Toll-like receptor 4 antagonist (E5564) prevents the chronic airway response to inhaled lipopolysaccharide


Toll-like receptor 4 antagonist (E5564) prevents the chronic airway response to inhaled lipopolysaccharide. Am J Physiol Lung Cell Mol Physiol 289: L329–L337, 2005. First published April 15, 2005; doi:10.1152/ajplung.00014.2005.—Although chronic inhalation of endotoxin or lipopolysaccharide (LPS) causes all of the classic features of asthma, including airway hyperreactivity, airway inflammation, and airway remodeling, the mechanisms involved in this process are not clearly understood. The objective of this study was to determine whether intratracheal treatment with LPS antagonist (E5564, a lipid A analog) prevented the development of chronic endotoxin-induced airway disease in a mouse model of environmental airway disease. Pretreatment with 10 and 100 μg of E5564 was found to inhibit the airway response (hyperreactivity and inflammation) for up to 48 h after the administration of the compound. Repeated dosing with 50 μg of E5564 intratracheally did not cause any measurable toxicity. Therefore, in a chronic experiment, mice were treated with either E5564 (50 μg) or vehicle three times weekly for 5 wk and simultaneously daily exposed to either LPS (4.65 ± 0.30 μg/m³) or saline aerosol. E5564 was effective in decreasing the airway hyperreactivity to methacholine, the air space neutrophilia, the interleukin-6 in the lung lavage fluid, and the neutrophil infiltration of the airways 36 h after 5 wk of LPS inhalation. Less collagen deposition was observed in the airways of E5564-treated mice compared with vehicle-treated mice after a 4-wk recovery period. Our results indicate that E5564, a Toll-like receptor 4 antagonist, minimizes the physiological and biological effects of chronic LPS inhalation, suggesting a therapeutic role for competitive LPS antagonists in preventing or reducing endotoxin-induced environmental airway disease.

endotoxin; asthma; airway inflammation; airway remodeling; airway pressure-time index; lipid A

ENVIRONMENTAL ENDOXIN is closely associated with asthma exacerbation (5, 21) and by itself can cause persistent lung disease in humans (17, 18) and mice (7, 12, 26) that shares many features in common with allergic asthma. Among agricultural workers, the concentration of endotoxin in the bioaerosol is the most important occupational exposure associated with the development (24, 29) and progression (27) of persistent environmental airway disease.

Although there is a growing understanding of the pathogenesis of endotoxin-induced airway disease, the full cascade of biological events leading to this condition initiated by endotoxin inhalation is not clearly understood. Four or eight weeks of exposure to inhaled lipopolysaccharide (LPS) causes chronic airway disease in mice that are sensitive to endotoxin (26). LPS-challenged mice demonstrate neutrophilic inflammation during the exposure, and airway remodeling continues to develop even after the end of the LPS challenge (26). A similar airway wall lesion is seen in endotoxin-responsive mice following chronic inhalation of grain dust (12), which is known to contain high concentrations of LPS. Prominent features of endotoxin-induced airway disease include increased airway hyperreactivity, which persists despite the removal of the stimulus (LPS) (26), and persistent expansion of the airway subepithelial cross-sectional area, characterized by subepithelial collagen deposition (7, 25).

Subepithelial fibrosis is also a key feature of airway remodeling in human asthma (23) and is more prevalent and more pronounced in individuals with severe or uncontrolled asthma (34). Thus therapeutic interventions that can limit asthma exacerbations could in turn reduce the chronic effects of this disease, reducing health care costs and improving quality of life for patients with asthma and environmental airway disease.

Toll-like receptor (TLR)-4 is an attractive target for novel anti-inflammatory and potentially antiasthmatic agents. TLR-4 is an essential receptor for LPS signaling and is also the main protein involved in recognition of gram-negative bacteria (15). CD14 assists monocyte/macrophage recognition of LPS-LBP (lipopolysaccharide binding protein) complexes and enhances the response of TLR-4 to endotoxin (9), and other components of this signaling cascade, such as MD-2, may be involved in facilitating the interaction of lipid A with TLR-4 (3). However, stimulation of TLR-4 is essential for downstream cellular activation events that in turn are mediated by mitogen-activated kinase p38 and c-Jun NH2-terminal kinase, leading to nuclear translocation of nuclear factor (NF)-κB (6, 13, 14, 33), resulting in cytokine mRNA transcription and release of proinflammatory cytokines (32).

A number of lipid A analogs have been demonstrated to act as LPS antagonists in vitro conditions (30, 31). E5564 (α-L-glucopyranose) is a second-generation synthetic lipodisaccharide synthesized by Eisai Research Institute (Andover, MA) designed to block the effects of endotoxin (10, 22) that has been shown to completely block the effects of experimental endotoxemia in humans (20). The purpose of the present study was to investigate the effects of E5564 on chronic LPS-induced airway disease in mice. We hypothesized that pretreatment with E5564 would diminish or block LPS-induced signaling through TLR-4 and prevent (or at least delay) the development of chronic LPS-induced airway disease.
least diminish) the chronic airway changes associated with persistent inhalation of LPS.

**METHODS**

**Study Design**

To optimize the effective dose of intratracheal (i.t.) instillation of E5564, we compared the physiological and biological effects of LPS inhalation in mice pretreated either with active substance (E5564, 1, 10, or 100 μg in 50 μl of sterile water per animal) or vehicle alone. Intratracheal instillation was performed at either 4 or 24 h before the start of LPS exposure, and results were assessed at 4, 24, and 48 h after the end of the acute LPS exposure (n = 6 per study group). In a second set of experiments E5564 toxicity was evaluated via repeated i.t. instillation of either 50 or 100 μg per mouse, administered three times per week, for 2 wk. Finally, to study whether E5564 alters Fig. 1. Airway responsiveness to methacholine (MCh). Four-hour pretreatment and assessment with whole body plethysmography (WBP) at 4, 24, and 48 h after an acute LPS exposure (A); 24-h pretreatment and WBP assessment at 4, 24, and 48 h after an acute LPS exposure (B); 24-h pretreatment with 100 μg, airway pressure-time index (APTI) assessment at 4 h after an acute LPS exposure (C). Penh, enhanced pause. *P < 0.05 and **P < 0.005 vs. vehicle-treated group.
chronic LPS-induced airway disease, we compared the effect of a 5-wk LPS inhalation challenge in mice treated with E5564 three times per week (50 μg per animal) or with vehicle. In each group (E5564-treated and vehicle-treated), 12 animals were exposed to LPS, and 12 were exposed to PBS. Mice were evaluated at the end of the 5-wk exposure (36 h after the final LPS or PBS aerosol challenge, n = 12 per study group) and 4 wk after the end of exposure (n = 12 per study group).

Animals and Treatment

Six- to eight-week-old male C3HeB/FeJ mice were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were anesthetized with ketamine (200 mg/kg) and xylazine (10 mg/kg) intraperitoneally. E5564 was delivered by gavage via PE-20 tubing. For the chronic study an initial doses of E5564 or vehicle alone was administered 24 h before the inhalation exposure and three times weekly for 5 wk. The study protocol was in accordance with guidelines set forth by the Duke University Animal Care and Use Committee. All procedures involving animals were reviewed and approved by the Duke University Medical Center Institutional Animal Care and Use Committee.

LPS Preparation and Aerosol Exposures

LPS (Escherichia coli serotype 0111:B4; lot #042K4120; Sigma-Aldrich, St. Louis, MO) was prepared as previously described (25). Mice were exposed to LPS or PBS in 60-L Hinnors-style inhalation chambers for 2.5 h for an acute exposure or 2.5 h/day, 5 days/wk, over a 5-wk period for a chronic exposure. Aerosol was delivered using a constant-output six-jet atomizer (9306 model; TSI, Shoreview, MN) that generates particles with a mean diameter of 0.3 μm at a flow rate of ∼3.3 l/min.

LPS Assay

The airborne concentration of LPS was assessed by sampling air drawn from the exposure chamber through a 37-mm binder-free glass fiber filter (Whatman, Maidstone, UK) held within a 47-mm in-line air sampling filter holder (Gelman model 2220). The airborne concentration of LPS was determined as previously described (25) using the chromogenic Limulus amebocyte lysate assay (QCL-1000; BioWhittaker, Walkersville, MD) according to the manufacturer’s instructions. The mean LPS concentration (4.65 ± 0.30 μg/m³) was determined and the corresponding concentration in endotoxin units/m³ was calculated according to the Sigma certificate of analysis.

Whole Body Plethysmography

For screening purposes, whole body barometric plethysmography was used to estimate respiratory response to methacholine (MCh) aerosol challenge. During MCh challenge, enhanced pause (P_{enh}) changes have been demonstrated empirically to correlate with changes in pulmonary resistance and are therefore believed to represent airway obstruction; however, this remains controversial (19). Assessments were performed before the LPS exposure and at 4, 24, and 48 h after the end of the acute exposure, as well as 36 h or 4 wk after the final LPS or PBS aerosol challenge from the chronic protocol. Individual mice were placed in 3-in-diameter chambers (Buxco Electronics, Troy, NY) that were ventilated by bias airflow at a rate of 0.5 l/min/chamber. Respiratory responses were evaluated at baseline.

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**Fig. 2.** Lung lavage fluid cellularity and differentials as determined at 3 different time points after acute LPS exposure (4, 24, and 48 h). Effect of a single administration of different doses E5564 (1, 10, and 100 μg i.t. per animal). Pretreatment 4 h before the exposure (A, B); pretreatment 24 h before the exposure (C, D). All values are presented as means ± SE. *P < 0.05 and **P < 0.005 vs. vehicle-treated group.
Airway Pressure-Time Index

To definitively assess airway hyperreactivity, the time-integrated change in tracheal pressure [airway pressure time index (APTI)] in response to intravenous MCh was measured. Briefly, mice were anesthetized with pentobarbital sodium (65 mg/kg, Nembutal; Abbott Laboratories, N. Chicago, IL). After tracheal and jugular vein cannulation, mice were paralyzed with doxacurium chloride (0.25 mg/kg, Neuromax; Abbott Labs) and ventilated at 125 breaths/min with a tidal volume of 6–8 ml/kg. Tracheal pressure was measured at a side port on the tracheal cannula using a differential pressure transducer (Validyne, Northridge, CA). After a baseline tracheal pressure was established, mice were challenged with MCh doses of 25, 100, and 250 µg/kg iv. Airway response was measured as the sum of the time-integrated change in airway pressure from baseline (cmH2O × s).

Whole Lung Lavage

Mice were killed by CO2 inhalation, and the lungs were lavaged through a PE-90 tube with 6.0 ml of sterile saline, 1 ml at a time at an infusion pressure of 25 cmH2O. Processing of the lavage fluid has been described previously (28). In brief, the lavage fluid was centrifuged for 5 min at 600 g. The supernatant was decanted and stored at -70°C for further use. The cell pellet was resuspended with Hanks’ balanced salt solution (without Ca or Mg) and washed twice. A small aliquot of resuspended cells was used for counting total number of cells using a Bright-Line hemocytometer (Hauser Scientific, Horsham, PA). Seventy-five to one hundred microliters of the cell suspension were spun onto a positively charged glass slide using a Shandon Cytospin centrifuge (Shandon, Southern Sewickley, PA). After cytocentrifugation, cells were stained with HEMA-3 stain, air-dried, and covered with a coverslip with Cytoseal (Stephens Scientific, Kalamazoo, MI). Differential counts of lavage cells were performed at ×400 (dry objective) using an Olympus microscope (Olympus). Two hundred random cells were counted from each slide.

Lung Lavage IL-6 Assay

The levels of IL-6 in lung lavage fluid were quantitated by enzyme-linked immunosorbent assay, using a mouse kit (Biosource International, Camarillo, CA).

Fig. 3. Quantification of inflammatory infiltration of the bronchial wall, expressed as number of polymorphonuclear neutrophils (PMN) per length of the bronchial basement membrane (BM). Four-hour pretreatment group (A); 24-h pretreatment group (B). *P < 0.05 and **P < 0.005 vs. the corresponding vehicle-pretreated group of mice.

Fig. 4. Airway responsiveness to MCh after chronic exposure. WBP at 36 h after 5-wk exposure (A); WBP 4 wk after 5-wk exposure (B). Vehicle-treated LPS-exposed groups are showing increased airway hyperrresponsiveness to 20 mg/ml MCh at both time points. *P < 0.05 vs. vehicle-treated group.
Immediately after collection of the lavage fluid, lungs were perfused with 0.9% saline through the pulmonary artery to remove blood from the vascular bed. The whole right lung was removed and snap-frozen in liquid nitrogen and stored at 

\(-70^\circ\text{C}\) for later use.

Freshly prepared ice-cold 4% paraformaldehyde (Fisher Scientific, Pittsburgh, PA) in 1/10 PBS (pH 7.4) was instilled through the tracheal cannula at a constant pressure of 25 cmH\(_2\)O to inflate and fix the left lung. The left lung was then removed and immersed in 4% paraformaldehyde at 4°C overnight and embedded in paraffin; 5-\(\mu\)m sections were cut for histological evaluation.

**Evaluation of Cellular Infiltration**

Neutrophils infiltrating the bronchial wall were counted on hematoxylin and eosin-stained sections. Images of cross-sectioned airways together with peribronchovascular connective tissue sheets were captured with the aid of Nikon Optiphot-2 light microscope (Nikon, Tokyo, Japan) equipped with Spot charge-coupled device camera system. On the calibrated digital images the perimeter of the bronchiolar basal lamina of each profile was traced, and the length was measured. Polymorphonuclear neutrophils (PMNs) were counted in the subtended area surrounding each bronchiole and in the extravascular connective tissue space (peribroncho-vascular zone). The results were expressed as the number of inflammatory cells per micrometer of airway basal lamina length for each profile. Two sections, representing approximately the same depth of each tissue block, were stained and evaluated. All present peribronchovascular connective tissue sheets in those sections were analyzed from each animal and then averaged per animal. A mean for each animal group (± SE) was calculated. E5564-treated mice were compared with vehicle-treated (control) group.

**Morphometric Quantification of Airway Fibrosis**

For quantification of airway fibrotic changes, Masson-Trichrome staining was performed. Slides were randomized, and a digitized picture of each bronchial profile in every whole lung tissue section was captured as described above and analyzed by Image-Pro Plus software (version 4.0; Media Cybernetics LP, Silver Spring, MD). Labeled areas in the subepithelial region of the airway wall were detected by color thresholding and the total collagen (blue) area was measured. Final results were expressed as stained area per length of the adjacent bronchial basal membrane. The advantage of this method over the total wall thickness measurements, used previously (25), was that the inflammatory cell and the nonfibrotic extracellular matrix were distinguished from the fibrotic changes and therefore excluded from the analysis.

**Statistical Analyses**

All data are expressed as means ± SE. We primarily compared the physiological and biological (bronchoalveolar lavage fluid cellularity and airway morphometry) responses in E5564-treated mice and vehicle-treated controls following three time points (4, 24, or 48 h) after a single LPS exposure and two time points (36 h and 4 wk) after a chronic LPS challenge. The difference between the two variables was analyzed by Mann-Whitney U-test (11). Probability values of \(P < 0.05\) (two-tailed) were considered statistically significant.
RESULTS

Acute LPS Exposure Studies

Airway physiology. Intratracheal instillation of 1, 10, or 100 μg of E5564 per animal 4 h before the LPS inhalation exposure significantly decreased LPS-induced airway hyperresponsiveness (AHR) to 0, 5, and 10 mg/ml aerosolized MCh as measured by whole body plethysmography (WBP) 4 h postexposure (Fig. 1A). Twenty-four hours after the end of the LPS exposure the group pretreated with 10 μg of E5564 per animal also had reduced LPS-induced AHR compared with animals treated with vehicle alone. The effect was no longer present at 48 h after the end of the exposure with any of the doses of E5564 used. When pretreated 24 h before the exposure with either 10 or 100 μg of E5564, the mice showed significant reduction of LPS induced airway hyperreactivity at 4 and 24 h post-LPS exposure (Fig. 1B). APTI also demonstrated that vehicle-pretreated, LPS-exposed group developed increased AHR to MCh (especially to doses of 100 and 250 μg/kg iv), whereas E5564 pretreatment (100 μg only) prevented the development of LPS-induced AHR (Fig. 1C). The only ineffective
dose, in terms of decreasing AHR, with this regimen was 1 μg of E5564 per animal. The beneficial effect of E5564 on LPS-induced airway hyperreactivity was no longer evident 48 h after the exposure to LPS, regardless of the dose of E5564.

Whole lung lavage cellularity. At 4 h after the acute LPS exposure, the total number of lavage cells was significantly reduced in mice pretreated with E5564 at a dose of 10 and 100 μg per mouse when compared with vehicle-treated mice (Fig. 2, A and C). This was most clearly observed in mice pretreated with E5564 24 h before the LPS challenge. At 24 h postexposure, E5564 was effective in decreasing LPS-induced airway inflammation; again, pretreatment with E5564 was most effective when given 24 h before the inhalation challenge (Fig. 2, B and D). The beneficial effect of E5564 on LPS-induced airway inflammation was no longer evident 48 h after the exposure to LPS, regardless of the dose of E5564.

Airway histopathology. Histological evaluation of bronchial profiles together with peribronchovascular zone from LPS-exposed mice revealed extensive bronchitis with neutrophil infiltration of airway wall. In mice pretreated with 100 μg of E5564 4 h before exposure, the number of PMNs calculated per length of the adjacent bronchial basement membrane was significantly (P < 0.05) reduced at 4 and 24 h postexposure (Fig. 3). However, these differences were even more pronounced in mice pretreated with E5564 24 h before the LPS inhalation challenge (Fig. 3). Again, the beneficial effects of E5564 were no longer evident 48 h after the exposure to LPS.

Repeated E5564 i.t. Instillation Studies

To evaluate the safety of E5564 in repeated i.t. instillation regimen, two doses were tested, 50 and 100 μg per mouse 3 days per week for 2 wk, and compared with the vehicle-administered mice. No inhalational exposure was performed during this treatment. Lavage cell counts (data not presented) showed significant neutrophilia in animals that received the 100-μg E5564 dose compared with both 50 μg per mouse E5564- and vehicle-treated controls. Therefore, we chose to treat the mice with 50 μg of E5564 given three times per week.

Chronic LPS Exposure Study

Airway physiology. Thirty-six hours after the chronic 5-wk LPS exposure, vehicle-treated mice showed significantly increased P_{oh} values in response to 20 mg/ml MCh compared with LPS-exposed E5564-treated mice (Fig. 4A). Saline-exposed mice from both treatment groups showed no change in airway reactivity from pre-exposure values. Importantly, E5564 also prevented the development of AHR 4 wk following the chronic exposure to LPS (Fig. 4B).

Whole lung lavage cellularity. Five weeks of LPS exposure induced a significant increase in the total concentration of lavage fluid cells in both vehicle-treated and E5564-treated mice compared with corresponding PBS-exposed control animals (Fig. 5A). However, mice treated with E5564 and exposed to LPS demonstrated a substantial reduction in the total concentration of lavage cells, as well as a decrease percentage of PMNs compared with the similarly exposed vehicle-treated group. After the completion of the 4-wk recovery period, there was no significant difference in total lavage fluid cell count or PMN percentage between the study groups (Fig. 5B).

Lung lavage IL-6 levels. Chronic LPS inhalational challenge increased significantly the concentration of IL-6 recovered in whole lung lavage fluid in vehicle-treated mice compared with PBS-exposed controls. E5564 i.t. treatment during the chronic experiment resulted in a twofold decrease in the concentration of IL-6 in the lavage fluid 36 h after the exposure (Fig. 6). Airway histopathology. Chronic exposure to LPS resulted in a significant peribronchial and perivascular neutrophil inflammation both in the vehicle-treated and TLR-4 antagonist-treated animals (Fig. 7, A and C) compared with the PBS-exposed mice. However, the peribronchial inflammation was significantly reduced in E5564-treated than in vehicle-treated mice (Fig. 7E).

Airway collagen deposition. Thirty-six hours after the chronic exposure to LPS, collagen staining was similar in vehicle-treated and TLR-4 antagonist-treated groups (Fig. 8A).

Fig. 8. Values obtained by morphometric analysis for collagen deposition in 3 sizes of airways: small (diameter ≤90 μm), medium (>90–129 μm), and large (>129 μm). A: collagen area per length of BM after completion of a 5-wk exposure. B: normalized measurements for the collagen area from 4 wk postexposure group of animals. All values are presented as means ± SE. *P < 0.05 vs. vehicle-treated group of mice.
The amount of collagen was compatible in PBS- and LPS-exposed animals. However, after the 4-wk recovery period, collagen deposition was markedly increased in the bronchial walls of medium- and large-size airways in LPS-exposed vehicle-treated group, but significantly less in the similarly exposed E5564-treated mice (Figs. 7, A and D, and 8B).

**DISCUSSION**

Our results indicate that pretreatment with the TLR-4 competitive antagonist E5564 prevents the development of AHR and airway remodeling in a murine model of chronic environmental airway disease. The ability of E5564 to suppress the acute response to inhaled LPS and to provide protection against the long-term effects of repeated LPS exposure supports our previous findings that recurrent episodes of LPS-induced airway injury and inflammation are important in the pathogenesis of chronic environmental airway disease (26). Moreover, since environmental endotoxin is a risk factor for uncontrolled allergic asthma (21), then E5564 may help to control the effects of this common environmental agent and may prove to be beneficial as a preventive therapy for postinflammatory airway remodeling resulting from LPS-induced asthma exacerbation.

Our results suggest that E5564 should be given at least 1 day before an endotoxin challenge and that its effects can last up to 48 h. In fact, mice pretreated 24 h before exposure developed less airway hyperreactivity and less airway inflammation than mice treated 4 h before exposure. One possible explanation for the effectiveness of pretreatment 24 h in advance of LPS exposure is that the effectiveness of E5564 may be dependent on its association with MD-2. E5564 was developed in tandem with E5531, and these LPS antagonists are structurally very similar (22). It has been shown that E5531 inhibits TLR-4-MD-2 binding of LPS directly (2), and it is likely that E5564 acts in the same way. Furthermore, it has recently been shown that LPS responsiveness of epithelial cells is limited by low levels of expression of MD-2 (16). Thus, if MD-2 is required for activity of E5531 (and therefore E5564), then very low endogenous levels of MD-2 could account for the delayed effectiveness of E5564. Furthermore, E5564, because it is an antagonist, likely does not induce MD-2, whereas LPS stimulation of epithelial cells does (1). Thus the ability of the airway to respond to LPS in the presence of E5564 with MD-2 limiting would be reduced dramatically when E5564 is given in advance of LPS exposure (2, 16). Moreover, E5564 appeared to be effective for up to 48 h after instillation, suggesting that the presumed interaction with MD-2/TLR-4 is stable over time. These results suggest that E5564 could be given intermittently and provide long-lasting protection.

E5564 has been shown to inhibit or ablate LPS responsiveness both in vitro (22) and in an in vivo model of human sepsis (20). In acute exposure experiments, E5564 at all doses in treatment 4 h before LPS exposure and at 10 and 100 μg in treatment 24 h before LPS exposure significantly attenuates AHR (Fig. 1, A and B) and at 10 and 100 μg in pretreatment significantly attenuates airway inflammation as well (Fig. 2, A and C). We have previously demonstrated attenuation of the response to inhaled LPS in LBP-deficient mice (8). Similar to mice treated with E5564, LBP-deficient mice had reduced neutrophilic inflammation and reduced AHR after an acute LPS inhalation challenge compared with wild-type control mice. Furthermore, LBP-deficient mice did not have significantly thickened submucosal region compared with wild-type mice (8). We conclude from this study that LBP deficiency reduced the efficiency of TLR-4 signaling in response to LPS, and our current findings with E5564 demonstrate a similar effect. These data taken together suggest that there is a threshold of TLR-4-mediated signaling required to activate the physiological response to inhaled LPS. This hypothesis is supported by a previous study from this laboratory that showed that a common missense mutation in human TLR-4 (Asp299Gly) renders individuals that harbor this mutation hyporesponsive to inhaled LPS (4). The mechanism by which this mutation modulates the response to inhaled LPS in humans is unclear, although the Asp299Gly mutation is in the extracellular domain of TLR-4, suggesting that it has an effect on the efficiency of LPS recognition rather than on the ability to transduce signal such as a cytoplasmic mutation would be expected to have. Our overall hypothesis is that genes, in addition to TLR-4, modulate the response to inhaled LPS and have an important impact on the susceptibility to environmental airway disease. Thus as with mutations in TLR-4 such as the Asp299Gly, or antagonists such as E5564, anything such as LBP that can modulate the efficiency of TLR-4 signaling will have a marked impact on both the acute and the chronic effects of inhaled LPS.

In conclusion, this study demonstrates for the first time that the TLR-4 competitive antagonist E5564 attenuates the long-term adverse effects induced by chronic LPS inhalation challenge in our murine model of chronic environmental airway disease. The ability of E5564 to prevent airway remodeling by reducing LPS-induced leukocyte influx further supports our previous finding (26) that recruitment of the neutrophil from the vascular space to the airway is a necessary step in the development of chronic LPS-induced airway disease.

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