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ENHANCED PULMONARY INFLAMMATORY RESPONSE TO INHALED ENDOTOXIN IN PREGNANT RATS

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Evidence suggests that pregnant animals are more sensitive than nonpregnant animals to the systemic administration of endotoxin. Studies were undertaken to assess whether an enhanced sensitivity of the pulmonary system to aerosolized endotoxin might exist during pregnancy. Pregnant Sprague-Dawley female rats (17 d of gestation) or age-matched virgin female rats were exposed to air or endotoxin (lipopolysaccharide) by inhalation for 3 h. At 18 h following exposure to endotoxin, lactate dehydrogenase activity levels in bronchoalveolar lavage (BAL) fluid samples from pregnant rats were 1.5-fold greater than those from endotoxin-exposed virgin rats. BAL polymorphonuclear leukocyte (PMN) numbers were also approximately twofold greater in pregnant rats than in virgins following the inhalation of endotoxin. The increases in BAL PMNs in pregnant rats following endotoxin exposure were observed just following exposure to endotoxin as well as at 18 h following exposure. These results indicate that an increased pulmonary inflammatory response to inhaled endotoxin occurs during pregnancy in rats. Additional findings suggest that these pregnancy-linked pulmonary responses to endotoxin cannot be explained by the following potential mechanisms: changes in the inhaled dose of endotoxin, or alterations in the responsiveness of alveolar macrophages to endotoxin. To our knowledge this is the first study that has evaluated pulmonary responses to inhaled endotoxin during pregnancy. Our finding that pregnancy is associated with an increased lung inflammatory response to aerosolized endotoxin raises the possibility that there may be a generalized enhancement of pulmonary responses to inhaled toxic agents during pregnancy.

Bacterial endotoxins (lipopolysaccharide, LPS) are derived from the cell walls of gram-negative bacteria, and exposure to endotoxin can produce sepsis, endotoxic shock, and multiorgan system failure (Welbourn & Young, 1992). It has been recognized for a number of years that pregnant animals are more sensitive than nonpregnant animals to systemic endotoxin infections. For instance, a generalized Shwartzman reaction, resulting in renal lesions and necrosis, can

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be elicited in pregnant rats or rabbits by a single injection of endotoxin, whereas nonpregnant animals require several doses of endotoxin for this response to occur (Apitz, 1935; McKay et al., 1960; Wong, 1962). Furthermore, pregnant rats show an enhanced response to a low-dose infusion of endotoxin, which is characterized by increases in systemic blood pressure, proteinuria, an activation of leukocytes, and the development of a condition that resembles clinical preeclampsia (Faas et al., 1994, 2000). This syndrome appears to be specific for pregnancy, since an identical endotoxin infusion into nonpregnant, cyclic rats is not associated with any of the effects described above (Faas et al., 1994, 2000).

While animals and humans may be exposed systemically to endotoxin, the inhalation of endotoxin may be a more common route of exposure. For instance, high levels of endotoxin can be found in airborne dusts in textile mills (Castellan et al., 1984; Rylander & Vesterlund, 1982). The potential for exposure to aerosolized endotoxin also exists in agricultural settings, such as grain handling and animal husbandry operations, as well as in industrial settings, such as metal-working and wood-processing operations (DeLorme et al., 2003; Iversen et al., 2000; Olenchock et al., 1987; Rylander, 2002). Endotoxin can also be found in domestic environments and may be present in house dust and/or contaminated humidifier water (Michel, 2001; Rylander, 2002). When endotoxin is inhaled, the lung is the main target organ, and a number of studies have characterized pulmonary responses to aerosolized endotoxin in both humans and animals. Foremost among these responses is the development of pulmonary inflammation. This is characterized by an activation of alveolar macrophages (AMs), the recruitment of polymorphonuclear leukocytes (PMNs) into alveolar spaces, lung cell damage, and an increase in the permeability of the alveolar–capillary barrier (Brigham & Meyrick, 1986; Thorn, 2001).

Studies concerning the effects of aerosolized endotoxin on pulmonary responses have almost exclusively been conducted on male and nonpregnant female humans and mammals. The results of these studies suggest that individual factors can be a significant determinant of pathophysiological responses to endotoxin. For example, atopic asthmatics exhibit augmented airway responses to endotoxin (Eldridge & Peden, 2000). Gender and the state of lactation also appear be factors affecting responses to aerosolized endotoxin. For instance, it has been reported that females are more sensitive than males to inhaled endotoxin (Kline et al., 1999). In addition, pulmonary responses to inhaled endotoxin are increased in lactating rats compared to those in virgin female rats (Gordon et al., 1993).

To the best of our knowledge, no study has assessed whether the physiologic state of pregnancy might be associated with an increased sensitivity of the pulmonary system to endotoxin. Therefore, the objective of this investigation was to determine whether pregnant rats are more susceptible than virgin rats to lung injury and pulmonary inflammation resulting from the inhalation of endotoxin. Specifically, studies were undertaken to assess how pregnancy affects lung cell damage, the permeability of the alveolar–capillary barrier, and

the influx of PMNs into alveolar regions following exposure to aerosolized endotoxin. In addition, we determined whether any effects of pregnancy on pulmonary responses to endotoxin could be explained by the following potential mechanisms: (1) changes in the inhaled dose of endotoxin, (2) differences in circulating corticosterone levels, or (3) alterations in the responsiveness of AMs to endotoxin.

METHODS

Animals

Timed-pregnant and age-matched virgin female Sprague-Dawley [Hla:(SD) CVF] rats were obtained from Hilltop Lab Animals (Scottdale, PA). The animals were housed in a AAALAC-accreditied, specific-pathogen-free facility. The rats were monitored to be free of endogenous viral pathogens, parasites, mycoplasms, *Helicobacter*, and CAR *Bacillus*. The rats were acclimated for at least 1 wk before use and were housed in ventilated cages that were provided with HEPA-filtered air. Alpha-Dri virgin cellulose chips and hardwood Beta-chips were used as bedding. The rats were provided Prolab RMH Rodent Diet and tap water ad libitum and housed in laminar-flow hoods under controlled light (12 h light, 12 h darkness) and temperature (22–24°C) conditions. Inhalation exposures (described later) for pregnant rats were conducted on d 17 of gestation (d 1 = day of vaginal plug). Age-matched virgin controls were also exposed on the same day.

Inhalation Exposures

A whole-body inhalation exposure system was used to expose the rats to endotoxin (lipopolysaccharide [LPS] from *Escherichia coli* serotype 055:B5, Difco Laboratories, Inc., Detroit, MI) as described previously (Huffman et al., 2000). In order to estimate actual endotoxin exposure levels, gravimetric samples were collected from the exposure chamber by drawing the aerosol through a filter (Gelman 37-mm PVC filter, Ann Arbor, MI) at a rate of 1 L/min. The filters were extracted for 6 h with pyrogen-free water, and a modified *Limulus* amebocyte lysate (LAL) assay (BioWhittaker, Walkersville, MD) was used to determine the total assayable endotoxin on the filter samples ($12.6 \pm 2.1 \times 10^4$ EU/m^3). Control rats were exposed to HEPA-filtered air in a whole-body inhalation exposure system that was similar to that used for endotoxin exposures. The inhalation exposures lasted 3 h. The animals were studied immediately (0 h postexposure) or 18 h following the end of the exposure (18 h postexposure). The 18-h postexposure time point was chosen based on previous work showing that endotoxin inhalation results in significant pulmonary inflammatory responses in rats at 18 h following the exposure (Huffman et al., 1997).

Ventilation Measurements

Breathing rate, tidal volume, and minute ventilation measurements were made just prior to the inhalation exposures and at the end of the exposure period as previously described (Huffman et al., 2001).

Collection of Blood and Bronchoalveolar Lavage Fluid and Cell Samples

The rats were first anesthetized with sodium pentobarbital (>100 mg/kg, ip; Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA). Blood was collected from the abdominal vein into a syringe and placed in a glass tube without anticoagulant. The left renal artery was then cut. A tracheal cannula was inserted and an initial bronchoalveolar lavage (BAL) was performed with 6 ml of cold Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS; 145 m*M* NaCl, 5 m*M* KCl, 9.4 m*M* Na_2HPO_4, 1.9 m*M* NaH_2PO_4, and 5.5 m*M* dextrose, pH 7.4). This lavage solution was introduced into and withdrawn from the lungs three times. The total return of the initial lavage averaged 4 ml per rat. Subsequent BALs were performed with 8 ml PBS each until a total volume of 80 ml of lavage fluid was collected. The initial and subsequent lavage samples were then centrifuged (500×*g*, 5 min, 4°C). The supernatants from the initial lavage were processed for analyses of lactate dehydrogenase (LDH) activity levels and albumin levels. The cell pellets from the initial and subsequent lavages were then combined and resuspended in 5 ml PBS. The samples were centrifuged to pellet the cells and the supernatants were aspirated to waste. This wash procedure was performed three times. Following the final wash, the cells were resuspended in 1 ml PBS.

Determination of BAL Cell Counts, Cell Profiles, and Cell Viabilities

BAL cell counts were determined using an electronic cell counter equipped with a cell-sizing attachment (Coulter Multisizer II, Coulter Electronics, Hialeah, FL). Portions of the harvested cells were then deposited on slides using a cytocentrifuge (Shandon Scientific, London) and stained with Wright stain (Volu-Sol, Henderson, NV). The percentages of alveolar macrophages and leukocytes present on the slides were determined using light microscopy. Greater than 99% of these cells were alveolar macrophages or PMNs. Viability of the cell population for each rat was assessed using a trypan blue exclusion test. The percentages of viable cells were then determined by placing aliquots of the treated cells in a hemocytometer and scoring 100 cells for the absence (viable cells) or presence (dead cells) of blue staining. The percentages of viable cells from pregnant and virgin rats exposed to air (group mean ranges from 89 to 92%) were similar to those of pregnant and virgin rats exposed to endotoxin (group mean ranges from 90 to 95%).

Analysis of Circulating Corticosterone Levels

Blood samples collected from the rats were centrifuged (1010×*g*, 10 min, 4°C). The serum was separated from the blood and stored at −20°C prior to analysis. Serum corticosterone levels were measured using a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). The results are expressed as nanograms per milliliter.

Analyses of LDH Activity Levels and Albumin Levels in BAL Fluid Samples

LDH activity levels in supernatants from initial BAL fluid samples were determined by measuring the oxidation of lactate to pyruvate coupled with the reduction of NAD at 340 nm (Roche Diagnostic Systems, Indianopolis, IN). The results are expressed as units per liter. Albumin levels in supernatants from initial BAL fluid samples were determined colorimetrically at 628 nm based on albumin binding to bromcresol green using an albumin BCG diagnostic kit (Sigma Diagnostic Procedure 631; Sigma Chemical Co., St. Louis, MO). The results are expressed as milligrams per milliliter. Measurements of LDH activity levels and albumin levels were performed using a COBAS MIRA auto-analyzer (Roche Diagnostic Systems).

Determination of the Effect of Endotoxin on the Release of Reactive Oxygen and Nitrogen Species and Cytokines by AMs from Pregnant and Virgin Rats

BAL cells were harvested from pregnant rats (17 d of gestation) or age-matched virgin rats. These cell populations consisted of 96 to 100% AMs with an average viability of 88%. BAL cells were suspended in culture medium (minimum essential medium, BioWhittaker, Walkersville, MD) supplemented with penicillin (100 U/ml; Invitrogen, Carlsbad, CA), streptomycin (100 U/ml; Invitrogen), and kanamycin (100 μg/ml; Invitrogen). The culture medium also contained 10% steroid-free fetal bovine serum (Gibco BRL, Grand Island, NY). The steroid-free fetal bovine serum was prepared using a dextran–charcoal procedure (Soto et al., 1995), which was modified slightly to include 2 U sulfatase/ml of fetal bovine serum in the procedure. The cells were then placed into wells of 24-well tissue culture plates (Costar Corp., Cambridge, MA). Each well contained 0.5×10^6 viable cells per 1 ml culture medium. The plates were then incubated at 37 °C in a humidified atmosphere (relative humidity 90%) of 95% air–5% CO_2. After the incubation period, the tissue culture plates were centrifuged ($500 \times g$, 10 min, 4 °C).

In one set of experiments, cells were incubated with or without endotoxin (0.1 μg LPS/ml). Cell-free culture supernatants were then harvested after 2, 4, 6, 12, or 24 h of incubation. In another set of experiments, cells were incubated with or without endotoxin at concentrations ranging from 0.01 to 1 μg LPS/ml. Cell-free culture supernatants were then harvested after 24 h of incubation. The culture supernatants were stored at −80 °C prior to analysis of tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2 (MIP-2), or nitric oxide (NO) levels. TNF-α and MIP-2 levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Inc.; Camarillo, CA). Results are expressed as nanograms per 0.5×10^6 AMs. The production of NO was assessed by measuring media nitrate and nitrite levels in the supernatant samples. Nitrate and nitrite were determined as previously described (Porter et al., 2002). Briefly, nitrate in the media samples was reduced

to nitrite, and then nitrite was determined by flow injection analysis colorimetry at 540 nm using the Griess reaction (Quick-Chem 8000, Lachat Instruments, Milwaukee, WI). The results are expressed as nanomoles per 0.5×10^6 AMs.

In the experiment where cells were incubated with or without endotoxin at concentrations ranging from 0.01 to 1 μg LPS/ml, superoxide anion release from alveolar macrophages was also measured. After the removal of the cell-free culture medium supernatant and prior to the determination of superoxide anion release, the cells were washed two times with HEPES-buffered medium (145 m*M* NaCl, 5 m*M* KCl, 10 m*M* HEPES, 1 m*M* $CaCl_2$, and 5.5 m*M* dextrose, pH 7.4). The method used to measure superoxide anion release was a modification of the one described by Johnston (1984). Briefly, the cultured AMs were preincubated for 10 min at 37 °C in HEPES-buffered medium containing 0.12 m*M* cytochrome *c* (Type VI; Sigma). Then either HEPES-buffered medium or unopsonized zymosan (2 mg/ml) was added to the wells. The samples were then incubated for 30 min at 37 °C. Following the incubation period, the tissue culture plates were centrifuged and the optical densities of the supernatants were measured at 550 nm with a spectrophotometer. Superoxide dismutase (SOD; 0.4 mg/ml) was added to some samples. The difference in the amount of cytochrome *c* reduced in the absence and presence of SOD was used to estimate superoxide anion release. The results are expressed as nmoles superoxide anion released per 0.5×10^6 AMs per 30 min by using an extinction coefficient of 21 $mM^{-1}\,cm^{-1}$ (Massey, 1959).

Statistical Analyses

Using SAS (SAS Institute, Inc., Cary, NC), two-way analyses of variance were conducted on response variables measured 18 h after the inhalation exposure to either air or endotoxin or immediately after the inhalation exposure to either air or endotoxin (Tables 1–4 and 6; Figures 1–3). A one-way analysis of variance was used to determine if respiratory measurements differed between virgin and pregnant rats before inhalation exposures (Table 5).In addition, several two-way analyses of covariance were conducted to determine if the factors of pregnancy and endotoxin inhalation would result in a significant difference in postinhalation respiratory measurement variables. In these cases,

TABLE 1. BAL Fluid Levels of Serum Albumin in Virgin or Pregnant Rats at 18 h Postexposure to Air or Endotoxin

	Virgin		Pregnant	
	Air	Endotoxin	Air	Endotoxin
Serum albumin (mg/ml)	0.24 ± 0.01	0.38 ± 0.05^a	0.32 ± 0.03^b	$0.47 \pm 0.05^{a,b}$

Note. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation as described under Methods. BAL fluid samples were obtained 18 h after the inhalation exposures. Serum albumin levels in the initial acellular bronchoalveolar lavage fluid samples were analyzed using commercially available reagents. Values are the means ± SE ($n = 6$ rats/group).

[a] Significant at ($p \leq .05$, main effect of endotoxin vs. air.

[b] Significant at $p \leq .05$, main effect of pregnant vs. virgin.

TABLE 2. Numbers of Total Cells and AMs Harvested by BAL from Virgin or Pregnant Rats at 18 h Postexposure to Air or Endotoxin

	Virgin		Pregnant	
	Air	Endotoxin	Air	Endotoxin
Total cells (10^{-6})	7.2 ± 0.5	19.5 ± 2.0^{a}	9.4 ± 0.3	$28.9 \pm 1.5^{a,b}$
Ams (10^{-6})	7.2 ± 0.5	11.6 ± 0.6^{a}	9.3 ± 0.4	12.1 ± 1.5^{a}

Note. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation as described under Methods. Bronchoalveolar cells were harvested 18 h after the inhalation exposures. BAL cell counts were determined using an electronic cell counter. The number of AMs were then determined following differential analysis of BAL cell populations. Values are the means ± SE ($n = 6$ rats/group).

[a] Significant at $p \leq .05$, main effect of endotoxin vs. air.

[b] Significant at $p \leq .05$, interaction pregnant/endotoxin vs. virgin/endotoxin.

TABLE 3. BAL Fluid LDH Activity Levels and Serum Albumin Levels in Virgin or Pregnant Rats a 0 h Postexposure to Air or Endotoxin

	Virgin		Pregnant	
	Air	Endotoxin	Air	Endotoxin
LDH (U/L)	70 ± 8	75 ± 9	73 ± 7	82 ± 8
Serum albumin (mg/ml)	0.25 ± 0.06	0.22 ± 0.01	0.36 ± 0.07^{a}	0.45 ± 0.07^{a}

Note. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$EU/m^3) by inhalation for 3 h as described under Methods. BAL fluid samples were obtained immediately after the inhalation exposures. LDH activity levels and serum albumin levels in the initial acellular BAL fluid samples were analyzed using commercially available reagents. Values are the means ± SE ($n = 6$ rats/group).

[a] Significant, $p \leq .05$ main effect of pregnant vs. virgin.

TABLE 4. Numbers of Total Cells and AMs Harvested by BAL from Virgin or Pregnant Rats at 0 h Postexposure to Air or Endotoxin

	Virgin		Pregnant	
	Air	Endotoxin	Air	Endotoxin
Total Cells (10^{-6})	9.4 ± 1.2	19.4 ± 1.3^{a}	15.3 ± 2.5^{b}	$35.6 \pm 5.3^{a,b}$
AMs (10^{-6})	9.4 ± 1.2	9.4 ± 1.1	14.8 ± 2.4^{b}	12.3 ± 1.2^{b}

Note. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation for 3 h as described under Methods. Bronchoalveolar cells were harvested immediately after the inhalation exposures. BAL cell counts were determined using an electronic cell counter. The number of AMs were then determined following differential analysis of BAL cell populations. Values are the means ± SE ($n = 6$ rats/group).

[a] Significant, $p \leq .05$ main effect of endotoxin vs. air.

[b] Significant, $p \leq .05$ main effect of pregnant vs. virgin.

TABLE 5. Breathing Rate, Tidal Volume, and Minute Ventilation in Virgin and Pregnant Rats

Measurement	Virgin	Pregnant
Breathing rate (breaths/min)	166 ± 10	152 ± 11
Tidal volume (ml/breath)	1.98 ± 0.17	2.26 ± 0.18
Minute ventilation (ml/min)	327 ± 30	344 ± 36

Note. Respiratory measurements were made just before exposure of pregnant rats (17 d of gestation) or age-matched virgin rats to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation as described under Methods. Values are the means ± SE ($n = 12$ rats/group).

TABLE 6. Serum Corticosterone Levels in Virgin or Pregnant Rats at 0 h or 18 h Post-exposure to Air or Endotoxin

	Virgin		Pregnant	
Serum corticosterone (ng/ml)	Air	Endotoxin	Air	Endotoxin
0 h postexposure	227 ± 65	253 ± 88	319 ± 54	235 ± 59
18 h postexposure	262 ± 47	153 ± 42	240 ± 83	237 ± 64

Note. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation as described under Methods. Blood samples were collected immediately after the exposure as well as 18 h after the inhalation exposures from separate groups of rats. The serum was separated for analysis of corticosterone levels using radioimmunoassay. Values are the means ± SE ($n = 6$ rats/group).

post breathing rate was investigated with pre breathing rate as the covariate, post tidal volume was investigated with pre tidal volume as the covariate, and post minute ventilation was investigated with pre minute ventilation as the covariate. Using the mixed model procedure of SAS (SAS Institute, Inc., 1999), several split-plot analyses of variance were also conducted. The main plot factor was pregnancy status with the subplot factors being time and endotoxin for Figure 4A and the subplot factor being dose of endotoxin for Figure 4B. In addition, a split-split plot analysis of variance was conducted to determine if the factors of pregnancy, endotoxin, and zymosan stimulation would result in a significant difference in superoxide anion release (Table 7). In this case, the main plot factor was pregnancy status with the subplot factor being dose of endotoxin and the split subplot factor being zymosan stimulation. The significance level was set at $p \leq .05$.

RESULTS

Lung Cell Damage, Permeability of the Alveolar–Capillary Barrier, and BAL Cell Numbers in Virgin and Pregnant Rats at 18 h Following Inhalation Exposure to Endotoxin

The effect of the physiologic state of pregnancy on LDH activities in BAL fluid samples at 18 h following exposure to air or aerosolized endotoxin is

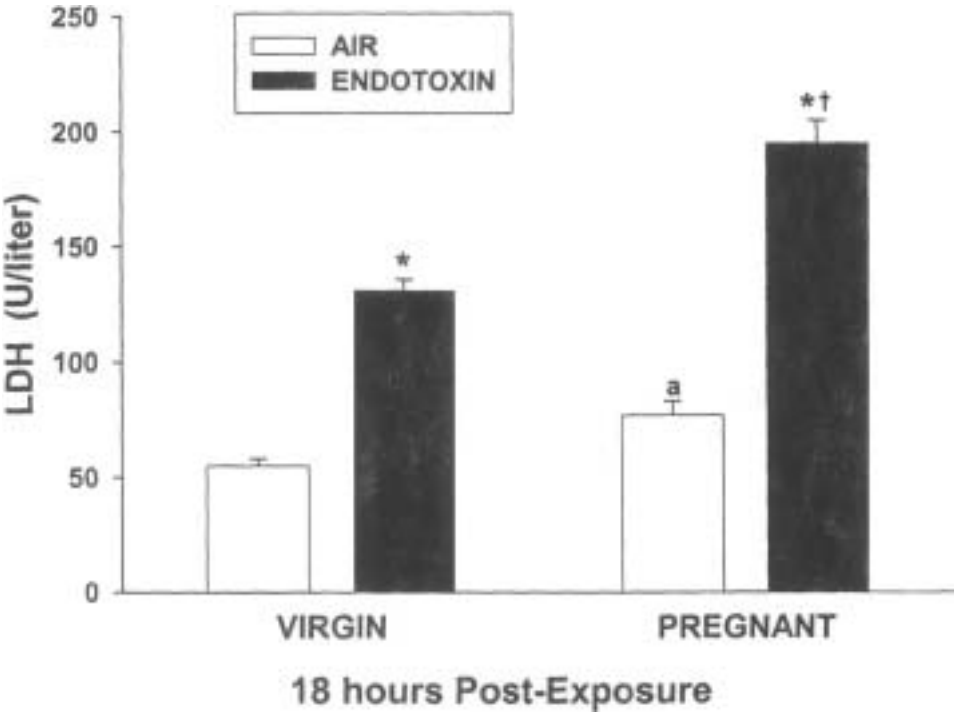

FIGURE 1. BAL fluid LDH activities in virgin or pregnant rats 18 h postexposure to air or endotoxin. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation for 3 h as described under Methods. BAL fluid samples were obtained 18 h after the inhalation exposures. LDH activity levels in the initial acellular BAL fluid samples were analyzed using commercially available reagents. Values are the means ± SE ($n = 6$ rats/group). Asterisk indicates significant at $p \leq .05$, main effect of endotoxin vs. air; a, significant at $p \leq .05$, pregnant/air vs. virgin/air; †, significant, $p \leq .05$, interaction pregnant/endotoxin vs. virgin/endotoxin.

shown in Figure 1. LDH is an intracellular enzyme and its presence in BAL fluid is an indicator of lung cell damage. In air-exposed rats, a significant increase in LDH activity levels in BAL fluid was observed in pregnant animals compared to virgin controls. Following endotoxin exposure, LDH activity levels in BAL fluid samples were increased in both virgin and pregnant rats. In the case of endotoxin-exposed virgins, LDH activity levels were 2.4-fold higher than air-exposed virgins. In pregnant rats, pulmonary damage following endotoxin inhalation was greater than that seen in virgins. In these animals, LDH activity levels in BAL fluid samples were increased 1.5-fold over levels seen in virgin rats exposed to endotoxin.

Serum albumin levels in BAL fluid samples from these animals are presented in Table 1. Albumin is normally confined to the intravascular space. However, serum albumin levels in BAL samples increase when the alveolar–capillary barrier is disrupted. The inhalation of endotoxin resulted in increased BAL serum albumin levels in both virgin and pregnant rats compared to levels in air-exposed animals.

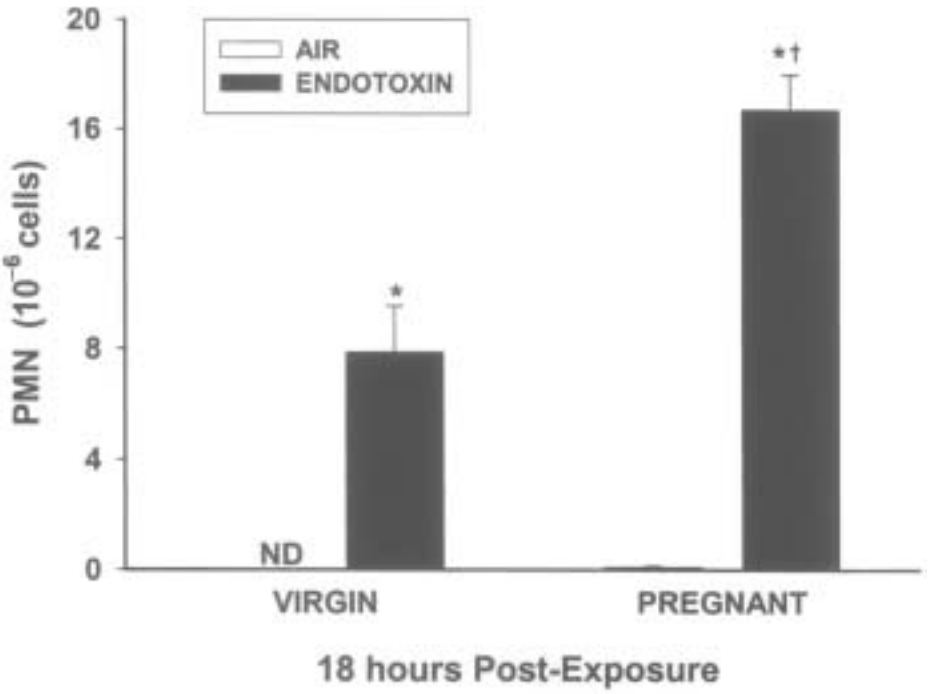

FIGURE 2. Number of PMNs harvested by BAL from virgin or pregnant rats 18 h postexposure to air or endotoxin. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation for 3 h as described under Methods. Bronchoalveolar cells were harvested 18 h after the inhalation exposures. BAL cell counts were determined using an electronic cell counter. The number of PMNs were then determined following differential analysis of BAL cell populations. Values are the means ± SE ($n = 6$ rats/group). ND, nondetectable. Asterisk indicates significant, $p \leq .05$, main effect of endotoxin vs. air; †, significant at $p \leq .05$, interaction pregnant/endotoxin vs. virgin/endotoxin.

In addition, pregnancy was associated overall with a slight elevation in BAL serum albumin levels compared to the levels in virgin rats.

The effect of pregnancy on the number of cells harvested by BAL was also examined in this experiment. The inhalation of endotoxin was associated with an increase in the total number of cells (i.e., AMs and/or PMNs) harvested by BAL from both virgin and pregnant rats (Table 2). Regarding the number of AMs harvested from virgin and pregnant rats following exposure to endotoxin, the numbers of cells were significantly increased (~1.4-fold) above those harvested from air-exposed animals (Table 2). However, no overall difference in AM number between similarly exposed virgin and pregnant rats was observed. The number of PMNs harvested by BAL are shown in Figure 2. Very few PMNs are normally found in bronchoalveolar areas, but the numbers of these cells that can be harvested by BAL increase during acute lung inflammation. The inhalation of endotoxin was associated with an increase in the number of PMNs harvested by BAL from virgin rats. BAL PMN numbers were also increased in pregnant rats

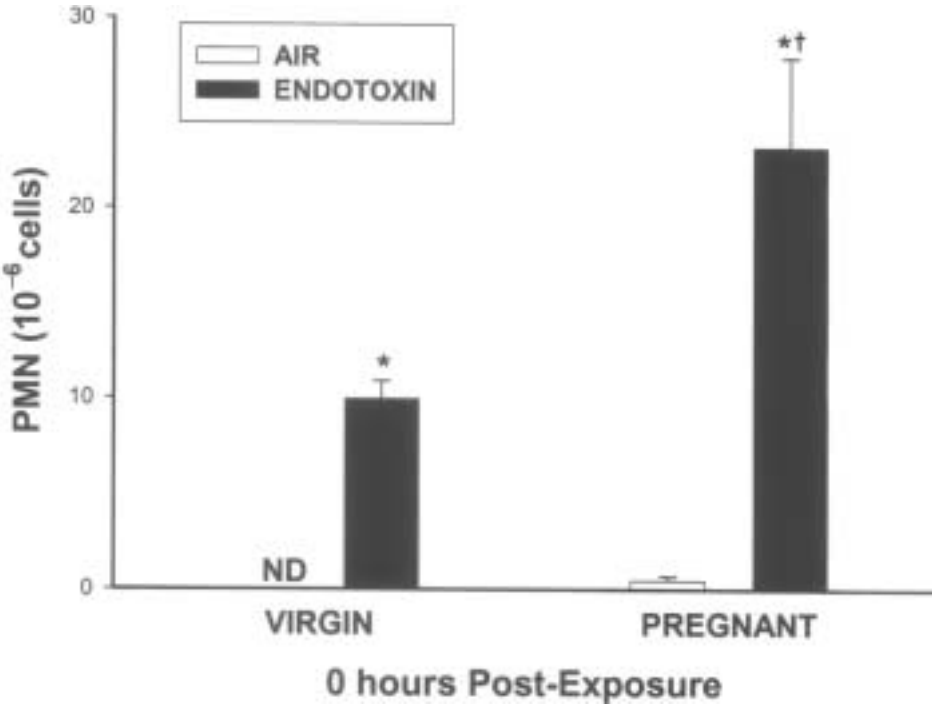

FIGURE 3. Number of PMNs harvested by BAL lavage from virgin or pregnant rats at 0 h postexposure to air or endotoxin. Pregnant rats (17 d of gestation) or age-matched virgin rats were exposed to air or endotoxin ($12.6 \pm 2.1 \times 10^4$ EU/m^3) by inhalation for 3 h as described under Methods. Bronchoalveolar cells were harvested immediately after the inhalation exposures. BAL cell counts were determined using an electronic cell counter. The number of PMNs were then determined following differential analysis of BAL cell populations. Values are the means ± SE ($n = 6$ rats/group). ND, nondetectable; asterisk indicates significant, $p \leq .05$, main effect of endotoxin vs. air; †, significant at $p \leq .05$ interaction pregnant/endotoxin vs. virgin/endotoxin.

exposed to endotoxin; furthermore, this rise was twofold greater than that in virgin rats exposed to endotoxin. Collectively, these findings indicate that greater lung cell damage and increased numbers of lung inflammatory cells in bronchoalveolar spaces are observed in pregnant rats relative to virgin rats at 18 h after exposure to aerosolized endotoxin.

Lung Cell Damage, Permeability of the Alveolar–Capillary Barrier, and BAL Cell Numbers in Virgin and Pregnant Rats Immediately Following Inhalation Exposure to Endotoxin

Early pulmonary events following endotoxin inhalation by virgin and pregnant rats were next studied. LDH activity levels and serum albumin levels in BAL fluid samples just following exposure to air or aerosolized endotoxin are presented in Table 3. At this time point, neither virgin nor pregnant rats showed overt lung cell damage or alveolar-capillary barrier disruption following endotoxin inhalation. However, serum albumin BAL fluid levels were significantly

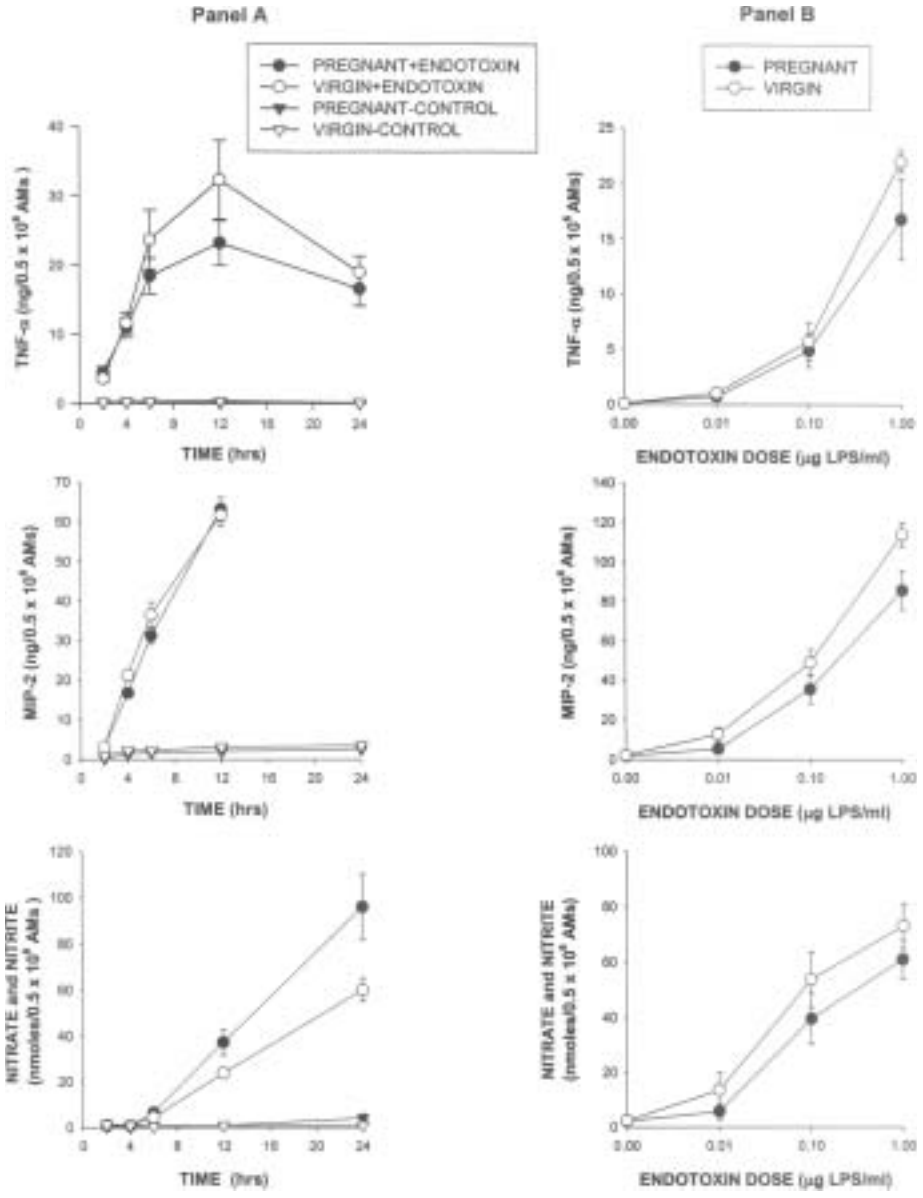

FIGURE 4. Effect of endotoxin on TNF-α, MIP-2, and NO release by AMs harvested from virgin or pregnant rats. AMs were harvested by BAL from pregnant rats (17 d of gestation) or age-matched virgin rats. Cells were cultured as described in the Methods. (A) Effect of endotoxin (0.1 µg/ml LPS) on cytokine and NO release by AMs after 2, 4, 6, 12, or 24 h of incubation; $n = 6$–8/group. (B) Effect of different doses of endotoxin (0.01 to 1.0 µg LPS/ml) on cytokine and NO release by AMs after 24 h of incubation; $n = 6$/group. I. (A), for TNF-α, the values for the pregnant-control and virgin-control groups overlap; for MIP-2, values are missing at the 24-h time point because assay values were greater than the upper limit of the standard curve of the ELISA, and insufficient samples remained for further analysis.

Table 7. Basal and Zymosan-Stimulated Superoxide Anion Production by AMs from Virgin or Pregnant Rats at 24 h following In Vitro Exposure to Endotoxin, Presented as Superoxide Anion Release (nmol/0.5× 10^6 cells/30 min)

	Virgin		Pregnant	
Endotoxin (μg LPS/ml)	Basal	Zymosan-stimulated	Basal	Zymosan-stimulated
0	0.51 ± 0.17	5.38 ± 0.64[a]	0.81 ± 0.36	4.90 ± 0.54[a]
0.01	0.86 ± 0.23	5.58 ± 0.53[a]	0.57 ± 0.20	4.74 ± 0.65[a]
0.1	0.47 ± 0.17	4.70 ± 0.24[a]	0.24 ± 0.13	3.98 ± 0.53[a]
1.0	0.51 ± 0.24	2.77 ± 0.30[a]	0.33 ± 0.08	3.48 ± 0.57[a]

Note. AMs were harvested by BAL from pregnant rats (17 d of gestation) or age-matched virgin rats and cultured as described under Methods. Basal and zymosan-stimulated superoxide anion release was then measured following a 24-h in vitro exposure to different concentrations of endotoxin. Values are the means ± SE ($n = 6$/group).

[a]Significant at $p \leq .05$, zymosan-stimulated vs. basal.

increased in pregnant animals compared to virgin females. The numbers of total cells and AMs harvested by BAL from these animals are presented in Table 4. The inhalation of endotoxin was associated with an increase in the number of cells harvested by BAL from both virgin and pregnant rats (Table 4). Furthermore, a greater total number of cells as well as AMs was harvested from pregnant rats, compared to the numbers harvested from virgin rats. The numbers of PMNs harvested by BAL are shown in Figure 3. In this case, pregnancy was associated with a twofold increase in the number of PMNs that could be harvested immediately after exposure to aerosolized endotoxin. These results suggest that an enhanced recruitment of PMNs into bronchoalveolar spaces is one of the initial events that occurs in pregnant rats following the inhalation of endotoxin.

Breathing Rate, Tidal Volume, and Minute Ventilation in Virgin and Pregnant Rats

One mechanism by which pregnancy could result in more severe pulmonary reactions to aerosolized endotoxin might be by altering the inhaled dose of this biogenic agent. In order to assess this possibility, breathing rate, tidal volume, and minute ventilation were measured. Measurements were made prior to exposure and at the end of the exposure period. Results for breathing rate, tidal volume, and minute ventilation for virgin or pregnant animals before the start of the exposure are shown in Table 5. No marked differences in these respiratory measurements were noted between virgin and pregnant rats. In addition, values for these respiratory variables were similar for virgin or pregnant rats following exposure to air or endotoxin with the exception that the inhalation of endotoxin was associated with a slight decrease in tidal volume (approximately 20% for both virgin and pregnant rats; data not shown). These results suggest that the increased pulmonary damage and inflammatory response that is observed in pregnant rats following the inhalation of endotoxin is not a consequence of the delivery of greater amounts of endotoxin to the lung.

Evaluation of Circulating Corticosterone Levels in Virgin and Pregnant Rats Following Inhalation Exposure to Endotoxin

There is some evidence that serum corticosterone levels can change following endotoxin exposure, and alterations in circulating corticosterone levels could potentially affect pulmonary responses to endotoxin. Therefore, studies assessed whether alterations in serum corticosterone levels might have occurred following the inhalation exposure and thereby possibly contribute to a change in the sensitivity of pregnant rats to aerosolized endotoxin (Table 6). However, no differences in serum corticosterone levels between virgin or pregnant rats exposed to air or endotoxin were observed.

Effect of Endotoxin on the Release of Reactive Oxygen and Nitrogen Species and Cytokine Production by AMs From Pregnant and Virgin Rats

In a final series of experiments, studies investigated the hypothesis that AMs from pregnant rats might be more responsive to endotoxin than AMs from virgin rats. The production of superoxide anion by AMs from virgin or pregnant rats following in vitro exposure to endotoxin (1 μg LPS/ml) for 24 h is shown in Table 7. It was found that treatment with unopsonized zymosan stimulated superoxide anion generation from both virgin and pregnant rat AMs. However, no marked differences in basal or stimulated superoxide production by AMs from virgin or pregnant rats were observed in the absence or presence of endotoxin.

The effect of endotoxin on TNF-α, MIP-2, and NO release from AMs harvested from virgin or pregnant rats is shown in Figure 4. In this investigation, both time- and dose-related responses of AMs to an in vitro challenge with endotoxin were explored. Treatment with endotoxin (0.1 μg LPS/ml) for 2 to 24 h resulted in time-dependent increases in TNF-α, MIP-2, and NO production by AMs from both virgin and pregnant rats (Figure 4A). Treatment with different amounts of endotoxin (0.01 to 1 μg LPS/ml) for 24 h was also associated with dose-dependent increases in TNF-α, MIP-2, and NO production by AMs from either virgin or pregnant rats (Figure 4B). Although a few slight differences were noted across these experiments, collectively the data suggest that AMs from virgin or pregnant rats respond very similarly to endotoxin with respect to TNF-α, MIP-2, and NO production.

DISCUSSION

The results from our study indicate that there is an enhanced pulmonary inflammatory response to aerosolized endotoxin during pregnancy in the rat. This was manifested by an increase in lung cell damage and a greater influx of PMNs into bronchoalveolar areas in pregnant rats relative to that in virgin controls following the inhalation of endotoxin. For example, at 18 h following exposure to endotoxin, LDH activity levels in BAL fluid samples were 1.5-fold greater

and BAL PMN numbers were approximately 2-fold greater in pregnant rats than virgins following exposure to endotoxin. These pregnancy-linked increases in BAL PMN numbers were observed both immediately following exposure and at 18 h following exposure to endotoxin. To our knowledge, this is the first report that has examined the effects of inhaled endotoxin on pulmonary responses during pregnancy. Endotoxin contamination exists in a number of settings, and the potential for inhalation exposure to endotoxin in domestic, environmental, and manufacturing settings has been well documented (Castellan et al., 1984; Iversen et al., 2000; Michel, 2001; Olenchock et al., 1987; Rylander, 2002; Rylander & Vesterlund, 1982). Our finding, in an animal model, that the physiologic state of pregnancy appears to sensitize the lung to endotoxin suggests that pregnant women may also be at greater risk to the adverse pulmonary effects of aerosolized endotoxin.

One mechanism by which pregnancy could result in more severe pulmonary reactions to endotoxin might be by altering the absolute amounts of inhaled endotoxin. However, this does not appear to be the case in the present study. Data showed that breathing rate, tidal volume, and minute ventilation were not different between pregnant and virgin rats. While it is known that an increase in minute ventilation occurs during human pregnancy (Lotgering et al., 1991; Pernoll et al., 1975), we are not aware of any previous reports on minute ventilation in pregnant rats. It should be noted that rats were studied on d 17 of gestation. The normal length of gestation in the rat strain used in the present study is 22 d. It may be that minute ventilation might increase in rats at a time closer to parturition. Nevertheless, our current results suggest that overall changes in minute ventilation at 17 d of gestation cannot account for the enhanced sensitivity of the pulmonary system of pregnant rats to aerosolized endotoxin.

In this study, the possibility that changes in circulating corticosterone levels might contribute to the increased sensitivity of pregnant rats to aerosolized endotoxin was also explored. This possibility was explored for the following reasons. It has been shown that the systemic administration of endotoxin to rats can activate the hypothalamo–pituitary–adrenal axis and result in increased serum levels of corticosterone (Suzuki et al., 1986). However, the responsiveness of the hypothalamo–pituitary–adrenal axis to some stimuli appears to be downregulated in pregnant rats (Neumann et al., 1998, 2000). Therefore, it was hypothesized that the inhalation of endotoxin might increase circulating corticosterone levels in both virgin and pregnant rats, but that the response might be lower during pregnancy. If this occurred, then the relatively lower corticosterone levels in pregnant rats compared to virgins might be associated with enhanced endotoxin-induced lung injury in pregnant rats, since it has been shown that glucocorticoid administration can attenuate endotoxin-associated lung injury (Yi et al., 1996). However, no differences in circulating corticosterone levels between virgin and pregnant rats, exposed to either air or endotoxin, were found in the present study. It should be noted that the circulating levels of corticosterone that were observed (overall mean range ~240 ng/ml) are higher

than levels reported for conscious virgin and pregnant rats implanted with jugular cannula (Atkinson & Waddell, 1995; Neumann et al., 2000). Circulating corticosterone levels following the induction of anesthesia were measured, and this procedure, that is, handling and ip injection, may have induced corticosterone release. Therefore, it is not possible to completely exclude the possibility that alterations in corticosterone levels are not a mechanism contributing to the enhanced endotoxin-induced pulmonary inflammatory response in pregnant rats. Further studies examining these levels in conscious, unrestrained virgin and pregnant rats exposed to aerosolized endotoxin may be warranted.

In a final series of experiments, it was determined whether differences in the responsiveness of AMs from virgin or pregnant rats to endotoxin might exist. Resident AMs serve as a first line of defense against pathogens invading the lung. Upon stimulation with foreign substances such as endotoxin, these cells produce reactive oxygen and nitrogen species as well as a number of cytokines that are involved in the coordination of subsequent inflammatory events (Monick & Hunninghake, 2002; Sharar et al., 1994). It has also been shown that reproductive hormones can alter the responsiveness of macrophages, including lung AMs (Miller & Hunt, 1996; Robert & Spitzer, 1997). It was hypothesized that endotoxin exposure might be associated with an increase in the release of inflammatory mediators by AMs from pregnant rats compared to the amounts released by AMs from virgin rats. If so, this could be an important mechanism contributing to the increased pulmonary inflammatory response to endotoxin which was observed in pregnant rats. To test this hypothesis, studies were conducted to determine whether AMs from pregnant rats released increased amounts of reactive oxygen or nitrogen species and/or cytokines following endotoxin stimulation compared to the amounts of these substances released by AMs from virgin rats. Specifically, how in vitro stimulation with endotoxin affected the production of superoxide anion, NO, TNF-α, and MIP-2 by AMs was examined. These endpoints were chosen for the following reasons. Superoxide radicals play an important role in host defense against invading pathogens, and there is some evidence to suggest that in vitro priming with endotoxin enhances superoxide anion generation by AMs (Meyer et al., 1991; Suzuki et al., 1993). NO is another free radical produced by AMs that has been shown play a significant role in pulmonary inflammatory reactions and the upregulation of NO production by AMs following stimulation with endotoxin has been well documented (Jorens et al., 1991; Prokhorova et al., 1994). It also has been shown that endotoxin is a potent stimulator of cytokine expression by AMs. TNF-α and MIP-2 are two specific cytokines that are produced by rodent AMs following endotoxin stimulation. TNF-α appears to play a major role in mediating endotoxin-induced lung inflammation (Simpson & Casey, 1989; Ulich et al., 1991). MIP-2 is a chemotactic cytokine that has been shown to play an important role in lung neutrophil recruitment in rodent animal models (Driscoll, 1994; Schmal et al., 1996).

In the present study, it was found that endotoxin was indeed a potent stimulator of NO, TNF-α, and MIP-2 release by AMs. For instance, treatment

with endotoxin resulted in both dose- and time-dependent increases in NO, TNF-α, and MIP-2 production by AMs from both virgin and pregnant rats. Also, as expected, stimulation with zymosan increased superoxide anion production by rat AMs from both virgin and pregnant rats, although in vitro stimulation of AMs with endotoxin did not enhance superoxide anion release. Despite overall increases in the stimulated production of superoxide anion by zymosan and enhanced NO, TNF-α, and MIP-2 release following endotoxin, our results indicate that AMs from virgin and pregnant rats respond very similarly to these stimuli. Therefore, a specific change in the responsiveness of pregnant rat AMs to endotoxin does not appear to be a mechanism mediating the enhanced pulmonary inflammatory response seen in these animals.

In summary, pregnancy is associated with an increased pulmonary inflammatory response to aerosolized endotoxin in a rat animal model. To our knowledge, this is the first study that has evaluated pulmonary responses to inhaled endotoxin during pregnancy. It has also been found that lactating rats are more sensitive to pulmonary responses to endotoxin (Gordon et al., 1993). Interestingly, in late pregnancy and during lactation, enhanced pulmonary responses to ozone also occur in rats (Gunnison et al., 1992). Collectively, these findings suggest that in late pregnancy and during lactation there may be a generalized enhancement of pulmonary responses to a variety of inhaled toxic agents.

The increase in sensitivity of the lung to endotoxin during pregnancy cannot apparently be attributed to changes in the inhaled dose of endotoxin or alterations in the responsiveness of AMs to endotoxin. One possible hypothesis is that alterations in pulmonary oxidant–antioxidant tone underlie the enhanced pulmonary inflammatory response to endotoxin in pregnancy. It has been shown that pregnancy in both rats and humans is associated with increases in oxidative stress (Morris et al., 1998; Sainz et al., 2000; Yoshioka et al., 1987; Zusterzeel et al., 2000). Further, it has been hypothesized that a pro-oxidant state in pregnancy may sensitize animals to endotoxin (Stark & Jackson, 1990). However, to our knowledge, the oxidant–antioxidant balance in lung tissue during pregnancy and how it may differ from that in the nonpregnant state has not been determined. Alternatively, it has been shown that endotoxin exposure results in an oxidative stress response in the lung and the upregulation of stress proteins, such as metallothionein and heme oxygenase (Hur et al., 1999, Repine & Parsons, 1994). These stress proteins have been proposed to play a role in free-radical scavenging and may function in an antioxidant capacity to limit pulmonary inflammatory responses to endotoxin (Choi & Alam, 1996; Hur et al., 1999; Sato & Bremner, 1993). In pregnancy, it may potentially be found that exposure of the lung to endotoxin is associated with a decreased ability to mobilize pulmonary antioxidant defense systems compared to responses in nonpregnant animals. If so, this could lead to an enhanced pulmonary inflammatory response to endotoxin in a pregnant state.

REFERENCES

Apitz, K. 1935. A study of the generalized Shwartzman phenomenon. *J. Immunol.* 29:255–266.

Atkinson, H. C., and Waddell, B. J. 1995. The hypothalamic–pituitary–adrenal axis in rat pregnancy and lactation: Circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. *Endocrinology* 136:512–520.

Brigham, K. L., and Meyrick, B. 1986. Endotoxin and lung injury. *Am. Rev. Respir. Dis.* 133:913–927.

Castellan, R. M., Olenchock, S. A., Hankinson, J. L., Millner, P. D., Cocke, J. B., Bragg, C. K., Perkins, H. H., Jr., and Jacobs, R. R. 1984. Acute bronchoconstriction induced by cotton dust: Dose-related responses to endotoxin and other dust factors. *Ann. Intern. Med.* 101:157–163.

Choi, A. M. K., and Alam, J. 1996. Heme oxygenase—1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am. J. Respir. Cell Mol. Biol.* 15:9–19.

DeLorme, M. P., Gao, X., Doyon-Reale, N., Barraclough-Mitchell, H., and Bassett, D. J. P. 2003. Inflammatory effects of inhaled endotoxin-contamined metal working fluid aerosols in rats. *J. Toxicol. Environ. Health A* 66:7–24.

Driscoll, K. E. 1994. Macrophage inflammatory proteins: Biology and role in pulmonary inflammation. *Exp. Lung Res.* 20:473–490.

Eldridge, M. W., and Peden, D. B. 2000. Airway response to concomitant exposure with endotoxin and allergen in atopic asthmatics. *J. Toxicol. Environ. Health A* 61:27–37.

Faas, M. M., Schuiling, G. A., Baller, J. F. W., Visscher, C. A., and Bakker, W. W. 1994. A new animal model for human preeclampsia: Ultra-low-dose endotoxin infusion in pregnant rats. *Am. J. Obstet. Gynecol.* 171:158–164.

Fass, M. M., Schuiling, G. A., Linton, E. A., Sargent, I. L., and Redman, C. W. G. 2000. Activation of peripheral leukocytes in rat pregnancy and experimental preeclampsia. *Am. J. Obstet. Gynecol.* 182:351–357.

Gordon, T., Weideman, P. A., and Gunnison, A. F. 1993. Increased pulmonary response to inhaled endotoxin in lactating rats. *Am. Rev. Respir. Dis.* 147:1100–1104.

Gunnison, A. F., Weideman, P. A., and Sobo, M. 1992. Enhanced inflammatory response to acute ozone exposure in rats during pregnancy and lactation. *Fundam. Appl. Toxicol.* 19:607–612.

Huffman, L. J., Judy, D. J., Robinson, V. A., and Castranova, V. 1997. Inhalation of cotton dust is associated with increases in nitric oxide production by rat bronchoalveolar lavage cells. *Inhal. Toxicol.* 9:567–579.

Huffman, L. J., Judy, D. J., Rao, K. M. K., Frazer, D. G., and Goldsmith, W. T. 2000. Lung responses to hypothyroidism, hyperthyroidism, and lipopolysaccharide challenge in rats. *J. Toxicol. Environ. Health A* 61:623–639.

Huffman, L. J., Judy, D. J., Brumbaugh, K., Frazer, D. G., Reynolds, J. S., McKinney, W. G., and Goldsmith, W. T. 2001. Hyperthyroidism increases the risk of ozone-induced lung toxicity in rats. *Toxicol. Appl. Pharmacol.* 173:18–26.

Hur, T., Squibb, K., Cosma, G., Horowitz, S., Piedboeuf, B., Bowser, D., and Gordon T. 1999. Induction of metallothionein and heme oxygenase in rats after inhalation of endotoxin. *J. Toxicol. Environ. Health A* 56:183–203.

Iversen, M., Kirychuk, S., Drost, H., and Jacobson, L. 2000. Human health effects of dust exposure in animal confinement buildings. *J. Agric. Safety Health* 6:283–288.

Johnston, R. B., Jr. 1984. Measurement of O_2^- secreted by monocytes and macrophages. *Methods Enzymol.* 105:365–369.

Jorens, P. G., Van Overveld, F. J., Bult, H., Vermeire, P. A., and Herman, A. G. 1991. L-Arginine-dependent production of nitrogen oxides by rat pulmonary macrophages. *Eur. J. Pharmacol.* 200:205–209.

Kline, J. N., Cowden, J. D., Hunninghake, G. W., Schutte, B. C., Watt, J. L., Wohlford-Lenane, C. L., Powers, L. S., Jones, M. P., and Schwartz, D. A. 1999. Variable airway responsiveness to inhaled lipopolysaccharide. *Am. J. Respir. Crit. Care Med.* 160:297–303.

Lotgering, F. K., Van Doorn, M. B., Struijk, P. C., Pool, J., and Wallenburg, H. C. S. 1991. Maximal aerobic exercise in pregnant women: Heart rate, O_2 consumption, CO_2 production, and ventilation. *J. Appl. Physiol.* 70:1016–1023.

Massey, V. 1959. The microestimation of succinate and the extinction coefficient of cytochrome *c*. *Biochem. Biophys. Acta* 34:255–256.

McKay, D., Wong, T., and Galton, M. 1960. Effect of pregnancy on the disseminated thrombosis caused by bacterial endotoxin. *Fed. Proc.* 19:246.

Meyer, K. C., Powers, C., Cornwell, R., and Borden, E. C. 1991. Priming of human alveolar macrophages and blood monocytes for superoxide anion release by interferons and lipopolysaccharide. *J. Interferon Res.* 11:283–290.

Michel, O. 2001. Role of house-dust endotoxin exposure in aetiology of allergy and asthma. *Mediators Inflam.* 10:301–304.

Miller, L., and Hunt, J. S. 1996. Sex steroid hormones and macrophage function. *Life Sci.* 59:1–14.

Monick, M. M., and Hunninghake, G. W. 2002. Activation of second messenger pathways in alveolar macrophages by endotoxin. *Eur. Respir. J.* 20:210–222.

Morris, J. M., Gopaul, N. K., Endresen, M. J. R., Knight, M., Linton, E. A., Dhir, S., Anggard, E. E., and Redman, C. W. G. 1998. Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia. *Br. J. Obstet. Gynaecol.* 105:1195–1199.

Neumann, I. D., Johnstone, H. A., Hatzinger, M., Liebsch, G., Shipston, M., Russell, J. A., Landgraf, R., and Douglas, A. J. 1998. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. *J. Physiol.* 508:289–300.

Neumann, I. D., Torner, L., and Wigger, A. 2000. Brain oxytocin: Differential inhibition of neuroendocrine stress responses and anxiety-related behaviour in virgin, pregnant and lactating rats. *Neuroscience* 95:567–575.

Olenchock, S. A., May, J. J., Pratt, D. S., and Morey, P. R. 1987. Occupational exposures to airborne endotoxins in agriculture. In *Detection of bacterial endotoxins with the* Limulus *amebocyte lysate test*, eds. S. W. Watson, J. Levin, and T. J. Novitsky, pp. 475–487. New York: Alan R. Liss.

Pernoll, M. L., Metcalfe, J., Kovach, P. A., Wachtel, R., and Dunham, M. J. 1975. Ventilation during rest and exercise in pregnancy and postpartum. *Respir. Physiol.* 25:295–310.

Porter, D. W., Millecchia, L., Robinson, V. A., Hubbs, A., Willard, P., Pack, D., Ramsey, D., McLaurin, J., Khan, A., Landsittel, D., Teass, A., and Castranova, V. 2002. Enhanced nitric oxide and reactive oxygen species production and damage after inhalation of silica. *Am. J. Physiol. Lung Cell Mol. Physiol.* 283:L485–L493.

Prokhorova, S., Lavnikova, N., and Laskin, D. L. 1994. Functional characterization of interstitial macrophages and subpopulations of alveolar macrophages from rat lung. *J. Leukocyte Biol.* 55:141–146.

Repine, J. E., and Parsons, P. E. 1994. Oxidant-antioxidant balance in endotoxin-induced oxidative injury and tolerance to oxidative injury. In *Endotoxin and the lungs*, ed. K. L. Brigham, pp. 207–227. New York: Marcel Dekker.

Robert, R., and Spitzer, J. A. 1997. Effects of female hormones (17β-estradiol and progesterone) on nitric oxide production by alveolar macrophages in rats. *Nitric Oxide* 1:453–462.

Rylander, R. 2002. Endotoxin in the environment—Exposure and effects. *J. Endotoxin Res.* 8:241–252.

Rylander, R., and Vesterlund, M. A. 1982. Airborne endotoxins in various environments. *Prog. Clin. Biol. Res.* 93:399–409.

Sainz, R. M., Reiter, R. J., Mayo, J. C., Cabrera, J., Tan, D.-X., Qi, W., and Garcia, J. J. 2000. Changes in lipid peroxidation during pregnancy and after delivery in rats: Effect of pinealectomy. *J. Reprod. Fertil.* 119:143–149.

SAS Institute, Inc., 1999. SAS/STAT User's Guide. Version 8. Cary, NC.

Sato, M., and Bremner, I. 1993. Oxygen free radicals and metallothionein. *Free Radical Biol. Med.* 14:325–337.

Schmal, H., Shanley, T. P., Jones, M. L., Friedl, H. P., and Ward, P. A. 1996. Role for macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J. Immunol.* 156:1963–1972.

Sharar, S. R., Winn, R. K., and Harlan, J. M. 1994. Endotoxin-induced interactions of inflammatory cells with the lungs. In *Endotoxin and the lungs*, ed. K. L. Brigham, pp. 229–265. New York: Marcel Dekker.

Simpson, S. Q., and Casey, L. C. 1989. Role of tumor necrosis factor in sepsis and acute lung injury. *Crit. Care Clin.* 5:27–47.

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., and Serrano, F. O. 1995. The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environ. Health Perspect.* 103:113–122.

Stark, J. M., and Jackson, S. K. 1990. Sensitivity to endotoxin is induced by increased membrane fatty-acid unsaturation and oxidant stress. *J. Med. Microbiol.* 32:217–221.

Suzuki, K., Yamamoto, T., Sato, A., Murayama, T., Amitani, R., Yamamoto, K., and Kuze, F. 1993. Lipopolysaccharide primes human alveolar macrophages for enhanced release of superoxide anion and leukotriene B_4: Self-limitations of the priming response with protein synthesis. *Am. J. Respir. Cell Mol. Biol.* 8:500–508.

Suzuki, S., Oh, C., and Nakano, K. 1986. Pituitary-dependent and -independent secretion of CS caused by bacterial endotoxin in rats. *Am. J. Physiol.* 250:E470–E474.

Thorn, J. 2001. The inflammatory response in humans after inhalation of bacterial endotoxin: A review. *Inflamm. Res.* 50: 254–261.

Ulich, T. R., Watson, L. R., Yin, S., Guo, K., Wang, P., Thang, H., and del Castillo, J. 1991. The intratracheal administration of endotoxin and cytokines. *Am. J. Pathol.* 138:1485–1496.

Welbourn, C. R. B., and Young, Y. 1992. Endotoxin, septic shock and acute lung injury: Neutrophils, macrophages and inflammatory mediators. *Br. J. Surg.* 79:998–1003.

Wong, T.-C. 1962. A study on the generalized Shwartzman reaction in pregnant rats induced by bacterial endotoxin. *Am. J. Obstet. Gynecol.* 84:786–797.

Yi, E. S., Remick, D. G., Lim, Y., Tang, W., Nadzienko, C. E., Bedoya, A., Yin, S., and Ulich, T. R. 1996. The intratracheal administration of endotoxin: X. Dexamethasone downregulates neutrophil emigration and cytokine expression in vivo. *Inflammation* 20:165–175.

Yoshioka, T., Motoyama, H., Yamasaki, F., Ando, M., Takehara, Y., and Yamasaki, M. 1987. Lipid peroxidation and vitamin E levels during pregnancy in rats. *Biol. Neonate* 52:223–231.

Zusterzeel, P. L. M., Mulder, T. P. J., Peters, W. H. M., Wiseman, S. A., and Steegers, E. A. P. 2000. Plasma protein carbonyls in nonpregnant, healthy pregnant and preeclamptic women. *Free Radical Res.* 33:471–476.