

Immunomodulatory effect of endotoxin on the development of latex allergy

Michael D. Howell, PhD,^a Vesna J. Tomazic, PhD,^b Tina Leakakos, PhD,^c
Wava Truscott, PhD,^d and B. Jean Meade, DVM, PhD^a Morgantown, WV, Rockville, Md,
San Diego, Calif, and Roswell, Ga

Background: Although numerous studies have been conducted delineating the clinical manifestations of latex allergy and characterizing the protein allergens, little is known regarding the natural history of the disease.

Objective: These studies were undertaken to investigate the immunomodulatory role of inhaled endotoxin on the development of latex-specific IgE-mediated responses to natural rubber latex (NRL) proteins by using a mouse model.

Methods: Female BALB/c mice were exposed to 25 µg of NRL proteins with or without increasing concentrations of endotoxin (50-25,000 EU) through the respiratory tract. Serum antibody levels were evaluated biweekly during the study. After sensitization, mice were challenged with methacholine or NRL proteins, and airway hyperreactivity (AHR) was evaluated with whole-body plethysmography. After NRL challenge, lungs were excised for histopathology, and lung-associated lymph nodes were removed for cytokine mRNA evaluation.

Results: When compared with mice exposed to latex alone, mice exposed to latex and endotoxin demonstrated up to 50% lower levels of latex-specific IgE and decreased latex-specific AHR and mucin production. Conversely, these same animals demonstrated increased levels of latex-specific serum IgG2a and IgA antibodies and an increase in IFN-γ and IL-12 mRNA levels in the draining lymph node cells. Concurrent exposure to LPS with nonammoniated latex resulted in increased alveolitis and nonspecific AHR on respiratory challenge with methacholine.

Conclusion: Coexposure with LPS and allergen decreased latex-specific IgE but augmented nonspecific AHR. These studies demonstrate that endotoxin associated with NRL gloves can modulate the development of allergic responses to NRL proteins. (*J Allergy Clin Immunol* 2004;113:916-24.)

Key words: Latex, endotoxin, mouse model, IgE, airway hyperactivity

Over the past 2 decades, natural rubber latex (NRL) gloves have been used extensively in health care settings

Abbreviations used

AHR: Airway hyperreactivity
AU: Arbitrary unit
NAL: Nonammoniated latex
NRL: Natural rubber latex
PenH: Enhanced pause

to prevent occupational exposure to blood-borne pathogens and hazardous chemicals. As a result, health care workers have experienced extensive dermal and respiratory NRL protein exposure, and an increased prevalence of latex allergy in this population has been reported.¹⁻⁴ Latex allergy manifests as urticaria, rhinitis, asthma, and potentially anaphylaxis.⁵⁻¹⁰

Significant levels of endotoxin have been found in NRL gloves, and it has been suggested that these levels are capable of inducing respiratory symptoms similar to those associated with latex allergy.¹¹ The inflammatory properties of endotoxin are well documented. Studies have shown that exposure to endotoxin induces (1) respiratory complications on inhalation,^{12,13} (2) macrophage and neutrophil infiltration into the lungs of exposed mice,^{14,15} and (3) increased proinflammatory (TNF-α, IL-1, IL-6, and IL-8) and T_H1 (IL-12 and IFN-γ) cytokine message levels in activated T cells and macrophages.¹⁶⁻¹⁹

Numerous studies have investigated the effects of endotoxin or NRL proteins on the immune system; however, few have examined the modulatory role of endotoxin in the development of latex sensitization. These studies were conducted to evaluate the role of endotoxin in the induction of IgE to nonammoniated latex (NAL) protein, nonspecific and specific airway hyperreactivity (AHR), and cytokine message levels in the lung-associated lymph nodes.

METHODS

Mice

Female BALB/c mice were purchased from Harlan Sprague Dawley, Inc (Indianapolis, Ind) and were received between 6 and 12 weeks of age. On arrival, mice were maintained under conditions specified within National Institutes of Health guidelines²⁰ and quarantined for at least 1 week before use, as previously described.²¹ Nitrile gloves were worn while handling mice during study procedures to prevent unscheduled latex exposure.

Test articles

LPS isolated from *Salmonella typhimurium* (3×10^6 EU/mg; 1×10^6 EU/mg), *Salmonella typhosa* (lot no. 78H4059, 5×10^6

From ^athe Agriculture and Immunotoxicology Group, National Institute for Occupational Safety and Health, Morgantown; ^bthe Center for Devices and Radiological Health, Food and Drug Administration, Rockville; ^cPreclinical Development, FerRx Inc, San Diego; and ^dScientific Affairs and Clinical Education, Kimberly Clark Corporation, Roswell.

Supported in part by the National Toxicology Program Interagency Agreement no. Y1-ES-0001-02 and a Cooperative Research and Development Agreement between the US Food and Drug Administration and Safeskin Corp, presently Kimberly-Clark Corp.

Received for publication December 3, 2003; revised January 16, 2004; accepted for publication February 2, 2004.

Reprint requests: B. Jean Meade, MS 4020, 1095 Willowdale Rd, Morgantown, WV 26505.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.02.017

EU/mg), and *Escherichia coli* (lot no. 684091, 3×10^6 EU/mg) was purchased from Sigma Chemical Co (St Louis, Mo). NAL was generously provided by Dr Che Hasma Hashim of the Rubber Research Institute of Malaysia.

Murine exposures

Before the initial exposure, mice were weighed and assigned to homogenous weight groups ($n \geq 5$). Mice were tail bled before the initial exposure and weekly or biweekly thereafter to monitor antibody levels. The dose of NAL to be administered (25 μ L) was based on previous dose-response studies conducted in this laboratory. A concentration in the middle of the dose-response curve was chosen so that either an augmentation or suppression of the response could be evaluated.²²

Intranasal exposures. Mice were manually restrained in a supine position while 10 μ L of vehicle or test article was applied to the external openings of both nares (5 μ L/nare).²² Mice were exposed to 25 μ g of NAL proteins, 50 to 25,000 EU of endotoxin, or both 5 days a week for up to 72 days. Endotoxin tested was from *S typhimurium*, *S typhosa*, or *E coli*. All dosing solutions were prepared in Goodyear Preservative (50% glycerol, 67 mmol/L NaHCO₃, and 2 mmol/L L-cysteine) and diluted 1:7 in PBS. Single-vehicle ($n = 10$) and latex-only ($n = 10$) exposure groups were used in the experiment evaluating the 3 different strains of bacterial endotoxin to reduce animal numbers.

Intratracheal exposures. Intratracheal aspirations were performed on mice to localize exposure to the trachea and lung.²³ Mice were lightly anesthetized with isoflurane (Abbott Laboratories, Chicago, Ill) inhalation by using a Bell jar and were restrained in a vertical position on a plexiglass stand by the upper and lower teeth. While withdrawing the tongue to prevent swallowing, 50 μ L of the dosing solution was pipetted into the oropharynx. The tongue was held withdrawn until the dose was aspirated. Mice were dosed every fifth day for up to 76 days with 25 μ g of NAL proteins, 50 to 25,000 EU of endotoxin, or both.

Total IgE ELISA

Total IgE serum levels were measured by using a standard sandwich ELISA, as described by Manetz and Meade.²⁴ The capture antibody (B1E3), secondary antibody (R1E4), and anti-DNP IgE standard antibodies were purified from hybridoma cell lines generously provided by Dr Daniel Conrad (Medical College of Virginia/Virginia Commonwealth University, Richmond, Va).

Latex-specific antibody levels

Latex-specific antibody levels were measured as previously described,²¹ with minor modifications. Wells of Immulon-2 plates (Dynex, Chantilly, Va) were coated overnight with 100 μ L of 5 μ g/mL rat anti-mouse IgE (R1E4), 2 μ g/mL rat anti-mouse IgG1 (A85-3; Pharmingen, San Diego, Calif), 2 μ g/mL rat anti-mouse IgG2a (Igh-1a, Pharmingen), or 2 μ g/mL rat anti-mouse IgA (C10-3, Pharmingen). After blocking, serum samples (100 μ L/well) were added (1:10 for IgE and IgA or 1:1000 for IgG1 and IgG2a) and incubated for 2 hours at 37°C. NAL, antibodies, enzyme, and substrate were subsequently added, as previously described. Arbitrary units (AUs) for the neat sera were determined by multiplying the dilution for each sample by the optical density. Average AU values were calculated for each mouse and then used to determine the overall mean AU $\pm$ SE for each exposure group.

Whole-body plethysmography

Nonspecific and antigen-specific AHR was evaluated by using whole-body plethysmography (Buxco Electronics, Sharon, Conn), as previously described.²¹ Before all respiratory challenges, mice were placed into plethysmography chambers and monitored for 5 minutes

to establish baseline enhanced pause (PenH) values. PenH, an indicator of bronchoconstriction, was calculated as follows²⁵:

$$\begin{aligned} & (\text{expiratory time/relaxation time} - 1) \\ & \times \text{peak expiratory flow/peak inspiratory flow.} \end{aligned}$$

Methacholine challenge. On day 47 after initial exposure, mice were nonspecifically challenged with increasing concentrations of methacholine (10, 25, and 50 mg/mL). After the baseline period, mice remained in the plethysmography chambers, and methacholine was delivered with a nebulizer (DeVilbiss HealthCare Inc, Somerset, Pa). Mice were exposed to each concentration of methacholine for 3 minutes, followed by exposure to dry air for 2 additional minutes. Changes in AHR were determined by calculating the percentage increase in PenH over baseline for each mouse at each concentration of methacholine. These data were then used to determine the mean $\pm$ SE for each exposure group at each concentration of methacholine.

NAL challenge. Animals were challenged with NAL on days 67 and 76. After baseline measurement, mice were removed from the plethysmograph chambers and lightly anesthetized (Isoflurane, Abbott Laboratories, North Chicago, Ill) to allow for intratracheal aspiration of 300 μ g of NAL in 50 μ L of PBS. Mice were returned to the Buxco chambers within 1 minute of challenge, and PenH values were monitored for an additional 25 minutes. Changes in AHR were determined by calculating the percentage increase in PenH over baseline for each mouse and determining the mean $\pm$ SE for each exposure group.

Histopathology

Twenty-four hours after the final intratracheal NAL challenge, mice were killed by means of exsanguination after CO₂ anesthesia. Lungs were insufflated through intratracheal instillation of approximately 1 mL of 10% buffered formalin and excised for histopathology. Paraffin-embedded tissues were cut in 5- μ m sections and stained with hematoxylin-eosin stain, 0.5% Chromotop 2R for evaluation of eosinophilia, or Alcian blue and periodic acid-Schiff to visualize mucous production. Pathologic changes were evaluated in the left lungs of all animals.

RNA isolation and cDNA amplification

After death, the lung-associated draining lymph nodes were excised, snap-frozen in liquid nitrogen, and stored at -80°C . RNA was isolated according to the manufacturer's recommendation by using RNeasy and QiaShredder Kits (Qiagen, Valencia, Calif). Complementary DNA was generated by using TaqMan reverse transcription reagents (Applied Biosystems, Branchburg, NJ), according to the manufacturer's recommendations. Each sample was prepared with 2 μ g of total RNA in a total volume of 50 μ L for each reaction, and reverse transcription was carried out by using the GeneAmp PCR system 6700 thermocycler (Applied Biosystems).

Real-time PCR

Cytokine mRNA (IL-4, IL-10, IL-12, and IFN- γ) levels were determined by using real-time PCR. Complementary DNA, PCR master mix (Applied Biosystems), and probe-primer kits (reduced glyceraldehydes-phosphate dehydrogenase, IL-4, IL-10, IL-12, and IFN- γ ; Applied Biosystems) were mixed in a total volume of 20 μ L and added to 96-well Bio-Rad iCycler iQ plates. The reaction was carried out on the iCycler iQ (Bio-Rad, Hercules, Calif), according to the manufacturer's recommendations.

mRNA levels were determined on the basis of the relative expression of the target gene compared with reduced glyceraldehyde-phosphate dehydrogenase. In brief, a cycle threshold was determined for both the endogenous control and target genes on the basis of nuclease degradation and the subsequent release of fluorescence. These

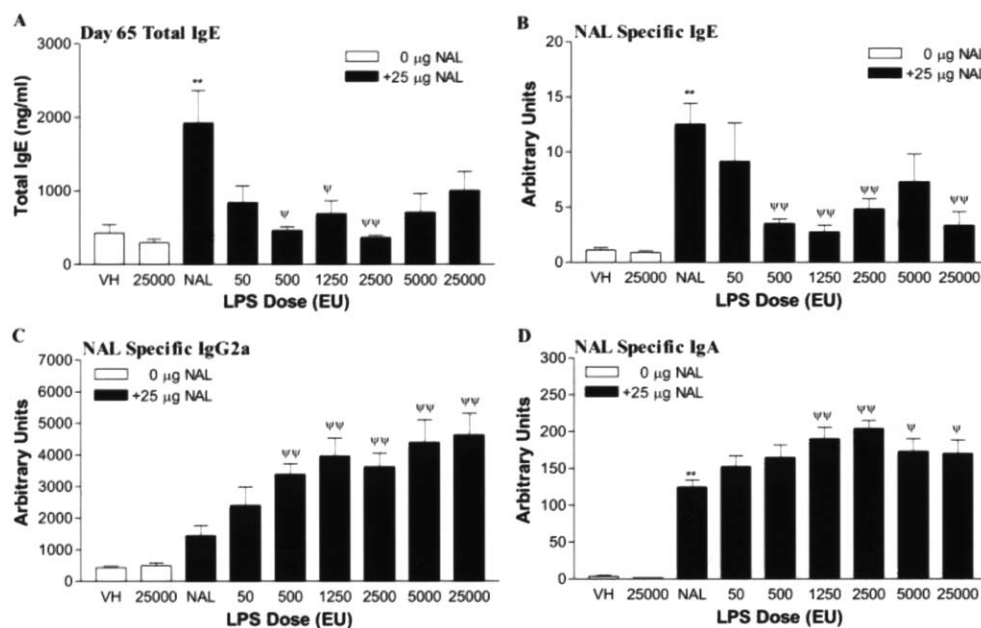


FIG 1. Serum antibody levels in mice intranasally exposed to NAL, endotoxin, or NAL and endotoxin from *S typhimurium*. Day 65 total serum IgE (A), latex-specific IgE (B), IgG2a (C), and IgA (D) levels were evaluated in mice concurrently exposed to NAL and concentrations of endotoxin ranging from 50 to 25,000 EU ($n = 5$ for all exposure groups except the vehicle-exposed and NAL-only groups, in which $n = 10$). Bars represent means $\pm$ SEs for each exposure group. Statistical significance was determined by using a Dunnett t test. ** $P < .01$ compared with vehicle alone; $\Psi P < .05$ and $\Psi\P P < .01$ compared with NAL alone.

cycle thresholds (Cts) were then used to calculate the relative expression of the target gene by using the following formula:

$$2^{-\Delta\Delta Ct} \cdot \Delta\Delta Ct = \Delta Ct(\text{Sample}) - \Delta Ct(\text{Vehicle}) \cdot \Delta Ct = Ct(\text{GAPDH}) - Ct(\text{Target})^{26,27}$$

Statistics

All statistical analyses were conducted with Graph Pad Prism, version 3.01 (San Diego, Calif). Body weights, serum immunoglobulin levels, and PenH data were analyzed by means of 1-way ANOVA. When significant differences were detected ($P < .05$), test groups were compared with the control groups by using a Dunnett test or an unpaired t test. Dose-response relationships were determined using linear regression.

RESULTS

Endotoxin downregulated total and NAL-specific IgE

In an initial experiment, mice were exposed through the intranasal route to 25 μ g of NAL alone or with increasing concentrations of 1 of 3 different endotoxins (*S typhimurium*, *S typhosa*, or *E coli*) for 65 days. Mice exposed to NAL alone demonstrated significantly increased levels of total IgE, reaching greater than 1500 ng/mL by day 65. Total IgE levels in mice exposed to NAL mixed with *S typhimurium* endotoxin were approximately 50% lower ($P < .05$; Fig 1, A). Analysis of the NAL-specific antibody levels revealed a decrease in latex-specific IgE levels to approximately one third (4 AU) of

those observed in mice exposed to NAL alone (12.5 AU; Fig 1, B). In contrast, endotoxin induced a dose-dependent increase in NAL-specific IgG2a, reaching a 3-fold increase in levels compared with those of mice exposed to NAL alone (Fig 1, C). Additionally, concurrent exposure to NAL and endotoxin concentrations of 1250 EU or greater resulted in a significant ($P < .01$) increase in latex-specific IgA compared with that seen in mice exposed to NAL alone (Fig 1, D). Similar trends in latex-specific serum antibody levels were observed in mice concurrently exposed to NAL with endotoxin from either *S typhosa* or *E coli* (data not shown).

In subsequent experiments mice were exposed intratracheally to NAL mixed with endotoxin from one source (*S typhimurium*) every fifth day for 76 days. Intratracheal exposures resulted in a greater IgE response, with significant increases ($P < .01$) detected as early as day 37 and peak production in mice exposed to NAL alone on day 58 reaching levels greater than 20,000 ng/mL. In mice exposed to NAL mixed with endotoxin (50–5000 EU), total IgE levels were approximately 50% to 70% less than in mice exposed to NAL alone (Fig 2, A). Sera obtained from mice on the final day of the study were analyzed for latex-specific antibodies. Mice exposed to NAL proteins alone demonstrated significant increases ($P < .01$) in latex-specific IgE, IgG2a, and IgA levels. Latex-specific IgE levels in NAL- and endotoxin-exposed mice were decreased by as much as 75% (Fig 2, B). Both IgG2a (Fig 2, C) and IgA (Fig 2, D) levels in mice exposed to NAL and as little as 500 EU reached greater than 2-fold

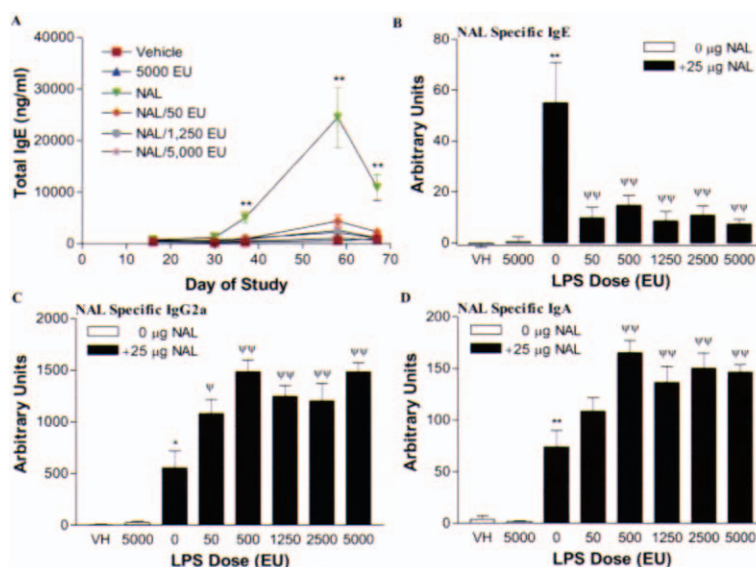


FIG 2. Serum antibody levels in mice intratracheally exposed to NAL, endotoxin, or NAL and endotoxin from *S typhimurium*. Total IgE time course (A) and day 79 levels of latex-specific IgE (B), IgG2a (C), and IgA (D) were evaluated in serum obtained from intratracheally exposed mice. Bars represent the means $\pm$ SEs for each exposure group ($n = 5$ for all exposure groups except the vehicle-only group, in which $n = 4$). ** $P < .01$ compared with vehicle alone; $\Psi P < .05$ and $\Psi\Psi P < .01$ compared with NAL alone.

increases over the levels found in animals exposed to NAL alone.

T_H1 cytokines were upregulated after administration of endotoxin

Twenty-four hours after the final respiratory challenge (day 76), mice were killed, and the lung-associated lymph nodes were excised for evaluation of cytokine mRNA levels. IL-4, IL-10, IL-12, and IFN- γ mRNA levels were evaluated by using semiquantitative real-time PCR. No significant differences in the levels of IL-4 or IL-10 mRNA were observed in mice from any exposure groups when compared with vehicle-treated animals (data not shown). In contrast, endotoxin (*S typhimurium*) exposure induced a greater than 50-fold increase in IL-12 and an 8-fold increase in IFN- γ mRNA levels. Mice exposed to latex alone showed negligible increases in T_H1 cytokines, whereas mice exposed to NAL and endotoxin demonstrated an endotoxin dose-dependent increase in IL-12 (40-fold) and IFN- γ (4-fold) production (Fig 3).

Increased nonspecific AHR in animals exposed to endotoxin

On day 47, mice were challenged with increasing concentrations of methacholine to assess nonspecific AHR. For each methacholine dose, the levels of AHR (percentage increase in PenH over baseline) in mice exposed to NAL, endotoxin, or both were compared with levels in the vehicle-exposed control group for significance. No significant increases in nonspecific AHR were observed in mice exposed to NAL alone or NAL mixed with either 50 or 1250 EU of endotoxin. However, exposure to NAL with 5000 EU of endotoxin resulted in

a significant ($P < .05$) increase in AHR on challenge with either 25 or 50 mg/mL methacholine compared with vehicle-exposed mice. Mean AHR in mice exposed to 5000 EU of endotoxin alone were similar to those observed for mice exposed to NAL and 5000 EU of endotoxin (Fig 4, A).

Decreased NAL-specific AHR in mice concurrently exposed to endotoxin

After methacholine challenge, mice continued to receive intratracheal exposures and were then challenged through intratracheal aspiration with NAL proteins on days 67 and 76 after initial exposure. The development of antigen-specific AHR was then evaluated. A 2-fold increase ($P < .05$) in AHR was observed in mice exposed to NAL alone (Fig 4, B) when compared with mice exposed to vehicle alone. Mice exposed to 5000 EU of endotoxin alone did not demonstrate increased AHR after latex challenge. Mice exposed to NAL and increasing concentrations (50, 1250, or 5000 EU) of endotoxin demonstrated up to 50% lower levels of AHR than mice exposed to NAL alone (Fig 4, B). The decrease observed in NAL-specific AHR on day 76 in mice concurrently exposed to NAL with increasing amounts of endotoxin corresponded with decreased total serum IgE levels for these animals on day 79 for each exposure group.

Decreased mucin production and increased cellular infiltration in coexposed mice

Sections of tissue from the left lung lobe were analyzed for the degree of cellular infiltration and mucin production. All vehicle-exposed animals demonstrated low levels of peribronchial histocytic alveolitis, presumably

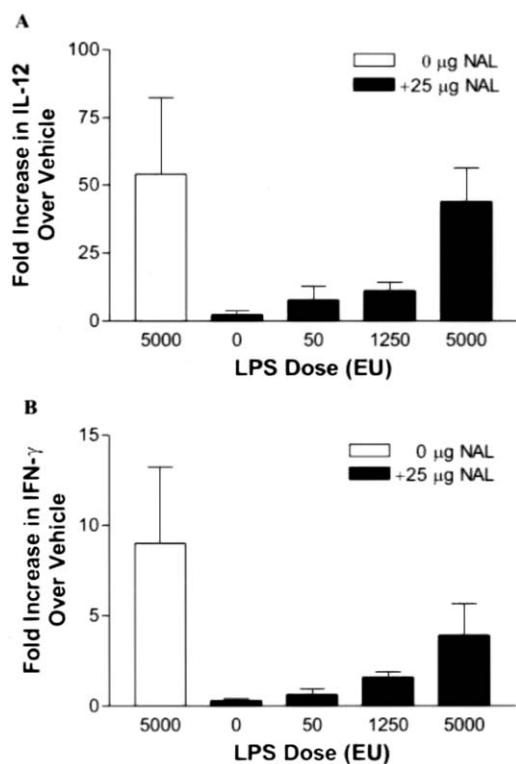


FIG 3. Cytokine gene expression on day 76 in mice exposed to endotoxin, NAL, or concurrently to NAL and endotoxin. Increases in expression of the genes encoding IL-12 (**A**) and IFN- γ (**B**) were determined for each exposure group by calculating the fold increase over vehicle levels, as described in the Methods section. Bars represent means $\pm$ SEs for each group ($n = 5$ for all exposure groups except the vehicle-only group, in which $n = 4$).

because of repeated intratracheal exposure to PBS. Mice exposed to endotoxin alone exhibited perivascular and peribronchial alveolitis with increased numbers of macrophages over those seen in control animals. Latex exposure resulted in multifocal peribronchial and perivascular alveolitis. The severity of pathology appeared to increase with endotoxin concentration as animals exposed to 5000 EU and NAL demonstrated severe diffuse alveolitis with large numbers of macrophages (Table I and Fig 5).

Whereas exposure to both NAL and endotoxin resulted in the infiltration of macrophages, lymphocytes, and neutrophils, an increase in eosinophils was not observed in mice from any of the exposure groups. Differential mucin responses were observed between exposure groups. Lung sections were scored on a +/– scale on the basis of the degree of mucin production in the bronchial epithelium, and the severity of the response is summarized in Table I. Although 3 of 4 and 3 of 5 lung sections from mice exposed to vehicle (Fig 6, A) and endotoxin alone (Fig 6, B), respectively, demonstrated positive (+) staining, increased production of mucin was observed in mice exposed to NAL alone, as evidenced by scoring of ++ or +++ in all mice (Fig 6, C). Mice exposed to endotoxin (50 EU) with latex demonstrated a decrease in

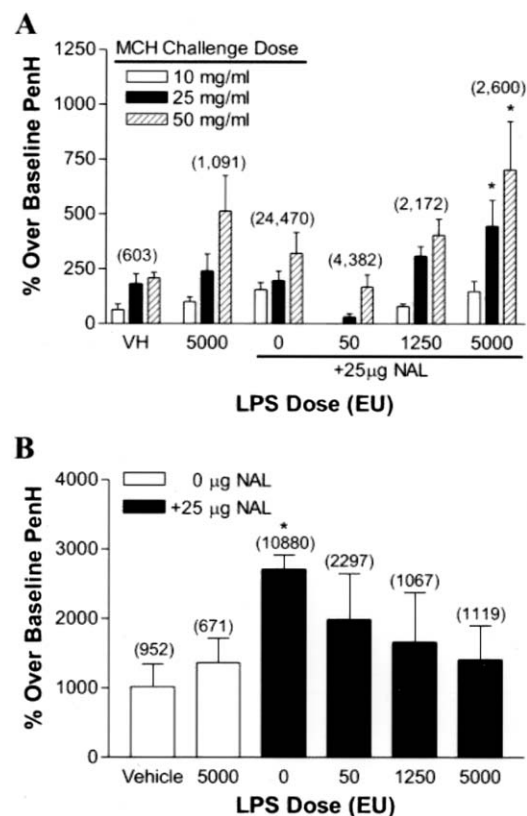


FIG 4. Levels of nonspecific and latex-specific AHR in mice exposed to NAL, endotoxin, or NAL and increasing concentrations of endotoxin. Numbers in parentheses represent the mean total serum IgE levels at the time of nonspecific (day 58; **A**) and latex-specific (day 79; **B**) challenge for each corresponding exposure group. Bars represent means $\pm$ SEs for each group ($n = 5$ for all exposure groups except the vehicle-only group, in which $n = 4$). For each challenge dose of methacholine, exposed mice were compared with vehicle-exposed animals, and statistical significance was determined by using a Dunnett t test. * $P < .05$.

the severity of the response as scores for mucin production were – to ++ (Fig 6, D). With coexposure, the mucin production score decreased to – or + for mice exposed to NAL and 1250 EU (Fig 6, E), and only one mouse scored positively for mucin production among the mice exposed to NAL and 5000 EU of endotoxin (Fig 6, F).

DISCUSSION

The role of endotoxin in the development of allergic disease has been the subject of considerable interest. Because of its presence in both environmental and occupational settings and its ability to alter the T_H1/T_H2 balance, endotoxin has the potential to significantly influence allergic disease. Studies have shown that acute exposure to endotoxin before ovalbumin challenge downregulates the allergen-specific serum IgE level and AHR response in previously sensitized mice.^{28,29} Recent evidence has also suggested an association between exposure to inhaled endotoxin early in life and a reduction

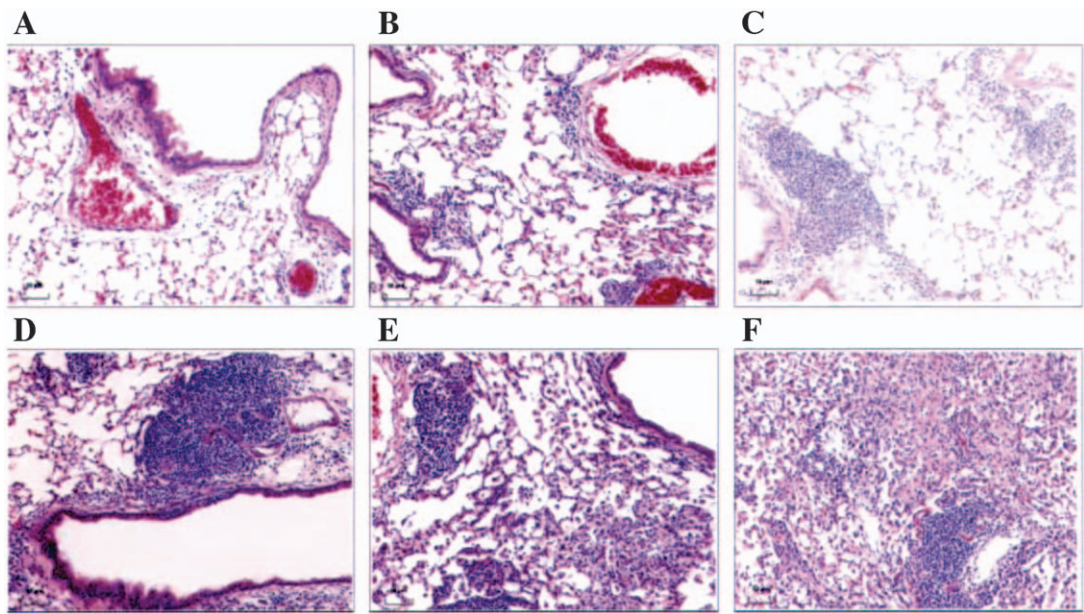


FIG 5. Histopathologic evaluation of cellular infiltration after respiratory exposure to NAL, endotoxin, or NAL and endotoxin from *S typhimurium*. Lungs were excised 24 hours after intratracheal challenge with latex proteins and stained with hematoxylin-eosin stain. Lung sections are representative of mice previously intratracheally exposed to vehicle (**A**), 5000 EU of endotoxin (**B**), NAL (**C**), NAL and 50 EU of endotoxin (**D**), NAL and 1250 EU of endotoxin (**E**), and NAL and 5000 EU of endotoxin (**F**; original magnification, 20×). Bars represent 50 μm.

TABLE I. Histopathologic analysis of the lung after concurrent exposure to latex and increasing concentrations of endotoxin from *S typhimurium*

Exposure group	Mucin score*	No. of responders†	Hematoxylin-eosin evaluation	No. of responders†
Vehicle	—	1/4	Mild peribronchial alveolitis	1/4
	+	3/4	Moderate peribronchial alveolitis	2/4
			Moderate peribronchial and perivascular alveolitis	1/4
5000 EU	—	2/5	Multifocal perivascular and peribronchial alveolitis	5/5
	+	3/5		
NAL	++	2/5	Multifocal peribronchial and pervascular alveolitis	5/5
	+++	3/5		
NAL and 50 EU	—	1/5	Multifocal peribronchial and pervascular alveolitis	5/5
	+	2/5		
	++	2/5		
NAL and 1250 EU	—	1/5	Multifocal peribronchial and pervascular alveolitis with areas of diffuse alveolitis	5/5
	+	4/5		
	—	4/5	Severe diffuse alveolitis	5/5
NAL and 5000 EU	—	4/5		
	+	1/5		

*Lung sections were scored — to +++ on the basis of the degree of mucin production, as visualized by staining with Alcian blue and periodic acid-Schiff stain.
†Represents the number of mice in each exposure group with the particular response.

in the incidence of allergic disease.^{30,31} The studies presented here demonstrate that endotoxin modulates the development of latex-specific IgE in BALB/c mice when mice are concurrently exposed to endotoxin and allergen. One other study has investigated the role of endotoxin in the development of latex-specific IgE. Slater et al³² demonstrated that coadministration of endotoxin augmented rHev b 5–specific IgE and IgG1 levels in

BALB/c mice. Although similar exposure methods were used in the 2 studies, in the present studies mice co-exposed to NAL and endotoxin demonstrated decreased latex-specific IgE levels. Additionally, increased mucin production was observed in mice exposed to NAL without endotoxin, whereas exposure to rHev b 5 did not influence mucin production.³² Key differences between the studies that might explain the divergent

Environmental and occupational respiratory disorders

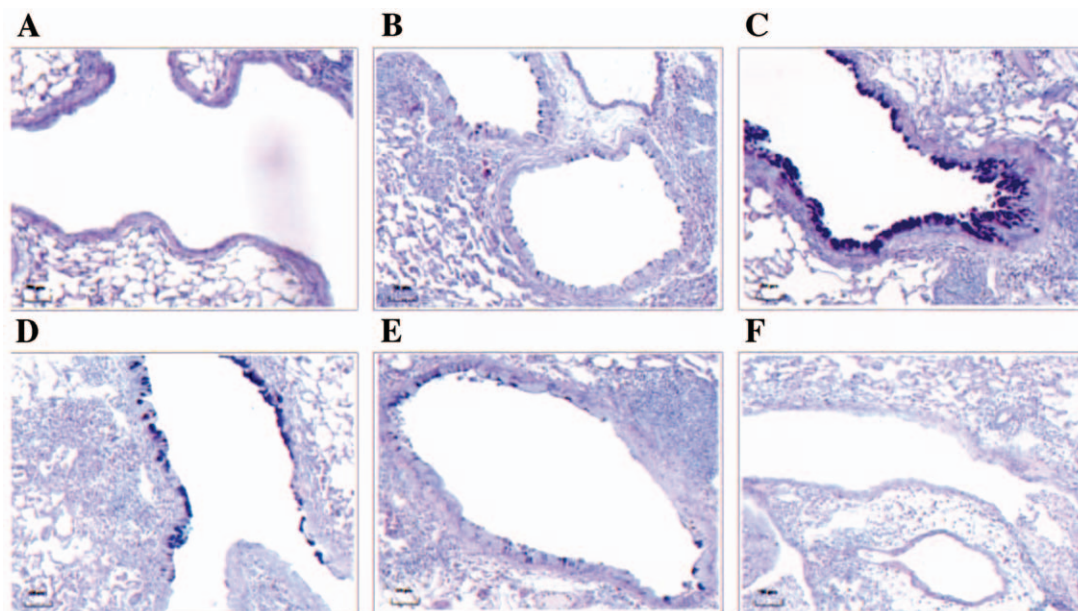


FIG 6. Mucin production in mice after respiratory exposure to NAL, endotoxin, or NAL and endotoxin from *S typhimurium*. Lungs were excised 24 hours after intratracheal challenge with latex proteins and stained with Alcian blue/periodic acid–Schiff stain for visualization of mucin production. Lung sections are representative of mice previously intratracheally exposed to vehicle (**A**), 5000 EU of endotoxin (**B**), NAL (**C**), NAL and 50 EU of endotoxin (**D**), NAL and 1250 EU of endotoxin (**E**), and NAL and 5000 EU of endotoxin (**F**; original magnification, 20 $\times$). Bars represent 50 μ m.

responses include the following: (1) the amount of endotoxin used for exposures in the current studies is approximately 10,000 times lower than those used by Slater et al³²; (2) rHev b 5 is a single antigenic protein, whereas the NAL used in these studies contains approximately 60 antigenic proteins, 12 of which have been identified as allergenic or IgE binding; (3) a different dosing regimen was used; and (4) there was a different time of histopathologic evaluation of the lungs after the final respiratory exposure.

Serum IgE levels are in part modulated by T_H2 cytokines, including IL-4. After intranasal exposure to 30 μ g of NAL proteins, Kurup et al³³ demonstrated increased IL-4 and IL-5 mRNA levels in the spleens of mice, which correlated with an increase in latex-specific serum IgE levels in the same animals. In the present study intratracheal exposure to latex alone did not significantly alter T_H2 cytokine expression in the draining lymph nodes. However, mice exposed to endotoxin alone or concurrently to latex and endotoxin demonstrated increased levels of T_H1 cytokines (IFN- γ and IL-12). Previous literature has described the downregulatory role of these 2 cytokines in the development of IgE.^{34–37} Additionally, increased levels of IL-12 and IFN- γ corresponded to increased levels of IgG2a and IgA and an increase in macrophage and neutrophil infiltration of the lungs. IL-12 produced by macrophages indirectly downregulates the IgE response by inducing IFN- γ production by natural killer cells and T cells.^{36,37} IFN- γ acts directly by inhibiting germline ϵ -chain transcription and inducing a class switch to IgG2a.³⁸ This suggests

a strong relationship between the induction of a T_H1 cytokine response after endotoxin exposure and the subsequent downregulation of the latex-specific IgE response.

In addition to downregulating the allergen-specific IgE response, IFN- γ and IL-12 have been shown to inhibit the development of allergen-specific AHR.^{39–41} In a study by Huang et al,⁴² Brown Norway rats treated with exogenous IFN- γ before ovalbumin sensitization demonstrated a significant reduction in AHR and decreases in IL-4, IL-5, and IL-10 mRNA levels on aerosol challenge with ovalbumin. Schwarze et al⁴³ demonstrated that local administration of IL-12 reduced the AHR and T_H2 cytokine (IL-4 and IL-5) production in previously ovalbumin-sensitized mice. Gerhold et al⁴⁴ observed a decrease in ovalbumin-specific AHR on sensitization with ovalbumin and endotoxin that could be reversed on administration of a neutralizing IL-12 antibody. Mice concurrently exposed to latex and increasing concentrations of endotoxin in these studies demonstrated decreased allergen-specific AHR that coincided with increases in both IL-12 and IFN- γ , as well as IgG2a and IgA antibody levels. The observed increase in latex-specific IgA might also modulate AHR in that antigen-specific IgA downregulates the specific airway response on subsequent respiratory challenge.³⁴

Unlike allergen-specific AHR, nonspecific AHR can occur in both sensitized and nonsensitized individuals. Inhalation of LPS has been shown to induce nonspecific bronchoconstriction in both nonatopic and atopic patients,¹³ and a positive relationship between the influx of neutrophils after endotoxin exposure and nonspecific

AHR to methacholine has been demonstrated.⁴⁵ After chronic exposure to endotoxin, Vernooy et al¹⁵ demonstrated the development of chronic lung inflammation with increased numbers of macrophages and lymphocytes in the peribronchial regions of the lung. Macrophages recruited to the site of exposure release excessive amounts of nitric oxide on exposure to endotoxin, which induces contraction of the smooth muscle, resulting in bronchoconstriction.⁴⁶ In the studies presented here, mice concurrently exposed to 5000 EU of endotoxin and latex exhibited increased levels of nonspecific AHR that correlated with an increase in the mixed cellular alveolitis (ie, macrophages and neutrophils).

In summary, these studies demonstrate that endotoxin is capable of downregulating the development of latex-specific IgE and AHR while enhancing nonspecific AHR and alveolitis. These data illustrate the importance of understanding the role of concurrent environmental or occupational exposures in modulating IgE responses to allergens and interpreting clinical observations attributed to allergic diseases.

We thank Ann Hubbs, DVM, PhD, DACVP, for comments on the histopathologic slides and Patsy Willard, HT, ASCP, for slide preparation and staining.

REFERENCES

- Zak HN, Kaste LM, Schwarzenberger K, Barry MJ, Galbraith GMP. Health-care workers and latex allergy. *Arch Environ Health* 2000;55:336-46.
- Phillips VL, Goodrich MA, Sullivan TJ. Health care worker disability due to latex allergy and asthma: a cost analysis. *Am J Public Health* 1999;89:1024-8.
- Kujala VM, Reijula KE. Glove-induced dermal and respiratory symptoms among health care workers in one Finnish hospital. *Am J Ind Med* 1995;28:89-98.
- Liss GM, Sussman GL. Latex sensitization: occupational versus general population prevalence rates. *Am J Ind Med* 1999;35:196-200.
- Sussman GL, Tarlo S, Dolovich J. The spectrum of IgE-mediated responses to latex. *JAMA* 1991;265:2844-7.
- Kagy L, Blaiss MS. Anaphylaxis in children. *Pediatr Ann* 1998;27:727-34.
- Tomazic VJ, Withrow TJ, Fisher BR, Dillard SF. Latex-associated allergies and anaphylactic reactions. *Clin Immunol Immunopathol* 1992;64:89-97.
- Kelso JM. Latex allergy. *Pediatr Ann* 1998;27:736-9.
- Alenius H, Turjanmaa K, Makinen-Kiljunen S, Reunala T, Palosuo T. IgE immune response to rubber proteins in adult patients with latex allergy. *J Allergy Clin Immunol* 1994;93:859-63.
- Tomei F, Martini A, Marcellini L, Riservato R. Latex allergy. *Panminerva Med* 2000;42:217-22.
- Williams PB, Halsey JF. Endotoxin as a factor in adverse reactions to latex gloves. *Ann Allergy Asthma Immunol* 1997;79:303-10.
- Lantz RC, Birch K, Hinton DE, Burrell R. Morphometric changes of the lung induced by inhaled bacterial endotoxin. *Exp Mol Pathol* 1985;43:305-20.
- Ginanni R, Michel O, Sergysels R, Duchateau J. Characteristics of the bronchial obstructive response induced by inhalation of endotoxins. *Eur Respir J* 1991;4:251-2.
- Yanagihara K, Seki M, Cheng P. Lipopolysaccharide induces mucus cell metaplasia in mouse lung. *Am J Respir Cell Mol Biol* 2001;24:66-73.
- Vernooy JH, Dentener MA, van Suylen RJ, Buurman WA, Wouters EFM. Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology. *Am J Respir Cell Mol Biol* 2002;26:152-9.
- Salkowski CA, Detore GR, Vogel SN. Lipopolysaccharide and monophosphoryl lipid A differentially regulate interleukin-12, Gamma interferon, and interleukin-10 mRNA production in murine macrophages. *Infect Immun* 1997;65:3239-47.
- Friedman H, Blanchard DK, Newton C, Klein T, Stewart W, Keler T, et al. Distinctive immunomodulatory effects of endotoxin and non-toxic lipopolysaccharide derivatives in lymphoid cell cultures. *J Biol Response Modifiers* 1987;6:664-77.
- Tobias PS, Tapping RI, Gegner JA. Endotoxin interactions with lipopolysaccharide-responsive cells. *Clin Infect Dis* 1999;28:476-81.
- Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Ann Rev Immunol* 1995;13:437-57.
- National Research Council. Guide for the care and use of laboratory animals. Washington (DC): National Research Council; 1996p. 125.
- Howell MD, Weissman DN, Meade BJ. Latex sensitization by dermal exposure can lead to airway hyper-reactivity. *Int Arch Allergy Immunol* 2002;128:204-11.
- Woolhiser MR, Munson AE, Meade BJ. Immunological responses of mice following administration of natural rubber latex proteins by different routes of exposure. *Toxicol Sci* 2000;55:343-51.
- Rao GV, Tinkle S, Weissman DN, Antonini JM, Kason ML, Salmen R, et al. Efficacy of a technique for exposing the mouse lung to particles aspirated from the pharynx. *J Toxicol Environ Health A* 2003;66:1441-52.
- Manetz TS, Meade BJ. Development of a flow cytometry assay for the potential identification and differentiation of chemicals known to elicit irritation, IgE-mediated, or T cell-mediated hypersensitivity responses. *Toxicol Sci* 1999;48:206-17.
- Drazen JM, Finn PW, De Sanctis GT. Mouse models of airway responsiveness: physiological basis of observed outcomes and analysis of selected examples using these outcome indicators. In: Hoffman JF, Weer PD, editors. Annual review of physiology. Palo Alto (CA): Annual Reviews; 1999. p. 593-625.
- Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods* 2001;25:386-401.
- Pfaffi MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:2002-7.
- Tulic MK, Holt PG, Sly PD. Modification of acute and late-phase allergic responses to ovalbumin with lipopolysaccharide. *Int Arch Allergy Immunol* 2002;129:119-28.
- Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;22:604-12.
- Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869-77.
- Gehring U, Bischof W, Fahlbusch B, Wichmann H, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002;166:939-44.
- Slater JE, Paupore EJ, Elwell MR, Truscott W. Lipopolysaccharide augments IgG and IgE responses of mice to the latex allergen Hev B 5. *J Allergy Clin Immunol* 1998;102:977-83.
- Kurup V, Kumar A, Choi H, Murali PS, Resnick A, Kelly KJ, et al. Latex antigens induce IgE and eosinophils in mice. *Int Arch Allergy Immunol* 1994;103:370-7.
- Schwarze J, Cieslewicz G, Joetham A, Sun LK, Sun WN, Chang TW, et al. Antigen specific immunoglobulin-A prevents increased airway responsiveness and lung eosinophilia after airway challenge. *Am J Respir Crit Care Med* 1998;158:519-25.
- Snapper CM, Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987;236:944-7.
- Morris SC, Madden KB, Adamovics JJ, Gause WC, Hubbard BR, Gately MK, et al. Effects of IL-12 on in vivo cytokine gene expression and Ig isotype selection. *J Immunol* 1994;152:1047-56.
- Metzger DW, Buchanan JM, Collins JT, Lester TL, Murray KS, Van Cleave VH, et al. Enhancement of humoral immunity by interleukin-12. *Ann N Y Acad Sci* 1996;795:100-15.
- Berton MT, Uhr JW, Vitetta ES. Synthesis of germ-line $\gamma 1$ immunoglobulin heavy-chain transcripts in resting B cells: induction by interleukin 4 and inhibition by interferon γ . *Proc Natl Acad Sci* 1989;86:2829-33.

39. Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and T_H2 cytokine expression in mice. *J Exp Med* 1995;182:1527-36.
40. Tang C, Inman MD, van Rooijen N, Yang P, Shen H, Matsumoto K, et al. T_H type 1-stimulating activity of lung macrophages inhibits T_H2 -mediated allergic airway inflammation by an IFN- γ -dependent mechanism. *J Immunol* 2001;166:1471-81.
41. Huang T, MacAry PA, Eynott P, Moussavi A, Daniel KC, Askenase PW, et al. Allergen-specific T_H1 cells counteract efferent T_H2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN- γ . *J Immunol* 2001;166:207-17.
42. Huang T, MacAry PA, Wilke T, Kemeny DM, Chung KF. Inhibitory effects of endogenous and exogenous interferon-gamma on bronchial hyperresponsiveness, allergic inflammation, and T-helper 2 cytokines in Brown-Norway Rats. *Immunology* 1999;98:280-8.
43. Schwarze J, Hamelmann E, Cieslewicz G, Tomkinson A, Joetham A, Bradley K, et al. Local treatment with IL-12 is an effective inhibitor of airway hyperresponsiveness and lung eosinophilia after airway challenge in sensitized mice. *J Allergy Clin Immunol* 1998;102:86-93.
44. Gerhold K, Blumchen K, Bock A, Seib C, Stock P, Kallinich T, et al. Endotoxins prevent murine IgE production, Th2 immune responses, and development of airway eosinophilia but not airway hyperreactivity. *J Allergy Clin Immunol* 2002;110:110-6.
45. Lefort J, Motreff L, Vargaftig BB. Airway administration of *Escherichia coli* endotoxin to mice induces glucocorticosteroid-resistant and vasopermeation. *Am J Respir Cell Mol Biol* 2001;24:345-51.
46. Toward TJ, Broadley KJ. Chronic lipopolysaccharide exposure on airway function, cell infiltration, and nitric oxide generation in conscious guinea pigs: effect of rolipram and dexamethasone. *J Pharmacol Exp Ther* 2001;298:298-306.