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Maternal endotoxin exposure attenuates allergic airway disease in infant rats

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Submitted 17 November 2009; accepted in final form 27 January 2010

Cao L, Wang J, Zhu Y, Tseu I, Post M. Maternal endotoxin exposure attenuates allergic airway disease in infant rats. *Am J Physiol Lung Cell Mol Physiol* 298: L670–L677, 2010. First published January 29, 2010; doi:10.1152/ajplung.00399.2009.—Prenatal exposures to immunogenic stimuli, such as bacterial LPS, have shown to influence the neonatal immune system and lung function. However, no detailed analysis of the immunomodulatory effects of LPS on postnatal T helper cell differentiation has been performed. Using a rat model, we investigated the effect of prenatal LPS exposure on postnatal T cell differentiation and experimental allergic airway disease. Pregnant rats were injected with LPS on day 20 and 21 (term = 22 days). Some of the offspring were sensitized and challenged with ovalbumin. Positive control animals were placebo exposed to saline instead of LPS, whereas negative controls were sensitized with saline. Expression of T cell-related transcription factors and cytokines was quantified in the lung, and airway hyperresponsiveness was measured. Prenatal LPS exposure induced a T helper 1 (T_H1) immune milieu in the offspring of rats [i.e., increased T-bet and T_H1 cytokine expression while expression of T_H2-associated transcription factors (GATA3 and STAT6) and cytokines was decreased]. Prenatal LPS exposure did not trigger T_H17 cell differentiation in the offspring. Furthermore, prenatal LPS exposure reduced ovalbumin-induced (T_H2-mediated) airway inflammation, eosinophilia, and airway responsiveness. Thus, in utero exposure to endotoxin promotes a T_H1 immune environment, which suppresses the development of allergic airway disease later in life.

asthma; lipopolysaccharide; pulmonary inflammation; ovalbumin; airway hyperresponsiveness

ALLERGIC AIRWAYS DISEASE USUALLY starts early in life (39). The early occurrence of asthma suggests that prenatal influences are of importance (12, 25). In utero exposures to environmental stimuli have shown to influence the prenatal and neonatal immune system (3). Epidemiological and clinical studies have provided compelling evidence for a link between the relative lack of infectious diseases and the increase in allergic disorders (7, 30). Based on these findings, it is theorized that children in rural environments are somewhat protected from the development of atopic disease and asthma, and this protection may be a result of early exposure to immunogenic stimuli including allergens and endotoxin (42, 44). Allergic airway disease is associated with an exaggerated T helper 2 cell (T_H2)-mediated immune response to environmental stimuli (17, 41). Bacterial infections downregulate the allergen-induced T_H2 response because they elicit a T_H1-based immunity. In addition, bacterial pathogens and fungi can trigger another T_H lineage, namely T_H17 cells (35). Several transcription factors play a pivotal role in T_H cell differentiation. Both the signal transducer and activator of transcription 6 (STAT6) and GATA

binding protein 3 (GATA3) play a crucial role in the selective T_H2 response of activated naive T helper cells to allergic stimuli (26, 38). STAT1, STAT4, and the transcription factor T-bet (T-box expressed in T cells) are required for the generation of interferon(IFN)γ-producing T_H1 cells (47, 48), whereas T_H17 cell lineage commitment involves the retinoic acid-related orphan receptor (ROR)γt (9, 14). The prenatal immune system is predisposed to T_H2 cell activation (2), but postnatal maturation of the immune response, driven by contact with an antigen, may take several different directions depending on the stimulus (22, 23). In utero events can also affect early life immune responses. Maternal smoking in pregnancy impairs neonatal cytokine responses (33, 34) and increases the risk of recurrent wheezing (29). Recently, we found that systemic maternal exposure to endotoxin results in a prolonged pulmonary inflammation in newborn rats (8). Whether systemic maternal infections also affect allergic airway disease in the offspring is unclear. Some epidemiological studies have revealed that prenatal exposure to endotoxin may be protective (15, 16), although chorioamnionitis may increase the risk of recurrent wheezing in combination with preterm birth (28). Here we tested whether a systemic maternal exposure to endotoxin attenuates experimental (OVA-induced) allergic airway disease in infant rats by promoting T_H1 differentiation.

METHODS

Animals. Timed pregnant Wistar rats (Charles River, Oakville, Quebec, Canada) were maintained on an ovalbumin (OVA)-free diet. Approval for the study was obtained from the Animal Care Review Committee of the Hospital for Sick Children Research Institute. All animal procedures were conducted according to the guidelines of the Canadian Council for Animal Care.

Maternal LPS exposure. Timed pregnant Wistar rats were randomly assigned to two different treatment strategies consisting of an intraperitoneal (ip) injection on fetal days 20 and 21 (term = 22 days) of either 2.5 mg/kg body wt of LPS from *Escherichia coli* serotype 026:B6 (Sigma-Aldrich, St. Louis, MO) or the same volume of saline. We have previously reported that this was the optimal of LPS to trigger a systemic maternal inflammation without inducing premature delivery or increasing the number of stillborns (8). On day 22 of pregnancy (term), pregnant rats from each group were killed by ether overdose, and lungs of offspring were recovered and processed for analysis [labeled as postnatal days (PN) 0 lungs]. Other pregnant rats were allowed to deliver, and lungs were collected and processed at PN2, 6, 10, and 14.

Postnatal OVA exposure. After maternal saline or LPS treatment, randomly picked rat pups were sensitized at PN1 and PN7 with OVA (ip injections of 50 μg OVA; Sigma) mixed with 20 μg Alum (Pierce, Rockford, IL) (S+O and L+O groups). The rats were then challenged every day starting on day 14 until day 21 (20 mg/ml OVA aerosolized for 30 min). Alum (ip) and prenatal LPS plus alum sensitization were administered to other rat pups as negative controls (S+A and L+A groups). At 24 h following the final exposure (day 21), the airway

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response to inhaled MCh was assessed, and tissue and bronchoalveolar lavage (BAL) samples were collected from each rat ($n = 8/\text{group}$) for assessment of airway inflammation.

Quantitative RT-PCR. Lung tissue was homogenized in TRIzol (Invitrogen Canada, Burlington, ON, Canada), and total RNA was extracted according to the manufacturer's protocol. Total RNA was treated with DNase I (Invitrogen Canada) at 37°C to remove residual genomic DNA. Total RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen Canada) and random hexamers (Applied Biosystems, Foster City, CA). The resulting templates were quantified by real-time PCR (ABI Prism 7900) using SYBRGREEN in conjunction with rat gene-specific primer sets for IL-2, IL-4, IL-5, IL-12a/b, IL-13, IFN γ , GATA-3, STAT4 and 6, ROR γ t, FoxP3, and T-bet (Qiagen, Mississauga, Canada). For relative quantification, PCR signals were compared among groups after normalization using 18S as internal reference. Fold change was calculated according to Livak and Schmittgen (31).

Cytokine measurement in lung tissue. Lungs were homogenized in RIPA lysis buffer (PBS containing 0.05% sodium azide, 2 mM phenylmethylsulfonyl fluoride, and 1 $\mu\text{g}/\text{ml}$ aprotinin, leupeptin, and pepstatin A). Homogenates were centrifuged (60 min at 23,000 g), and supernatant was collected and stored at -80°C . Total protein content was measured according to Bradford (6). Cytokines were measured in the tissue lysates using multiplex immunoassays for Luminex technology from Linco Research (St. Charles, MO) as previously described (8).

Western blotting. Fifty micrograms of total whole lung protein was separated by 10% SDS-PAGE and blotted onto nitrocellulose membrane. The membranes were then incubated in blocking solution (5% dry milk in PBS containing 0.05% Tween 20) and subsequently exposed overnight to either mouse monoclonal anti-GATA3 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti-STAT6 (1:500; Santa Cruz Biotechnology), or anti-phospho-STAT6 (1:200; Santa Cruz Biotechnology) antibodies. The next day, the membrane was washed and incubated with anti-mouse peroxidase-conjugated IgG

(1:1,000; EMD Chemical, Gibbstown, NY). Protein bands were visualized using an enhanced chemiluminescence detection kit (Amersham, Piscataway, NJ). Band densities were quantified using Scion Image software (version 1.6; National Institutes of Health, Bethesda, MD). Equal protein loading was confirmed by β -actin immunoblotting of same membrane.

Inflammatory cells in BAL. BAL using PBS was performed through a catheter inserted into the trachea by a tracheotomy as recently described (43) and adapted for newborn rat pups. Total cell number was determined using a cell counter (Beckman). To differentiate inflammatory cell types, cytospin smears were prepared and stained with Kwik-Diff Stain (Thermo Shandon, Pittsburgh, PA). At least 400 cells from each smear were counted under a light microscope. The proportion of each cell population was expressed as a percentage of total cells, and this ratio together with the total cell count was used to calculate the number of each cell type.

Histology. Whole lungs were infused in situ with 4% (wt/vol) paraformaldehyde (PFA) in PBS with a constant pressure of 20 cmH_2O to have equalized filling pressure over the whole lung. Under these constant pressure conditions, the cannula was removed and the trachea immediately ligated. The lungs were excised and immersed in 4% PFA in PBS overnight, dehydrated, and embedded in paraffin. Lung sections were stained with hematoxylin and eosin or stained for either eosinophils using Giemsa (Sigma, Steinheim, Germany) or mucus using periodic acid-Schiff (Sigma-Aldrich).

Lung function measurements. The enhanced pause (Penh) was assessed at baseline and after increasing doses of aerosolized MCh using whole body barometric plethysmography (WBP) as described (13, 45, 53). Briefly, PN21 rat pups were placed in plethysmograph chambers that contain built-in individually connected pneumotachographs (Buxco, Sharon, CT). The pups were ventilated by a regulated bias airflow (1 l/min preconditioned to body temperature and humidity) through the plethysmograph and exposed to nebulized saline or MCh for 2 min. Data are recorded every 3 min at baseline and after stimulation with each concentration of MCh. Transducer signals are

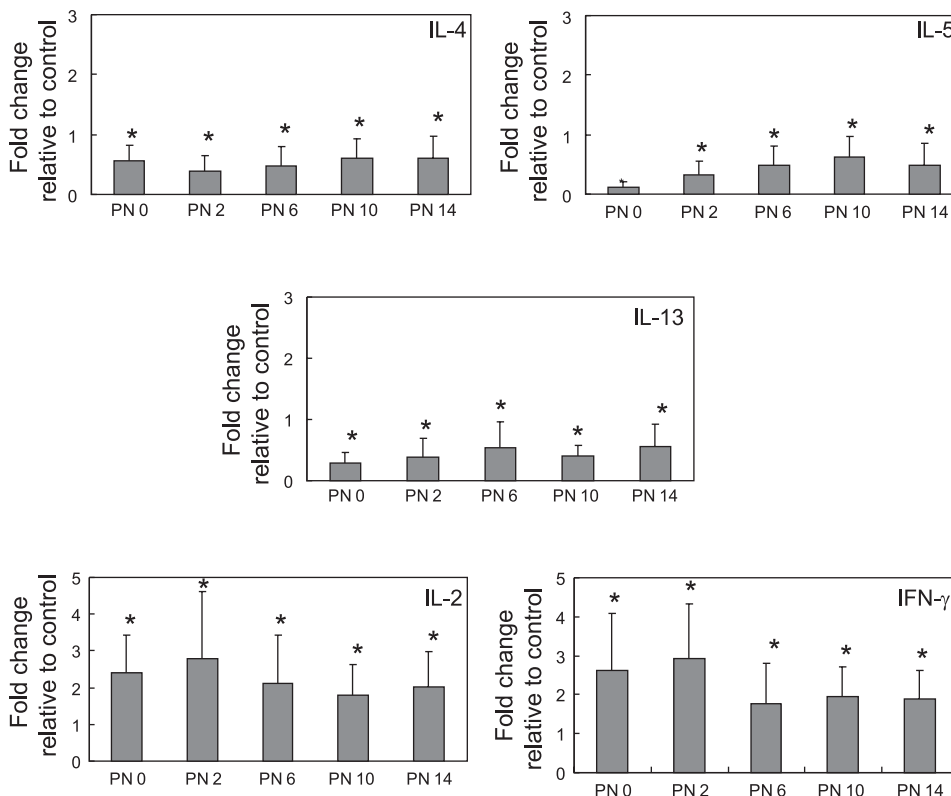


Fig. 1. Maternal endotoxin exposure decreases T helper 2 (T_H2) and increases T_H1 cytokine mRNA expression in neonatal rat lungs. IL-4, IL-5, IL-13, IL-2, and IFN γ mRNA expression was quantified by real-time PCR. Data are means $\pm$ SD, $n = 6$ animals per control (set at 1) and LPS (gray bar) group. * $P > 0.05$ vs. control (saline-treated) group.

conditioned, digitized, processed in real time, and computer recorded (Biosystem XA software, Buxco Electronics). Airway resistance was expressed as $\text{Penh} = (\text{expiratory time}/40\% \text{ of relaxation time} - 1) \times \text{peak expiratory flow/peak inspiratory flow} \times 0.67$. In preliminary experiments, we measured airway resistance using a rodent ventilator (SciReq flexiVent, Montreal, PQ) in S+O- and S+A-treated animals to validate the WBP Penh values. The decreased resistance (R_n) in the S+O vs. S+A groups confirmed the airway hyperresponsiveness suggested by WBP.

Statistical analysis. Data are presented as means $\pm$ SD. Student's *t*-test or one-way ANOVA followed by post hoc Student-Newman-Keuls tests were used. Statistical analysis was performed with the JMP statistical software, and significance was accepted at $P < 0.05$.

RESULTS

Maternal LPS exposure promotes T_H1 cytokine expression in postnatal lungs. Maternal exposure to LPS significantly decreased lung mRNA levels of T_H2 cytokines (IL-4, IL-5, IL-13), whereas T_H1 cytokine (IL-2 and IFN γ) mRNAs were increased at PN0, 2, 6, 10, and 14 (Fig. 1). IL-12 message levels were not altered in lungs of maternal LPS-exposed pups, whereas T_H17 cytokine (IL-17F and IL-21) mRNAs were undetectable (not shown). IL-4 and IL-13, but not IL-5, protein levels were significantly decreased in the lungs of prenatal LPS-exposed animals compared with saline controls at PN0, 2, and 14 (Fig. 2). IL-2 and IFN γ proteins were increased in lungs of prenatal LPS-exposed pups compared with saline controls at PN0, 2 and 14 (Fig. 2).

Transcription factors for T cell differentiation are modulated by maternal LPS. T cell differentiation is regulated by the transcription factors including STAT4 and T-bet (T_H1), STAT6 and GATA3 (T_H2), and ROR γ t (T_H17). In line with maternal LPS stimulating T_H1 cell differentiation, qRT-PCR and Western blotting showed reduced expression of both T_H2 transcription factors STAT6 and GATA3 in maternal LPS-exposed neonatal lung tissues (Figs. 3 and 4). In contrast, gene expression of T-bet, a T_H1 transcription factor, was significantly increased in lungs of maternal LPS-exposed rat pups compared with saline-exposed (control) animals. No pulmonary STAT4 mRNA expression was detected in any of the groups, whereas message levels of ROR γ t, a T_H17 transcription factor, were not altered by prenatal LPS exposure (not shown).

Maternal LPS reduces T_H2 cytokine expression in lungs of OVA-sensitized rat pups. OVA sensitization and challenge (S+O group) significantly increased in mRNA expression of T_H2 transcription factors (STAT6 and GATA3) and cytokines (IL-4, IL-5, and IL-13) in lung of PN21 pups compared with S+A control group (Figs. 5 and 6). Rat pups that had been exposed to maternal LPS exhibited hardly any increase in pulmonary mRNA expression of T_H2 transcription factors and cytokines after OVA priming and challenge (L+O group vs. L+A and S+A groups). OVA sensitization and challenge did not change the pulmonary expression of T-bet and T_H1 cytokines (IL-2, IFN γ) at PN21 (Figs. 5 and 6). However, T-bet expression was increased in maternal LPS-exposed animals at PN21 independently of alum or OVA treatment (Fig. 6). No significant change was noted in pulmonary IL-2 expression following OVA exposure in LPS-exposed animals (Fig. 5). However, IFN γ mRNA levels were significantly increased after OVA sensitization and challenge (L+O). Alum treatment

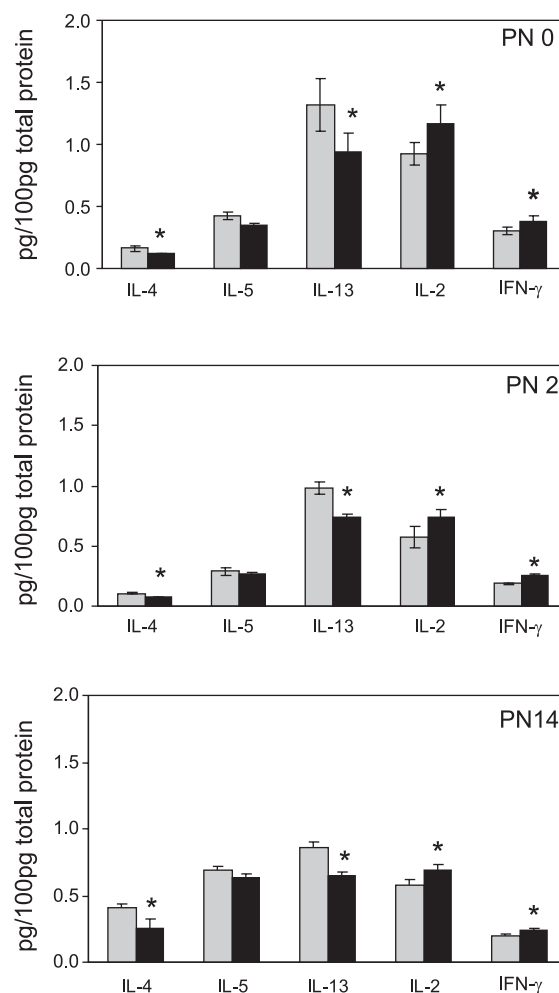


Fig. 2. Maternal endotoxin exposure decreases T_H2 and increases T_H1 cytokines in neonatal rat lungs. IL-4, IL-5, IL-13, IL-2, and IFN γ protein were quantified in tissue lysates by multiplex immunoassays and corrected for total protein. Data are means $\pm$ SD, $n = 6$ animals per control (gray bar) and LPS (black bar) group. * $P < 0.05$ vs. control (saline-treated) group.

(L+A) of maternal LPS-exposed pups did also increase IFN γ expression at PN21 (Fig. 5).

Inflammatory cells. The total number of inflammatory cells was significantly greater in BAL fluid of S+O and L+O groups vs. S+A and L+A groups (Table 1). The majority of inflammatory cells in BAL fluid of all four groups were lymphocytes and alveolar macrophages. Only a few polymorphonuclear cells (PMNs) were detected. BAL fluid of both S+O and L+O groups contained significantly more lymphocytes and eosinophils compared with their control (S+A and L+A, respectively) groups (Table 1). However, airway eosinophilia was significantly reduced in rat pups that had been exposed to maternal LPS compared with saline-exposed control animals (Table 1 and Supplemental Fig. E1A. Supplemental data for this article is available online at the AJP-Lung web site.). Histological staining of lung tissue confirmed these findings (Supplemental Fig. E1B). We also assessed the thickness of the bronchial wall and mucus in the bronchus. However, no obvious differences of thickness of the bronchial wall and mucus formation within the bronchus were observed among groups (not shown).

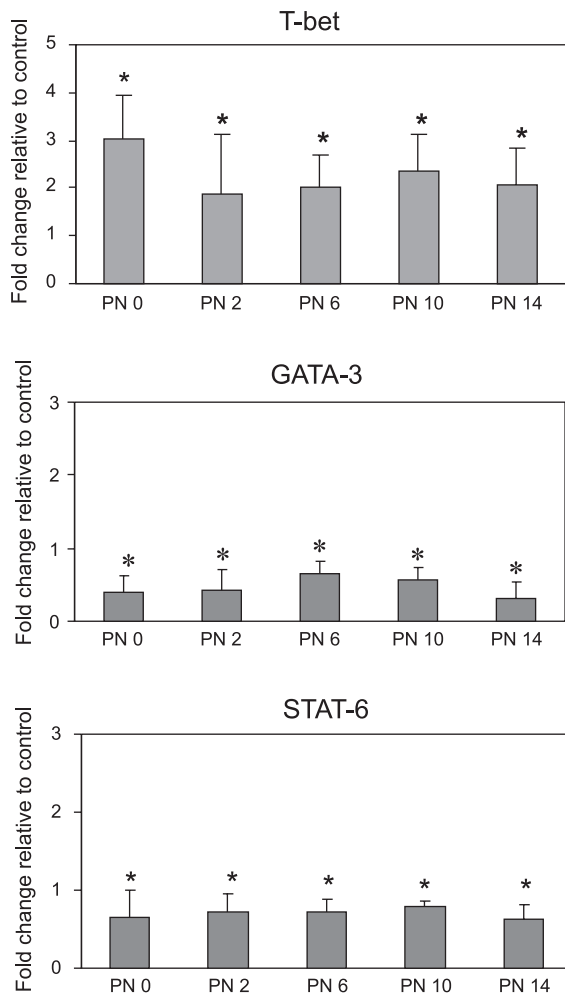


Fig. 3. Maternal endotoxin exposure increases T-bet and reduces GATA-3 and STAT6 mRNA expression in neonatal rat lungs. T-bet, GATA-3, and STAT6 mRNA expression was quantified by real-time PCR. Data are means $\pm$ SD, $n = 6$ animals per control (set at 1) and LPS (gray bar) group. * $P > 0.05$ vs. control (saline-treated) group.

Maternal LPS attenuates OVA-induced postnatal airway hyperresponsiveness. Independently of maternal treatment, no differences in Penh values at any dose of aerosolized MCh were found in nonchallenged PN21 rat pups (not shown). Alum priming alone (S+A and L+A groups) also did not affect Penh values (Fig. 7). In contrast, Penh values were significantly increased in response to MCh in OVA-sensitized rat pups from saline-injected mothers (S+O group). Maternal LPS exposure significantly reduced the Penh values in OVA-sensitized and -challenged rat pups (L+O group).

DISCUSSION

Epidemiological studies have suggested that prenatal farm exposure is protective against allergic airway diseases (15, 16). Experimental studies have shown that systemic maternal (4) and neonatal (51) LPS exposure before allergen (OVA) sensitization has strong immunomodulatory effects in mice, specifically with respect of reducing OVA-specific IgE, airway inflammation, and eosinophilia. However, preexposure to systemic LPS did not reduce airway hyperresponsiveness in these studies (4, 21, 51). Aerosolized LPS exposure also failed to

induce airway unresponsiveness to allergen sensitization in infant mice (20). A combination of prenatal initiated and sustained postnatal aerosolized LPS exposure of infant mice is required to suppress later OVA-induced airway inflammation and hyperresponsiveness (19). In contrast, we observed that systemic maternal exposure to LPS alone is sufficient to induce a T_H1 immune milieu in the offspring of rats and to reduce OVA-induced airway inflammation, eosinophilia, and airway hyperresponsiveness (AHR). Our findings support the concept that in utero exposure to bacterial endotoxins protects against allergic disease later in life.

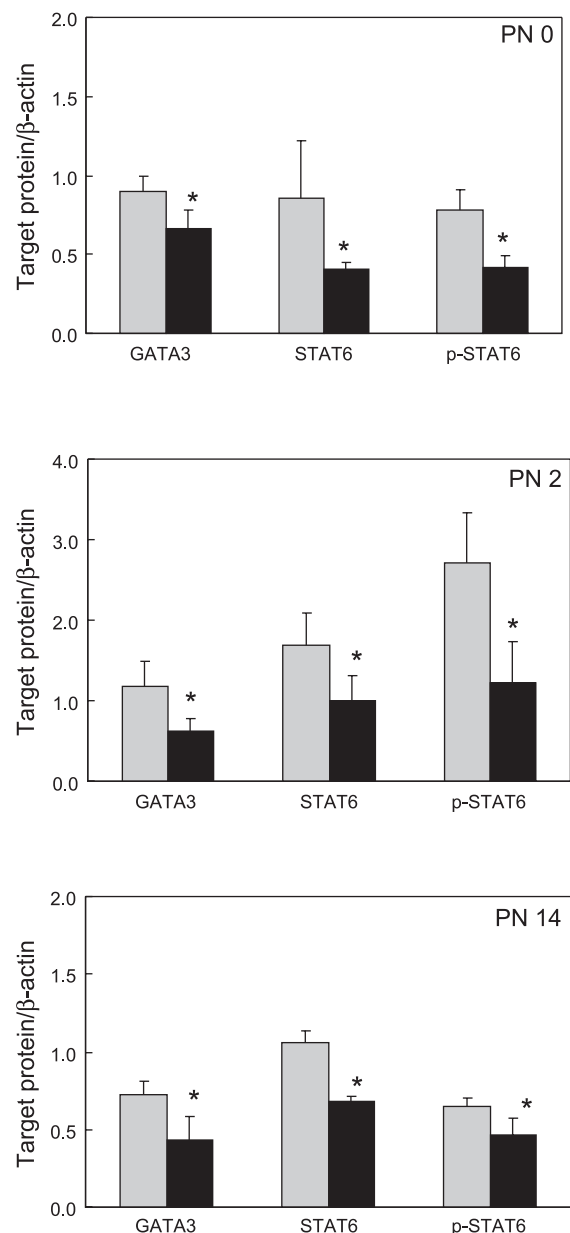


Fig. 4. GATA-3, STAT6, and p-STAT6 protein content is decreased in neonatal lungs of antenatal endotoxin-exposed animals. GATA-3, STAT6, and p-STAT6 were quantified in tissue lysates by Western blotting followed by densitometric analysis. β -actin immunoblotting was used to correct for protein loading differences. Data are means $\pm$ SD, $n = 6$ animals per control (gray bar) and LPS (black bar) group. * $P > 0.05$ vs. control (saline-treated) group.

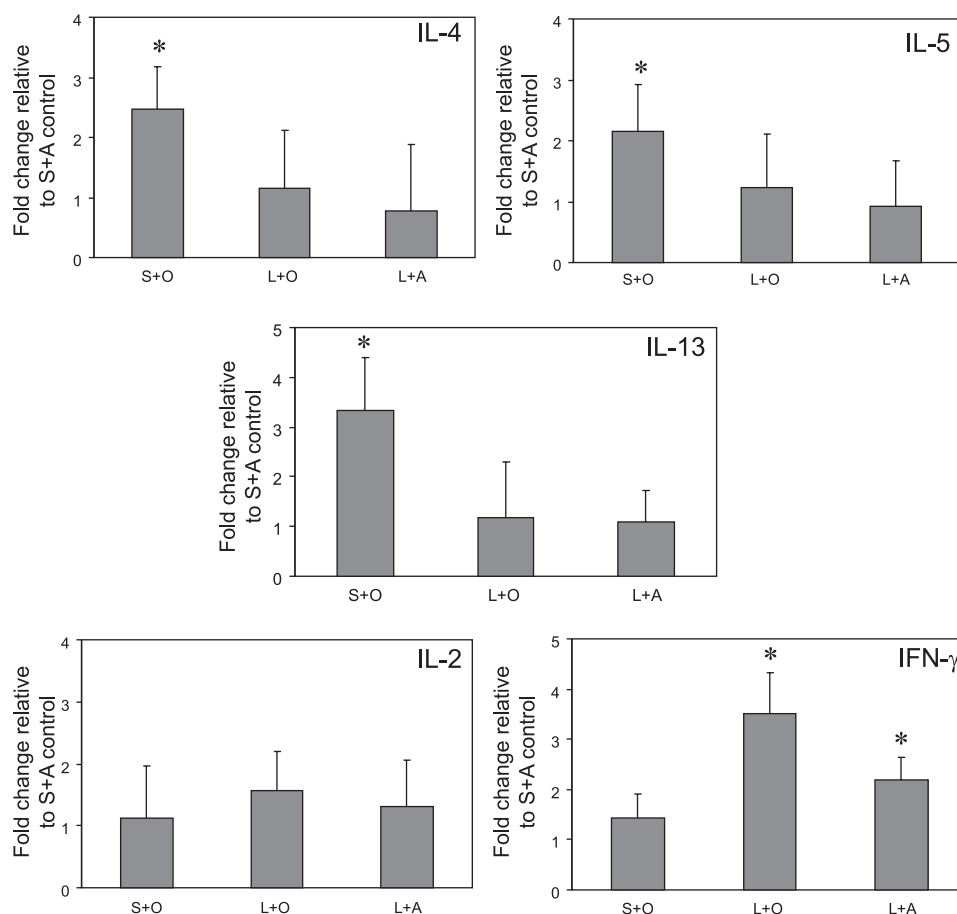


Fig. 5. Maternal endotoxin exposure attenuates increases in T_H2 cytokine mRNA expression in OVA-sensitized and -challenged neonatal rat lungs. IL-4, IL-5, IL-13, IL-2, and IFN γ mRNA expression was quantified by real-time PCR. Data are means $\pm$ SD, $n = 6$ animals per control (S+A, set at 1), S+O, L+O, and L+A group. * $P < 0.05$ vs. control (S+A) group. S+A, prenatal saline + alum sensitization and aerosolized saline challenge; S+O, prenatal saline + OVA sensitization and aerosolized OVA challenge; L+A, prenatal LPS + alum sensitization and aerosolized saline challenge; L+O, prenatal LPS + OVA sensitization and aerosolized OVA challenge.

Recently, we showed that maternal LPS administration increased the levels of IL-6, CXCL2, IL-1 β , and TNF α in the placenta and fetal placental membranes at term (8). In addition, we showed that maternal LPS exposure caused a mild, but transient, pulmonary inflammation after birth and a temporary delay in alveolar development (8). Here we show that maternal LPS exposure alone did not alter the baseline Penh values in offspring compared with saline-exposed control pups, implying that AHR was not affected by the postnatal proinflammatory milieu triggered by LPS. We observed that prenatal exposure to endotoxin induced a predominant T_H1 cytokine milieu, in agreement with previous studies with mice (19). T cell responses are typically primed in utero and subsequently reshaped during postnatal allergen exposure via immune deviation, leading to the eventual emergence of stable allergen-specific T cell memory. In general, the fetal compartment is characterized by a predominant T_H2 cytokine milieu (5, 41). The T_H2 immune responses are preserved during the neonatal period and are perpetuated in those who develop allergic airways disease (1, 22, 23). Our study shows that maternal LPS suppressed T_H2 development during prenatal and early postnatal life, but the exact mechanism through which maternal LPS modulates T cell immunity is not fully understood. Prenatal LPS did not change IL-10 expression in rat pups (8), indicating that IL-10, a well-known suppressor of T cell-mediated immune responses, is not involved. It is thought that LPS activates Toll-like receptors (TLRs) such as TLR4 present on dendritic cells and macrophages, which results in NF- κ B

translocation and induction of IL-12, thereby stimulating T_H1 cytokine production (37). However, we did not observe any change in pulmonary IL-12 expression upon prenatal LPS administration. It is also possible that maternal LPS stimulates the hypothalamic-pituitary-adrenal axis and elevates cortisol levels in the fetus, which may affect T_H cell differentiation (50). However, maternal stress has mainly been associated with an increased risk of allergic T_H2 -mediated disease (11).

We found that maternal LPS exposure reduced pulmonary STAT6 and GATA3 mRNA expression as well as STAT6 phosphorylation in the offspring later in life. Both transcription factors have been shown to be critical for differentiation of naïve T_H cells into T_H2 cells (36). Their importance in asthma has been derived primarily from genetically engineered mice. Deficiency in STAT6 and GATA3 protein or activity has been shown to prevent T_H2 cell development, eosinophilia, and hyperresponsiveness in allergen-sensitized and challenged mice (54). GATA3 expression is found to be increased in human asthmatic airways, and this increase correlates with T_H2 cytokine expression and AHR (32). In contrast to our rat findings, prenatal plus postnatal LPS exposure did not change GATA3 mRNA expression in allergen-sensitized and challenged infant mice (19). Prenatal plus postnatal LPS exposure did, however, stimulate pulmonary T-bet mRNA expression in infant mice (19). Similarly, T-bet gene expression was increased in the offspring later in life in our rat model of systemic maternal infection. T-bet is a key regulator of T_H1 development and function. Mice that lack T-bet fail to develop T_H1

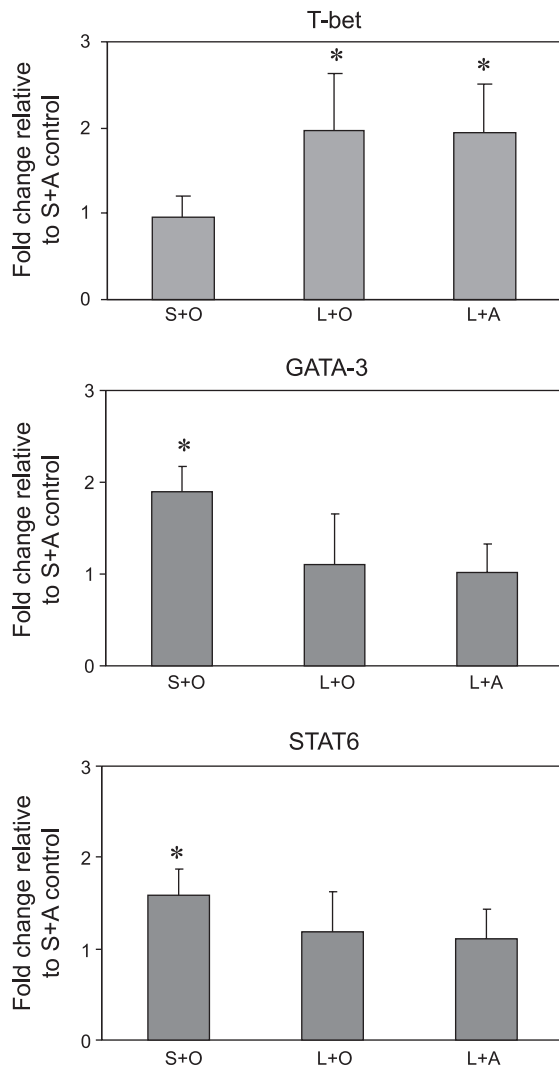


Fig. 6. Maternal endotoxin exposure abolishes increases in GATA-3 and STAT6 mRNA expression in OVA-sensitized and -challenged neonatal rat lungs. T-bet and GATA-3 and STAT6 mRNA expression was quantified by real-time PCR. Data are means $\pm$ SD, $n = 6$ animals per control (S+A, set at 1), S+O, L+O, and L+A group. * $P < 0.05$ vs. control (S+A) group. S+A, prenatal saline + alum sensitization and aerosolized saline challenge; S+O, prenatal saline + OVA sensitization and aerosolized OVA challenge; L+A, prenatal LPS + alum sensitization and aerosolized saline challenge; L+O, prenatal LPS + OVA sensitization and aerosolized OVA challenge.

cells and exhibit airway eosinophilia, AHR, and airway remodeling in the absence of allergen sensitization and inhaled challenge (18). In addition, fewer activated T helper cells expressing T-bet are found in airways of human asthmatic patients compared with control subjects (18). It is generally

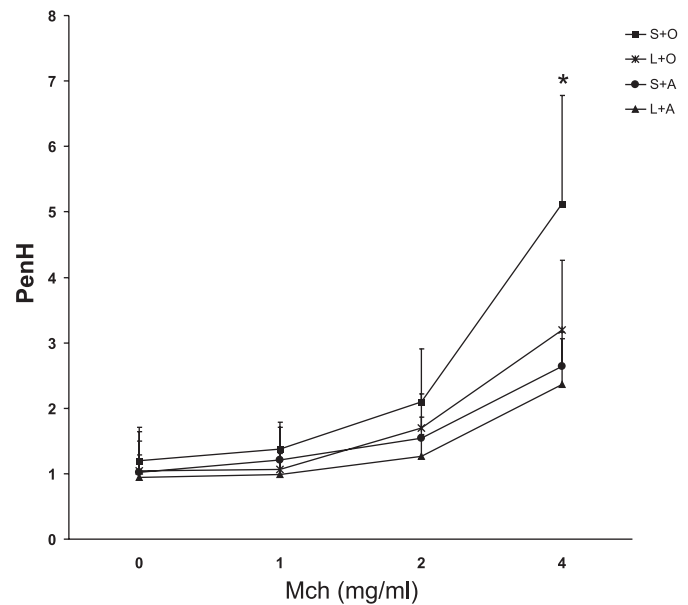


Fig. 7. Maternal endotoxin exposure reduces airway hyperresponsiveness in OVA-sensitized and -challenged neonatal rats. Penh was assessed at baseline and after increasing doses of aerosolized MCh using whole body barometric plethysmography. Data are means $\pm$ SD, $n = 8$ animals in each group. S+A, prenatal saline + alum sensitization and aerosolized saline challenge; S+O, prenatal saline + OVA sensitization and aerosolized OVA challenge; L+A, prenatal LPS + alum sensitization and aerosolized saline challenge; L+O, prenatal LPS + OVA sensitization and aerosolized OVA challenge.

thought T_H1 cell development is initiated by STAT1, which induces T-bet (40). Subsequent expression of IL-12 receptor allows for further T_H1 differentiation by IL-12, which activates STAT4 and upregulates the expression of T-bet (40). As mentioned earlier, we found no evidence that systemic maternal LPS altered pulmonary IL-12 signaling (no changes IL-12 and IL-12R expression) in the offspring. Moreover, we were not able to detect any STAT4 expression. Our earliest time point of measurement was at birth, and it is possible that the naïve T_H cell response of the fetus to systemic maternal LPS occurred primarily during the first 24–48 h after LPS administration. In addition, parenchymal cells of the lung may not contribute to T_H cell differentiation, and we should have analyzed prenatal LPS effects on fetal spleen T cells. However, a recent report showed that the development of allergic pulmonary inflammation requires the activation of STAT6 not only in T_H cells, but also in the parenchymal cells of the lung (55). Our observation of opposite T-bet (increased) and GATA-3 (decreased) expression upon prenatal LPS exposure supports the idea that T-bet may negatively regulate GATA-3 expression (49). Although IL-6 is upregulated by prenatal LPS,

Table 1. *Inflammatory cells in bronchoalveolar lavage fluid*

Treatment	Total Cell (10^6 cells/ml)	%Macrophages	%Lymphocytes	%Eosinophils	%PMNs
S+O	$0.11 \pm 0.02^*$	57.73	29.81*	$12.11^{*\dagger}$	0.35
L+O	$0.12 \pm 0.03^*$	63.25	30.95*	5.42*	0.38
S+A	0.08 ± 0.01	89.82	10.64	0.31	0.23
L+A	0.09 ± 0.02	88	11.46	0.23	0.31

Means $\pm$ SD, $n = 6$ pups/group. * $P < 0.05$ vs. S+A and L+A, $\dagger P < 0.05$ vs. L+O. PMNs, polymorphonuclear granulocytes; S+A, prenatal saline + alum sensitization and aerosolized saline challenge; S+O, prenatal saline + OVA sensitization and aerosolized OVA challenge; L+A, prenatal LPS + alum sensitization and aerosolized saline challenge; L+O, prenatal LPS + OVA sensitization and aerosolized OVA challenge.

no change in pulmonary ROR γ t expression was noted (52). Together with the observation of absence of IL-17F and IL-21 cytokine expression, prenatal LPS exposure does not trigger TH17 cell differentiation in the offspring.

AHR is a common feature of allergic asthma in human and rodent models. AHR is a result of a combination of increased contractility of the airway smooth muscle due to increased muscle mass and/or alterations in muscle function, increased airway wall thickness, reduced airway caliber, and increased mucus secretion in airways (10). However, AHR in our neonatal rats after sensitization and airway challenge with OVA was not associated with an increase in bronchial wall thickness. A recent study demonstrated that neonatal BCG vaccination had a long-term effect on inhibiting allergen-induced AHR in mice (46). Similarly, we observed that a systemic maternal (LPS) infection provided long-term protection against allergen-induced AHR in postnatal rats. Prenatal LPS had no influence on the expression of Foxp3 (not shown), a key regulatory gene for the development of regulatory T cells (24), making mucosal tolerance unlikely. Compared with adult rodent models (24, 27), we needed higher dosages of OVA for sensitization and challenge to induce AHR in neonatal rats. Also, the thickness of the bronchial wall, the eosinophilia, as well as mucus secretion in the airways after OVA sensitization and challenge, was not as profound as seen in adult mice and rat. Why neonatal rat pups are more resistant to experimental allergic airway disease remains to be investigated.

ACKNOWLEDGMENTS

M. Post is the holder of a Canadian Research Chair in Fetal, Neonatal, and Maternal Health.

GRANTS

This work was supported by an operating grant (MOP-86472) from the Canadian Institutes of Health Research and infrastructure grants (CCURE, CSCCD) from the Canadian Foundation for Innovation.

DISCLOSURES

No conflicts of interest are declared by the author(s).

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