbor, Michigan

²Current Affiliation: University of Colorado School of Medicine, Denver, Colorado

³Current Affiliation: The Ohio State College of Medicine, Columbus, Ohio

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2002; Adkins and Du, 1998), more pronounced Th2 responses (Adkins and Du, 1998; Li et al., 2004), and more abundant populations of cells with immunosuppressive properties such as regulatory T cells (T_{Reg}) (Mold et al., 2010; Takahata et al., 2004) and CD71⁺ erythroid cells (Elahi et al., 2013). Neonatal immune cells produce more of the immunomodulatory cytokine interleukin (IL)-10 and less interferon (IFN)- γ and IL-12 in response to Toll like receptor (TLR) stimulation (Kollmann et al., 2009). At the same time, dendritic cell (DC) maturation and recruitment to the lung is impaired in neonates (Garvy and Qureshi, 2000; Nelson and Holt, 1995), and neonatal DC produce less type I IFN following stimulation with TLR ligands (Stetson and Medzhitov, 2006). Other studies of neonatal immune function describe a lack of preexisting memory and both a low frequency and impaired function of effector B cells (Belderbos et al., 2009; Levy, 2007; PrabhuDas et al., 2011). In general, the neonatal immune system is thereby thought to be skewed towards an anti-inflammatory and immunomodulatory environment.

Prostaglandin E₂ (PGE₂) is a lipid mediator derived from the oxidation of arachidonic acid by cyclooxygenase (COX) enzymes. PGE₂ regulates immune function in many ways that are likely to affect viral pathogenesis (reviewed in McCarthy and Weinberg, 2012). High concentrations of PGE₂ inhibit the production of the Th1 cytokines IFN- γ and IL-12 (Betz and Fox, 1991; Snijdwint et al., 1993), although lower (nanomolar) concentrations of PGE₂ have been shown to enhance Th1 function *in vivo* (Bloom et al., 1999; Yao et al., 2009). PGE₂ also enhances the production of the anti-inflammatory cytokine IL-10 (Harizi et al., 2002). COX inhibitors suppress antibody production in activated human B lymphocytes (Bancos et al., 2009; Ryan et al., 2005), and PGE₂ can promote isotype switching to IgE or IgG1 (Fedyk and Phipps, 1996; Roper et al., 1995; Roper et al., 2002), suggesting that PGE₂ also makes essential contributions to humoral immune function. PGE₂ inhibits phagocytosis and bacterial killing by neutrophils and alveolar macrophages (AM) (Aronoff et al., 2004; Aronoff et al., 2005; Serezani et al., 2007).

Several studies have demonstrated increased PGE₂ production by neonatal AM compared to adult AM (Ballinger et al., 2011; Chakraborti et al., 1999; Lu et al., 1996), raising the possibility that increased PGE₂ production in the lung may increase susceptibility to infection in neonates. Differences such as these may help to explain why respiratory viruses cause substantial disease in infants (Crowe and Williams, 2003; Nair et al., 2010; Pavia, 2011). Lung PGE₂ production is induced by infection with a variety of respiratory viruses, including respiratory syncytial virus (RSV) (Liu et al., 2005; Richardson et al., 2005), influenza (Lee et al., 2008; Woo et al., 2010), and rhinovirus (Seymour et al., 2002). Likewise, we have previously demonstrated that mouse adenovirus type 1 (MAV-1, also known as MAdV-1) induces production of PGE₂ in the lung during acute respiratory infection (McCarthy et al., 2013). Neonates are more susceptible than adults to MAV-1 respiratory infection, and lung IFN- γ responses are blunted and delayed in neonates (Procario et al., 2012). PGE₂ deficiency does not dramatically affect the pathogenesis of MAV-1 respiratory infection in adult mice (McCarthy et al., 2013). However, we hypothesized that PGE₂ overproduction contributes to altered immune responses to MAV-1 and increased susceptibility to MAV-1 in neonatal mice. In this study, we demonstrate that MAV-1-induced lung PGE₂ production is exaggerated in neonatal mice compared to adults.

However, key host immune responses to acute MAV-1 infection and lung viral loads were not affected by PGE₂ deficiency in neonatal mice, suggesting that increased PGE₂ concentrations do not confer an increased susceptibility to MAV-1 infection.

2. Materials and Methods

2.1 Mice

All animal work was conducted according to relevant national and institutional guidelines and was approved by the University Committee on Use and Care of Animals. Microsomal PGE₂ synthase (mPGES)-1 heterozygous mice on a DBA/1LacJ background (Trebino et al., 2003) were originally obtained from Pfizer, Inc. (Groton, CT) and then backcrossed onto a C57BL/6 (B6) background. Homozygous mPGES-1^{-/-} mice, which are PGE₂-deficient, and homozygous wild type mPGES-1^{+/+} mice derived from the same heterozygous mPGES-1^{+/-} parents were bred at the University of Michigan. Four- to 6-week old wild type B6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were maintained under specific pathogen-free conditions.

2.2 Virus and Infections

Wild-type MAV-1 was grown and passaged in NIH 3T6 fibroblasts as previously described (Cauthen et al., 2007). Lysates from infected 3T6 cells were centrifuged to remove cellular debris, and titers of the resulting virus stocks were determined by plaque assay on 3T6 cells (Cauthen et al., 2007). Conditioned media used for mock infections was prepared using uninfected 3T6 cells in an otherwise identical manner. Adult mice were anesthetized with ketamine and xylazine and infected intranasally (i.n.) with 10⁵ plaque forming units (pfu) of MAV-1 in 40 µl of sterile phosphate-buffered saline (PBS). Unanesthetized neonatal mice (7 days of age) were manually restrained and infected i.n. with 10⁵ pfu of MAV-1 in a total volume of 10 µl sterile PBS. Control mice were administered conditioned media at an equivalent dilution in sterile PBS. A subset of mice infected as neonates were anesthetized with ketamine and xylazine and rechallenged i.n. with MAV-1 at 28 days post initial infection (dpi). Mice were euthanized via pentobarbital overdose at the indicated time points. Organs were harvested, snap frozen in dry ice, and stored at -80°C until processed further.

2.3 Isolation of DNA and RNA

DNA was extracted from the middle lobe of the right lung using the DNeasy® Tissue Kit (Qiagen Inc.). Total RNA was extracted from lungs as previously described (Nguyen et al., 2008). In brief, approximately one third of each lung was homogenized using sterile glass beads in a mini Beadbeater (Biospec Products) for 30 seconds in 1 mL of TRIzol (Invitrogen). RNA was then isolated from the homogenates according to the manufacturer's protocol.

2.4 Analysis of Viral Loads

MAV-1 viral loads were measured in lungs using quantitative real-time polymerase chain reaction (qPCR) as previously described (Nguyen et al., 2008). Primers and probe used to detect a 59-bp region of the MAV-1 early region 1A (E1A) gene are described in Table 1.

Five µl of extracted DNA were added to reactions containing TaqMan Universal PCR Mix (Applied Biosystems), forward and reverse primers (each at 200 nM final concentration) and probe (40 nM final concentration) in a 25 µl reaction volume. Analysis on an ABI Prism 7300 machine (Applied Biosystems) consisted of 40 cycles of 15 s at 90 °C and 60 s at 60 °C. Standard curves generated using known amounts of plasmid containing the MAV-1 EIA gene were used to convert cycle threshold values for experimental samples to copy numbers of EIA DNA. Results were standardized to the ng amount of input DNA. Each sample was assayed in triplicate.

2.5 Analysis of Cytokine and EP Receptor Gene Expression

Cytokine and EP receptor gene expression was quantified using reverse transcriptase (RT)-qPCR. First, 2.5 µg of RNA were reverse transcribed using MMLV reverse transcriptase (Invitrogen) in 20 µl reactions according to the manufacturer's instructions. Water was added to the cDNA product to bring the total volume to 50 µl. cDNA was amplified using duplexed gene expression assays for mouse CCL5 and GAPDH (Applied Biosystems). Five µl of cDNA were added to reactions containing TaqMan Universal PCR Mix and 1.25 µl each of 20X gene expression assays for the target cytokine and GAPDH. Primers used to detect IFN-γ, IL-17A, EP1, EP2, EP3, and EP4 are described in Table 1. For these measurements, 5 µl of cDNA were added to reactions containing Power SYBR Green PCR Mix (Applied Biosystems) and forward and reverse primers (each at 200 nM final concentration) in a 25 µl reaction volume. When SYBR green was used to quantify cytokine gene expression, separate reactions were prepared with primers for mouse GAPDH (Table 1). In all cases, RT-qPCR analysis consisted of 40 cycles of 15 s at 90°C and 60 s at 60°C. Quantification of target gene mRNA was normalized to GAPDH and expressed in arbitrary units as 2^{-C_t} , where C_t is the threshold cycle and $C_t = C_t(\text{target}) - C_t(\text{GAPDH})$.

2.6 Lung Tissue Homogenization

Lung tissue was homogenized using sterile glass beads in a mini Beadbeater (Biospec Products) for 3 cycles of 30 s each. Tissue was homogenized at 50 mg per 1 ml of homogenization buffer. Buffer contained sterile phosphate-buffered saline (PBS), 0.1% Triton® X-100 (Fisher Scientific) and protease inhibitor (complete, Mini, EDTA-free tablets; Roche Applied Science). For samples that were to be assayed for PGE₂ concentration, indomethacin (Sigma-Aldrich) was added to the buffer for a final concentration of 10 µM. Samples were centrifuged in a refrigerated microcentrifuge at 13,500 rpm ($17,136 \times g$) for 10 min at 4 °C to pellet debris, and the remaining supernatant was stored at -80°C.

2.7 Measurement of Lung PGE₂

PGE₂ concentrations in lung homogenates were measured using a high sensitivity PGE₂ ELISA kit (Enzo Life Sciences) according to the manufacturer's protocol.

2.8 Measurement of Virus-specific Antibody

MAV-1-specific IgG was measured using enzyme-linked immunosorbent assay as previously described (Moore et al., 2004). Plates were coated overnight with polyethylene

glycol-precipitated MAV-1 and then washed and blocked with 1% bovine serum albumin. Serial dilutions of serum in PBS were added to wells. Plates were incubated 1 h at 4°C and then washed. Mouse anti-MAV-1 antibody was detected with secondary peroxidase-conjugated goat anti-mouse IgG serum (Amersham) using Turbo-TMB (Pierce) as the substrate.

2.9 Statistics

Analysis of data for statistical significance was conducted using Prism 6 for Macintosh (GraphPad Software, Incorporated). Differences between two groups at a single time point were analyzed using Mann-Whitney rank sum test. Differences between more than two groups at a single time point were analyzed using one-way analysis of variance (ANOVA). Differences between more than two groups at multiple time points were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1 Age-Based Differences in Lung PGE₂ Production and EP Receptor Expression

Neonatal AM produce more PGE₂ than adult AM (Ballinger et al., 2011; Chakraborti et al., 1999; Lu et al., 1996). Age-based variations in whole lung PGE₂ concentrations have not been described. To determine whether baseline lung PGE₂ production varies with age, we harvested lungs from uninfected B6 mice at increasing ages and measured PGE₂ concentrations in whole lung homogenates. In the absence of infection or other stimulus, PGE₂ was readily detectable in whole lung homogenates from mice at 3, 7, 14, 21, 28, and 49 days of life (Figure 1A). Lung PGE₂ concentrations increased significantly with age, with the most substantial increases occurring over the first two weeks of life.

PGE₂ signals through four G protein-coupled E prostanoid (EP) receptors (McCarthy and Weinberg, 2012; Peters-Golden and Henderson, 2007). The EP1 receptor mediates PGE₂-induced increases in intracellular Ca²⁺. PGE₂ signaling through EP2 and EP4 receptors induces increases in intracellular cAMP, while signaling through EP3 largely mediates decreases in intracellular cAMP. AM from young rats are more responsive than AM from adult rats to PGE₂ stimulation (as assessed by measurement of intracellular cAMP), but this is not due to differences in EP receptor expression (Ballinger et al., 2011). To assess possible age-based differences in EP receptor expression in the lungs that might influence responses to PGE₂, we used RT-qPCR to quantify EP receptor expression in the lungs of young (12 days old) and adult (48 days old) mice (Figure 1B). EP2 and EP3 mRNA levels were significantly lower in lungs of adult mice compared to young mice. EP1 mRNA levels were slightly lower in lungs of adult mice compared to young mice, and EP4 mRNA levels were slightly higher in lungs of adult mice compared to young mice, although those differences were not statistically significant. Thus, some aspects of baseline PGE₂ responsiveness may change with increasing age.

3.2 PGE₂ Production in Lungs of Infected Neonatal Mice

To determine whether age affects virus-induced PGE₂ production in the lungs, we infected neonatal and adult B6 mice with MAV-1. We harvested lung tissue and measured the concentration of PGE₂ in whole lung homogenate at 7 dpi, corresponding to the time of peak viral replication (Procario et al., 2012), and at 14 dpi. Lung PGE₂ concentrations were somewhat greater in the lungs of neonatal mice at 7 dpi than at 14 dpi, but there were no significant differences between mock-infected and infected mice at either time point (Figure 2A), PGE₂ concentrations were similar in lungs of mock-infected and infected adult mice at 7 dpi (Figure 2B). However, in accord with our previous findings (McCarthy et al., 2013), PGE₂ concentrations were significantly greater in lungs of infected adult mice compared to mock-infected mice at 14 dpi. Of note, relative increases in PGE₂ concentrations (compared to age-matched uninfected mice) in the lungs of both mock-infected and infected neonatal mice were significantly greater than in adult mice at 7 dpi (Figure 2C). Thus, neonatal mice appeared to be primed to produce PGE₂ in an exaggerated manner, although MAV-1 infection itself did not specifically increase PGE₂ concentrations in the lungs of neonatal mice.

3.3 Acute MAV-1 Respiratory Infection in PGE₂-Deficient Neonatal Mice

We previously demonstrated that lung viral loads are greater in neonatal mice than in adult mice (Procario et al., 2012). Overall lung PGE₂ concentrations following mock or MAV-1 infection were greater in neonates than in adults (Figure 1). To assess the possibility that increased PGE₂ production in the lungs, even if not virus-specific, altered susceptibility to MAV-1 in neonatal mice, we infected neonatal wild type (mPGES-1^{+/+}) and PGE₂-deficient (mPGES-1^{-/-}) mice with MAV-1 to assess whether the absence of PGE₂ would alter their susceptibility to MAV-1. We previously demonstrated that PGE₂ production is not induced by MAV-1 infection in adult mPGES-1^{-/-} mice, and that compensatory overproduction of PGI₂ (which signals through a receptor that could lead to cAMP increases similar to those triggered by PGE₂ signaling) does not occur following MAV-1 infection of mPGES-1^{-/-} mice (McCarthy et al., 2013). Lung viral loads were variable in both mPGES-1^{+/+} and mPGES-1^{-/-} mice during the early stages of infection at 4 dpi, but there were no statistically significant differences between groups (Figure 3A). There were clusters of mPGES-1^{+/+} and mPGES-1^{-/-} mice with high or low viral loads at 4 dpi. These variations in viral load were present in each of 3 independent experiments and did not represent systematic differences between experiments. Lung viral loads were also similar between groups at the peak of viral replication in the lungs (7 dpi) and decreased to a similar extent in both groups by 21 dpi. Thus, PGE₂ deficiency had no effect on viral replication in neonatal mice.

Acute MAV-1 infection induces the expression of multiple cytokines and chemokines in the lungs of both neonatal and adult mice, but expression of some mediators is lower and/or delayed in neonates compared to adult mice (Procario et al., 2012). To determine whether increased PGE₂ concentrations in neonates contributed to this effect, we measured expression of representative mediators in the lungs of neonatal mPGES-1^{+/+} and mPGES-1^{-/-} mice. We included measurement of prototypic Th1 (IFN- γ) and Th17 (IL-17A) cytokines. As another measure of virus-induced inflammation, we measured expression of CCL5, a chemokine that is substantially upregulated in the lungs of adult and neonatal mice

during MAV-1 respiratory infection (McCarthy et al., 2013; Procario et al., 2012; Weinberg et al., 2005). Consistent with our previous findings (Procario et al., 2012), IFN- γ mRNA levels were significantly greater in infected neonatal wild type mice than in mock infected wild type mice at 7 dpi and then decreased through 21 dpi (Figure 3B). There were no significant differences in IFN- γ mRNA levels between mPGES-1^{+/+} and mPGES-1^{-/-} mice at any time point. IL-17A was not consistently upregulated in the lungs of infected mice, and there were no significant differences in IL-17A mRNA levels between mPGES-1^{+/+} and mPGES-1^{-/-} mice at any time point (data not shown). CCL5 mRNA levels were increased in lungs of infected mice compared to mock infected controls (Figure 3C). There was a small but statistically significant difference in CCL5 mRNA levels between mPGES-1^{+/+} and mPGES-1^{-/-} mice at 10 dpi, with less CCL5 mRNA in mPGES-1^{-/-} mice. There were no differences in CCL5 mRNA levels between mPGES-1^{+/+} and mPGES-1^{-/-} at other time points. No substantial differences were detected between mPGES-1^{+/+} and mPGES-1^{-/-} mice in concentrations of IFN- γ , IL-17A, or CCL5 protein measured in lung homogenate at any time point (data not shown). The data therefore indicate that PGE₂ production in neonatal mice has little effect on the expression of representative inflammatory mediators in the lung induced during acute MAV-1 respiratory infection.

3.4 Adaptive Immune Function in PGE₂-Deficient Neonatal Mice

Antibody production is protective following intraperitoneal MAV-1 infection of adult mice (Moore et al., 2004). Because PGE₂ contributes to appropriate antibody production (Roper et al., 1995; Roper et al., 2002), we evaluated virus-specific antibody responses following MAV-1 infection of neonatal mPGES-1^{+/+} and mPGES-1^{-/-} mice. Despite PGE₂ deficiency having no effect on control of viral replication and little effect on virus-induced lung inflammation, we reasoned that it was possible that the absence of PGE₂ would alter MAV-1-specific antibody production and the establishment of protective immunity in neonatal mice. We measured MAV-1-specific IgG in the serum of mice that had been infected as neonates. Virus-specific IgG was readily detected in the serum of both mPGES-1^{+/+} and mPGES-1^{-/-} mice at 21 dpi (Figure 4A). We detected slightly less virus-specific IgG in mPGES-1^{-/-} than in mPGES-1^{+/+} mice.

Given the slight decrease in virus-specific IgG production in mPGES-1^{-/-} mice, we sought to determine whether protective immunity was established following primary infection in the absence of PGE₂. We infected mPGES-1^{+/+} and mPGES-1^{-/-} mice at 7 days of age and allowed them to recover. At 28 dpi, mice were rechallenged with another dose of MAV-1. As controls, separate groups of age-matched naïve mPGES-1^{+/+} and mPGES-1^{-/-} mice were infected with MAV-1 for the first time. At 7 days following rechallenge (or following primary infection of control mice), we harvested lungs and quantified lung viral loads. There were no differences between lung viral loads in mPGES-1^{+/+} and mPGES-1^{-/-} mice, whether during acute infection or following rechallenge (Figure 4B). Lung viral loads were significantly decreased in rechallenged mice compared to mice infected for the first time, and the magnitude of this decrease was similar in mPGES-1^{+/+} and mPGES-1^{-/-} mice. These data indicate that PGE₂ is not necessary to establish protective immunity following neonatal MAV-1 infection.

4. Discussion

Many factors influencing host susceptibility to infection differ in neonates compared to adults. In this study, we addressed the possibility that overproduction of an immunomodulatory host factor, PGE₂, in the neonatal lung contributed to the increased susceptibility of neonatal mice to MAV-1. We demonstrate that baseline production of PGE₂ in uninfected lungs increased with age. Expression of some EP receptors was greater in young mice than in adult mice, suggesting that PGE₂ responsiveness may also change with increasing age. MAV-1-induced PGE₂ production was not greater in the lungs of neonatal mice than in adults. PGE₂ deficiency had no effect on viral replication or virus-induced inflammation in the lungs during acute MAV-1 infection of neonatal mice, and PGE₂ deficiency in neonatal mice had no effect on the establishment of protective immunity. Thus, PGE₂ does not appear to make significant contributions to the pathogenesis of MAV-1 respiratory infection in neonates, and altered PGE₂ production or PGE₂ responsiveness does not contribute to increased susceptibility of neonatal mice to MAV-1 infection.

Other reports have indicated that PGE₂ production may be exaggerated in the neonatal lung compared to adults, although many of these focus on PGE₂ production by specific cell types, such as AM (Ballinger et al., 2011; Chakraborti et al., 1999; Lu et al., 1996), instead of whole lung PGE₂ production. We detected increasing PGE₂ concentrations in whole lung homogenates that correlated with increasing age of uninfected mice. This measure is likely a summation of PGE₂ production by many cell types in the lung, and it may therefore be more relevant for questions of viral pathogenesis. We did observe exaggerated lung PGE₂ production in the lungs of neonates compared to adults. It is important to note that this was not a virus-specific effect, because PGE₂ concentrations in lungs of both mock-infected and infected neonatal mice were greater than those in uninfected neonates of the same age and greater than mock-infected and infected adult mice. It is possible that neonatal lungs are primed to respond to any stimulus with exaggerated PGE₂ production. For instance, neonatal rat AM produce more PGE₂ than adult AM both at baseline and following exposure to lipopolysaccharide (Ballinger et al., 2011). Although neonatal immune function is often considered to be less responsive to infection than that of adults, there are other instances in which immune function and inflammation are more pronounced in neonates than in adults. For instance, previous studies have demonstrated enhanced TLR stimulation in neonates compared to older animals (Martino et al., 2012; Zhao et al., 2008). It is possible that these differences could occur in an organ-specific manner. Consistent with that possibility, we have demonstrated that MAV-1-induced IFN- γ production is lower in the lungs of neonatal mice compared to adults (Procario et al., 2012), while IFN- γ production is substantially greater in hearts of infected neonates than in adults (McCarthy et al., 2015).

Regardless of the specificity of the response, we detected more PGE₂ in the lungs of infected neonates than in infected adults. We reasoned that increased PGE₂ concentrations, with a corresponding increase in the immunomodulatory effects exerted by PGE₂, conferred the increased susceptibility to infection in neonates that we have previously described (Procario et al., 2012). Data from our work here with PGE₂-deficient neonatal mice indicate that this is not likely to be the case. We did detect slightly less virus-specific IgG in

mPGES-1^{-/-} mice than in mPGES-1^{+/+} controls following infection, consistent with reported effects of PGE₂ on B cell function (Bancos et al., 2009; Roper et al., 2002; Ryan et al., 2005). However, PGE₂ deficiency had no overall effect on protective immunity following infection of neonatal mice, suggesting that any effect of PGE₂ on antibody production has little overall relevance for the MAV-1 pathogenesis. It is possible that differential expression of other lipid mediators not measured in our study, such as the leukotrienes, could contribute to age-based differences in susceptibility to MAV-1 infection. However, deficiency of 5-lipoxygenase, the primary enzyme responsible for leukotriene synthesis (Peters-Golden and Henderson, 2007), has no effect on lung viral loads or virus-induced inflammation in adult mice (McCarthy and Weinberg, unpublished data), making that possibility less likely.

In summary, PGE₂ contributes little to the pathogenesis of MAV-1 respiratory infection in neonatal mice, similar to our previous findings in adult mice (McCarthy et al., 2013). PGE₂ overproduction is not likely to explain enhanced susceptibility to MAV-1 in neonates. It is possible that PGE₂ production has more pronounced effects on virus-induced inflammatory responses and virus-specific immune functions that we did not directly assess in this study. However, because PGE₂ deficiency did not affect control of viral replication during acute infection or the establishment of protective immunity, any such effect would seem unlikely to have substantial biological relevance. While our results suggest that PGE₂ does not play an important role in the pathogenesis of MAV-1 respiratory infection, this may not hold true for other respiratory viruses. For instance, decreased PGE₂ production as the result of COX inhibition or deficiency has been associated with decreased immunopathology and improved survival following infection of mice with RSV and influenza (reviewed in McCarthy and Weinberg, 2012), although recent work also indicates that PGE₂ may inhibit important innate and adaptive immune responses to influenza infection (Coulombe et al., 2014). To our knowledge, no existing studies directly address age-specific contributions of PGE₂ to the pathogenesis of these viruses. It remains to be seen whether strategies to suppress PGE₂ production may prove beneficial for neonates infected with RSV, influenza, or other respiratory viruses.

Acknowledgments

We thank Kathy Spindler and Beth Moore for helpful review of the manuscript. This research was supported by R01 AI083334 and a University of Michigan Elizabeth E. Kennedy Children's Research Fund Award.

References

- Adkins B, Bu Y, Guevara P. Murine neonatal CD4+ lymph node cells are highly deficient in the development of antigen-specific Th1 function in adoptive adult hosts. *Journal of immunology*. 2002; 169(9):4998–5004.
- Adkins B, Du RQ. Newborn mice develop balanced Th1/Th2 primary effector responses in vivo but are biased to Th2 secondary responses. *Journal of immunology*. 1998; 160(9):4217–4224.
- Aronoff DM, Canetti C, Peters-Golden M. Prostaglandin E2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP. *Journal of immunology*. 2004; 173(1):559–565.

- Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: macrophage inhibition by cyclic AMP (cAMP): differential roles of protein kinase A and exchange protein directly activated by cAMP-1. *Journal of immunology*. 2005; 174(2):595–599.
- Ballinger MN, Peters-Golden M, Moore BB. Impaired neonatal macrophage phagocytosis is not explained by overproduction of prostaglandin E2. *Respiratory research*. 2011; 12:155. [PubMed: 22141755]
- Bancos S, Bernard MP, Topham DJ, Phipps RP. Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. *Cellular immunology*. 2009; 258(1):18–28. [PubMed: 19345936]
- Belderbos M, Levy O, Bont L. Neonatal innate immunity in allergy development. *Current opinion in pediatrics*. 2009; 21(6):762–769. [PubMed: 19770766]
- Betz M, Fox BS. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *Journal of immunology*. 1991; 146(1):108–113.
- Bloom D, Jabrane-Ferrat N, Zeng L, Wu A, Li L, Lo D, Turck CW, An S, Goetzl EJ. Prostaglandin E2 enhancement of interferon-gamma production by antigen-stimulated type 1 helper T cells. *Cell Immunol*. 1999; 194(1):21–27. [PubMed: 10357877]
- Cauthen AN, Welton AR, Spindler KR. Construction of mouse adenovirus type 1 mutants. *Methods in molecular medicine*. 2007; 130:41–59. [PubMed: 17401163]
- Chakraborti T, Mandal M, Das S, Chakraborti S. Age-dependent change in arachidonic acid metabolic capacity in rat alveolar macrophages. *Biochemistry and molecular biology international*. 1999; 47(3):501–507. [PubMed: 10204087]
- Coulombe F, Jaworska J, Verway M, Tzelepis F, Massoud A, Gillard J, Wong G, Kobinger G, Xing Z, Couture C, Joubert P, Fritz JH, Powell WS, Divangahi M. Targeted prostaglandin E2 inhibition enhances antiviral immunity through induction of type I interferon and apoptosis in macrophages. *Immunity*. 2014; 40(4):554–568. [PubMed: 24726877]
- Crowe JE Jr, Williams JV. Immunology of viral respiratory tract infection in infancy. *Paediatr Respir Rev*. 2003; 4(2):112–119. [PubMed: 12758048]
- Elahi S, Ertelt JM, Kinder JM, Jiang TT, Zhang X, Xin L, Chaturvedi V, Strong BS, Qualls JE, Steinbrecher KA, Kalfa TA, Shaaban AF, Way SS. Immunosuppressive CD71+ erythroid cells compromise neonatal host defence against infection. *Nature*. 2013; 504(7478):158–162. [PubMed: 24196717]
- Fedyk ER, Phipps RP. Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93(20):10978–10983. [PubMed: 8855294]
- Garvy BA, Qureshi MH. Delayed inflammatory response to *Pneumocystis carinii* infection in neonatal mice is due to an inadequate lung environment. *Journal of immunology*. 2000; 165(11):6480–6486.
- Harizi H, Juzan M, Pitard V, Moreau JF, Gualde N. Cyclooxygenase-2-induced prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. *Journal of immunology*. 2002; 168(5):2255–2263.
- Kollmann TR, Crabtree J, Rein-Weston A, Blimkie D, Thommai F, Wang XY, Lavoie PM, Furlong J, Fortuno ES 3rd, Hajjar AM, Hawkins NR, Self SG, Wilson CB. Neonatal innate TLR-mediated responses are distinct from those of adults. *Journal of immunology*. 2009; 183(11):7150–7160.
- Lee SM, Cheung CY, Nicholls JM, Hui KP, Leung CY, Uiprasertkul M, Tipoe GL, Lau YL, Poon LL, Ip NY, Guan Y, Peiris JS. Hyperinduction of cyclooxygenase-2-mediated proinflammatory cascade: a mechanism for the pathogenesis of avian influenza H5N1 infection. *The Journal of infectious diseases*. 2008; 198(4):525–535. [PubMed: 18613795]
- Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nature reviews Immunology*. 2007; 7(5):379–390.
- Li L, Lee HH, Bell JJ, Gregg RK, Ellis JS, Gessner A, Zaghoulani H. IL-4 utilizes an alternative receptor to drive apoptosis of Th1 cells and skews neonatal immunity toward Th2. *Immunity*. 2004; 20(4):429–440. [PubMed: 15084272]

- Liu T, Zaman W, Kaphalia BS, Ansari GA, Garofalo RP, Casola A. RSV-induced prostaglandin E2 production occurs via cPLA2 activation: role in viral replication. *Virology*. 2005; 343(1):12–24. [PubMed: 16153673]
- Lu MC, Peters-Golden M, Hostetler DE, Robinson NE, Derksen FJ. Age-related enhancement of 5-lipoxygenase metabolic capacity in cattle alveolar macrophages. *The American journal of physiology*. 1996; 271(4 Pt 1):L547–554. [PubMed: 8897901]
- Martino D, Holt P, Prescott S. A novel role for interleukin-1 receptor signaling in the developmental regulation of immune responses to endotoxin. *Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology*. 2012; 23(6):567–572. [PubMed: 22435759]
- McCarthy MK, Levine RE, Procario MC, McDonnell PJ, Zhu L, Mancuso P, Crofford LJ, Aronoff DM, Weinberg JB. Prostaglandin E2 induction during mouse adenovirus type 1 respiratory infection regulates inflammatory mediator generation but does not affect viral pathogenesis. *PloS one*. 2013; 8(10):e77628. [PubMed: 24147040]
- McCarthy MK, Procario MC, Twisselmann N, Wilkinson JE, Archambeau AJ, Michele DE, Day SM, Weinberg JB. Proinflammatory effects of interferon gamma in mouse adenovirus 1 myocarditis. *Journal of virology*. 2015; 89(1):468–479. [PubMed: 25320326]
- McCarthy MK, Weinberg JB. Eicosanoids and respiratory viral infection: coordinators of inflammation and potential therapeutic targets. *Mediators of inflammation*. 2012; 2012:236345. [PubMed: 22665949]
- Mold JE, Venkatasubrahmanyam S, Burt TD, Michaelsson J, Rivera JM, Galkina SA, Weinberg K, Stoddart CA, McCune JM. Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans. *Science*. 2010; 330(6011):1695–1699. [PubMed: 21164017]
- Moore ML, McKissic EL, Brown CC, Wilkinson JE, Spindler KR. Fatal disseminated mouse adenovirus type 1 infection in mice lacking B cells or Bruton's tyrosine kinase. *Journal of virology*. 2004; 78(11):5584–5590. [PubMed: 15140955]
- Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O'Brien KL, Roca A, Wright PF, Bruce N, Chandran A, Theodoratou E, Sutanto A, Sedyaningsih ER, Ngama M, Munywoki PK, Kartasmita C, Simoes EA, Rudan I, Weber MW, Campbell H. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet*. 2010; 375(9725):1545–1555. [PubMed: 20399493]
- Nelson DJ, Holt PG. Defective regional immunity in the respiratory tract of neonates is attributable to hyporesponsiveness of local dendritic cells to activation signals. *Journal of immunology*. 1995; 155(7):3517–3524.
- Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 2011; 52(Suppl 4):S284–289. [PubMed: 21460286]
- Peters-Golden M, Henderson WR Jr. Leukotrienes. *The New England journal of medicine*. 2007; 357(18):1841–1854. [PubMed: 17978293]
- PrabhuDas M, Adkins B, Gans H, King C, Levy O, Ramilo O, Siegrist CA. Challenges in infant immunity: implications for responses to infection and vaccines. *Nature immunology*. 2011; 12(3):189–194. [PubMed: 21321588]
- Procario MC, Levine RE, McCarthy MK, Kim E, Zhu L, Chang CH, Hershenson MB, Weinberg JB. Susceptibility to acute mouse adenovirus type 1 respiratory infection and establishment of protective immunity in neonatal mice. *Journal of virology*. 2012; 86(8):4194–4203. [PubMed: 22345470]
- Richardson JY, Ottolini MG, Pletneva L, Boukhvalova M, Zhang S, Vogel SN, Prince GA, Blanco JC. Respiratory syncytial virus (RSV) infection induces cyclooxygenase 2: a potential target for RSV therapy. *Journal of immunology*. 2005; 174(7):4356–4364.
- Roper RL, Brown DM, Phipps RP. Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE. *Journal of immunology*. 1995; 154(1):162–170.
- Roper RL, Graf B, Phipps RP. Prostaglandin E2 and cAMP promote B lymphocyte class switching to IgG1. *Immunology letters*. 2002; 84(3):191–198. [PubMed: 12413736]

- Ryan EP, Pollock SJ, Murant TI, Bernstein SH, Felgar RE, Phipps RP. Activated human B lymphocytes express cyclooxygenase-2 and cyclooxygenase inhibitors attenuate antibody production. *Journal of immunology*. 2005; 174(5):2619–2626.
- Serezani CH, Chung J, Ballinger MN, Moore BB, Aronoff DM, Peters-Golden M. Prostaglandin E2 suppresses bacterial killing in alveolar macrophages by inhibiting NADPH oxidase. *American journal of respiratory cell and molecular biology*. 2007; 37(5):562–570. [PubMed: 17585108]
- Seymour ML, Gilby N, Bardin PG, Fraenkel DJ, Sanderson G, Penrose JF, Holgate ST, Johnston SL, Sampson AP. Rhinovirus infection increases 5-lipoxygenase and cyclooxygenase-2 in bronchial biopsy specimens from nonatopic subjects. *The Journal of infectious diseases*. 2002; 185(4):540–544. [PubMed: 11865407]
- Snijdwint FG, Kalinski P, Wierenga EA, Bos JD, Kapsenberg ML. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *Journal of immunology*. 1993; 150(12):5321–5329.
- Stetson DB, Medzhitov R. Type I interferons in host defense. *Immunity*. 2006; 25(3):373–381. [PubMed: 16979569]
- Takahata Y, Nomura A, Takada H, Ohga S, Furuno K, Hikino S, Nakayama H, Sakaguchi S, Hara T. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. *Experimental hematology*. 2004; 32(7):622–629. [PubMed: 15246158]
- Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson PJ, Carty TJ, Perez JR, Audoly LP. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(15):9044–9049. [PubMed: 12835414]
- Weinberg JB, Stempfle GS, Wilkinson JE, Younger JG, Spindler KR. Acute respiratory infection with mouse adenovirus type 1. *Virology*. 2005; 340(2):245–254. [PubMed: 16054189]
- Woo PC, Tung ET, Chan KH, Lau CC, Lau SK, Yuen KY. Cytokine profiles induced by the novel swine-origin influenza A/H1N1 virus: implications for treatment strategies. *The Journal of infectious diseases*. 2010; 201(3):346–353. [PubMed: 20030555]
- Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, Sugimoto Y, Narumiya S. Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nature medicine*. 2009; 15(6):633–640.
- Zhao J, Kim KD, Yang X, Auh S, Fu YX, Tang H. Hyper innate responses in neonates lead to increased morbidity and mortality after infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(21):7528–7533. [PubMed: 18490660]

Highlights

- Neonatal mice are more susceptible than adult mice to mouse adenovirus type 1 (MAV-1) respiratory infection.
- We demonstrate that prostaglandin E₂ (PGE₂) is overproduced in lungs of neonatal mice compared to adults, although not in a virus-specific manner.
- Using PGE₂-deficient mice, we show that PGE₂ is not essential for control of viral replication, induction of virus-induced inflammation, or the establishment of protective immunity to MAV-1 in neonatal mice.
- Excess PGE₂ production therefore does not contribute to age-based differences in susceptibility to MAV-1.

Figure 1.

Baseline lung PGE₂ production and EP receptor expression. A) ELISA was used to quantify PGE₂ concentrations in lung homogenates from uninfected mice at the indicated ages.

Combined data from $n=3-6$ mice per group are presented as means $\pm$ S.E.M. Statistical analysis of the trend over ages was performed using one-way ANOVA. B) RT-qPCR was used to quantify expression of EP receptors in lungs of uninfected mice at the indicated ages. Combined data from $n=4-8$ mice per group are presented as means $\pm$ S.E.M. Statistical comparison was made using the Mann-Whitney rank sum test. * $P<0.05$ and ** $P<0.01$, comparing young to old mice.

Figure 2.

Virus-induced lung PGE₂ production. A) Neonatal (7 days of age) mice were infected i.n. with MAV-1 or mock infected with conditioned media. ELISA was used to quantify PGE₂ concentrations in lung homogenates at the indicated time points. Combined data from n=22–29 mice per group at 7 dpi and n=3 mice per group at 14 dpi are presented as means ± S.E.M. B) Adult (6–8 weeks of age) mice were infected i.n. with MAV-1 or mock infected with conditioned media. ELISA was used to quantify PGE₂ concentrations in lung homogenates at the indicated time points. Combined data from n=10–13 mice per group are presented as means ± S.E.M. C) Data at 7 dpi from Figures 2A and 2B are presented as lung PGE₂ concentrations relative to concentrations in age-matched uninfected mice from Figure 1. Statistical comparisons were made using two-way ANOVA followed by Bonferroni's multiple comparison tests. ***P*<0.01, comparing mock to MAV-1. ††*P*<0.01 comparing neonatal and adult mice within the same condition.

Figure 3.

Effects of PGE₂ deficiency on acute MAV-1 respiratory infection in neonatal mice. Neonatal mPGES-1^{+/+} and mPGES-1^{-/-} mice were infected i.n. with MAV-1 or mock infected with conditioned media. A) DNA was extracted from lungs harvested at the indicated time points. qPCR was used to quantify MAV-1 genome copies in lung DNA. DNA viral loads are expressed as copies of MAV-1 genome per 100 ng of input DNA. Individual circles represent values for individual mice and horizontal bars represent means for each group. B, C) RNA was extracted from lungs harvested at the indicated time points and RT-qPCR was used to quantify cytokine expression, which is shown in arbitrary units. Combined data from 3 independent experiments, n=3–10 mice per group (2 mock-infected mPGES-1^{-/-} mice at 14 dpi), are presented as means $\pm$ S.E.M. Statistical comparisons were made using two-way ANOVA followed by Bonferroni's multiple comparison tests. *** P <0.001 comparing mock to MAV-1 for a given genotype. $\dagger\dagger P$ <0.01, comparing mPGES-1^{+/+} to mPGES-1^{-/-} mice within the same condition.

Figure 4.

Effects of PGE₂ deficiency on adaptive immune responses to MAV-1 respiratory infection in neonatal mice. A) Neonatal mPGES-1^{+/+} and mPGES-1^{-/-} mice were infected i.n. with MAV-1. ELISA was used to quantify virus-specific IgG in the serum at 21 dpi. Data are presented as means ± S.E.M. Statistical comparisons were made using two-way ANOVA followed by Bonferroni's multiple comparison tests. ***P* < 0.01 comparing mPGES-1^{+/+} (n=5) to mPGES-1^{-/-} (n=7) mice. B) Neonatal mPGES-1^{+/+} and mPGES-1^{-/-} mice were infected i.n. with MAV-1. At 28 dpi, mice were rechallenged i.n. with MAV-1. Age-matched naïve mice infected for the first time were included as controls. Lungs were harvested at 7 days following rechallenge (or following primary infection of control mice). DNA was extracted from lungs and qPCR was used to quantify DNA viral loads, which are

expressed as copies of MAV-1 genome per 100 ng of input DNA. Individual circles represent values for individual mice and horizontal bars represent means for each group. Statistical comparisons were made using the Mann-Whitney rank sum test for differences between conditions within a given genotype. * $P < 0.05$ and ** $P < 0.01$ comparing primary to secondary infection within a genotype.

Table 1

Primers and probes used for real-time PCR analysis

Target	Oligonucleotide	Sequence (5' to 3')
MAV-1 E1A	Forward primer	GCACTCCATGGCAGGATTCT
	Reverse primer	GGTCGAAGCAGACGGTTCTTC
	Probe	TACTGCCACTTCTGC
IFN- γ	Forward primer	AAAGAGATAATCTGGCTCTGC
	Reverse primer	GCTCTGAGACAATGAACGCT
IL-17A	Forward primer	GGGTCTTCATTGCGGTGG
	Reverse primer	CTCCAGAAGGCCCTCAGACTAC
EP1	Forward primer	GTGCCAAGGGTGGTCCA
	Reverse primer	AACCACTGTGCCGGGAATA
EP2	Forward primer	TGCGCTCAGTCCTCTGTTGT
	Reverse primer	TGGCACTGGACTGGGTAGAAC
EP3	Forward primer	TCAGATGTCGGTTGAGCAATG
	Reverse primer	AGCCAGGCGAACTGCAATTA
EP4	Forward primer	ACGTCCCAGACCCTCCTGTA
	Reverse primer	CGAACCTGGAAGCAAATTCC
GAPDH	Forward primer	TGCACCACCAACTGCTTAG
	Reverse primer	GGATGCAGGGATGATGTTC