

Dose-dependent effects of endotoxins on allergen sensitization and challenge in the mouse

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Summary

Background Levels of endotoxins greatly differ according to environmental settings.

Objective To study the effect of lipopolysaccharide (LPS) at increasing doses (0.1–1000 ng) on allergen sensitization and challenge in the mouse.

Methods Mice were sensitized systemically and challenged locally with ovalbumin (OVA) in the presence or absence of LPS. Inflammation was assessed by determining total and differential cell counts and T-helper type 2 (Th)2 cytokine (IL-4 and IL-5) levels in bronchoalveolar lavage fluid (BALF). Total and OVA-specific IgE levels were quantified in serum. Airway hyper-responsiveness (AHR) was assessed by whole-body barometric plethysmography.

Results Administered prior to sensitization, LPS at 100 or 1000 ng dose-dependently decreased allergen-induced total and OVA-specific IgE, airway eosinophilia and Th2 cytokines in BALF, without changing AHR. Administered during OVA challenge, LPS at 1 ng (an infra-clinical dose) or 100 ng (a dose triggering neutrophilia) enhanced airway eosinophilia, without affecting IgE levels or AHR.

Conclusion Our data clearly demonstrate that exposure to LPS influences allergen-induced IgE production and airway eosinophilia in a time and dose-dependent manner, preventing IgE production and development of eosinophilia when administered during allergen sensitization at high doses, and inducing exacerbation of eosinophilia when administered upon allergen challenge at low doses, including infra-clinical doses.

Keywords airway hyper-responsiveness, allergy, asthma, endotoxin, eosinophil, IgE, lipopolysaccharide, mouse

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Introduction

Allergic asthma is a chronic inflammatory disease of the airways characterized by airway hyper-responsiveness (AHR) associated with elevated allergen-specific IgE in serum and infiltration of CD4⁺ T-helper type 2 (Th2) cells and eosinophils in the airways [1]. Th2 cells play a central role in the pathogenesis of asthma, as they secrete IL-4 that switches B cells towards IgE synthesis, and IL-5 that promotes eosinophil recruitment [2, 3].

Endotoxins, composed of lipopolysaccharides (LPS), are cell-wall components of Gram-negative bacteria that are ubiquitous in the environment. Endotoxins exhibit potent immune-stimulatory and pro-inflammatory activities when inhaled [4], and have received therefore a lot of attention in the past few years to tentatively explain the increased incidence and severity of asthma observed in developed countries. Growing up on farms, where endotoxins are known to be abundant because of the presence of farming animals, has been associated with lower prevalence of atopic

sensitization, hayfever and asthma in children, suggesting that exposure to high levels of endotoxins in early life may protect from the development of allergic asthma [5–8]. In consistency with this hypothesis, LPS administered at a high dose (10 µg) during sensitization prevented allergen-induced IgE production and airway eosinophilia in the mouse [9]. On the other hand, exposure to elevated endotoxin levels was associated with increased risk of wheeze in the first year of life [10]. As well, subjects with allergic asthma are more sensitive to the bronchoconstrictive and pro-inflammatory effects triggered by inhalation of LPS than normal subjects [11, 12]. Therefore, endotoxin exposure was proposed to exacerbate wheeze and asthma. In agreement with this hypothesis, sensitized rats exposed to LPS exhibited increased inflammation in response to allergen [13]. However, administration of LPS during challenge was also reported to decrease allergen-induced airway inflammation in both rat and mice [9, 13, 14].

Importantly, environmental exposure to endotoxins greatly differs according to settings. Indeed, whereas mean airborne levels ranging from 3.7 to 1200 ng/m³ have been measured in rural settings such as farms, levels found so far in urban homes are considerably lower, namely in the range of 0.01–30 ng/m³ [15–22]. Taking into account a mean ventilation rate of 10 L/min, these levels correspond to a daily

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cumulated exposure to endotoxins of 50–17 000 ng in settings such as farms, compared with 0.14–432 ng in urban homes. However, although environmental exposure to endotoxins covers a wide range, no studies involved any dose–response assessment of the effect of endotoxins in allergen-sensitized and challenged animals.

We have therefore assessed the effect of LPS at increasing doses in a mouse model of allergen sensitization and challenge characterized by production of allergen-specific IgE, airway eosinophilic inflammation and AHR. LPS at doses ranging from 0.1 to 1000 ng was given to mice during allergen sensitization or challenge.

Methods

Reagents

Chicken egg albumin (OVA, Grade V), aluminium hydroxide, LPS (*Escherichia coli*, serotype 055:B5), methacholine (MCh), bovine serum albumin (BSA), Tween-20 and extravidin-horseradish peroxidase conjugate were purchased from Sigma Chemical (Saint Quentin Fallavier, France). Monoclonal anti-mouse IgE (clone R35–72) and biotinylated anti-mouse IgE (clone R35–118) antibodies, mouse IgE standard (clone C38–2) and 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from PharMingen (San Diego, CA, USA). Diff-Quik was purchased from Dade Behring AG (Düdingen, Switzerland). Ketamine (Imalgene, Merial, Lyon, France) and xylazine (Rompum, Bayer, Ruteaux, France) were purchased from Centravet (Velaine-en-Haye, France).

Animals

BALB/c mice were purchased from Charles River Laboratories (Saint-Germain-sur-l'Arbresle, France) and used at the age of 9 weeks. Animals were maintained under controlled environmental conditions with a 12 h/12 h light/dark cycle. Food (UAR-Alimentation, Villemoisson, France) and tap water were available *ad libitum*. Animal care, handling and experimentation complied with the European Union guide for use of laboratory animals and the current law of France.

Allergen sensitization and challenge

Mice were sensitized on days 1 and 7 by intraperitoneal (i.p.) injections of 50 µg OVA adsorbed with 2 mg aluminium hydroxide in saline. Unsensitized animals received i.p. injections of aluminium hydroxide only. Mice were challenged on days 18, 19, 20 and 21 by intranasal (i.n.) instillations of 10 µg OVA in saline or of saline alone for unsensitized animals (Fig. 1a). Intranasal administrations (12.5 µL/nosril) were performed under anaesthesia (50 mg/kg ketamine and 3.33 mg/kg xylazine given i.p.). AHR, inflammation and IgE measurements were performed 18–24 h after the last challenge.

Exposure to lipopolysaccharide

Mice were exposed to LPS during either allergen sensitization or challenge. During sensitization, LPS (10, 100 or 1000 ng in saline) was injected i.p. 1 h prior to each administration of

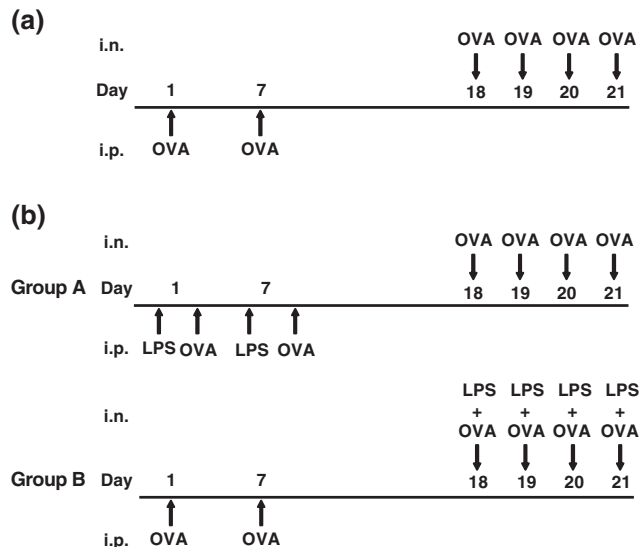


Fig. 1. Experimental protocols. (a) Allergen sensitization and challenge. Mice were sensitized on days 1 and 7 by intraperitoneal (i.p.) injection (50 µg ovalbumin (OVA) adsorbed with 2 mg aluminium hydroxide) and challenged on days 18, 19, 20 and 21 by intranasal (i.n.) instillations (10 µg OVA). (b) Exposure to lipopolysaccharide (LPS). Group A: mice exposed to LPS during allergen sensitization. LPS (10, 100 or 1000 ng) was given i.p. 1 h prior to OVA. Group B: mice exposed to LPS during allergen challenge. LPS (0.1, 1 or 100 ng) was given i.n. together with OVA. All mice were assessed 18–24 h after the last i.n. challenge.

OVA (Fig. 1b, group A). During allergen challenge, LPS (0.1, 1 or 100 ng) was administered by i.n. instillation together with OVA (Fig. 1b, group B). The response to LPS alone was assessed in unsensitized mice instilled i.n. once daily for 4 consecutive days and investigated 18–24 h after the last LPS administration.

Assessment of airway reactivity

Airway reactivity (AR) was assessed in unrestrained, conscious and spontaneously breathing animals by whole-body barometric plethysmography (Emka Technologies, Paris, France), using enhanced pause (Penh) as an index of airway obstruction [23]. Mice were stabilized in the chambers for 30 min until stable baseline, and then exposed to aerosolized saline (30 s) as a control. Then, MCh (0.2 M) was aerosolized for 30 s.

Collection of fluids

Mice were anaesthetized by i.p. injection of ketamine (150 mg/kg) and xylazine (10 mg/kg). Blood was drawn from the abdominal vein for serum determination of total and OVA-specific IgE levels. Sera were collected by centrifugation (3000 × *g* for 15 min at 4 °C) and stored at –20 °C until assay. After blood collection, trachea was cannulated to perform bronchoalveolar lavages (BAL). Lungs were lavaged by 10 instillations of 0.5 mL ice-cold saline supplemented with 2.6 mM ethylenediaminetetraacetic acid (EDTA) (saline-EDTA). Fluids recovered from the first two instillations were centrifuged (3000 × *g* for 5 min at 4 °C), and the resulting supernatant was stored at –20 °C for cytokine measurements.

Fluids recovered from the eight last instillations were dedicated to total and differential cell counts.

Serum immunoglobulin assay

Serum levels of total and OVA-specific IgE were measured by ELISA. Briefly, microtitre plates were coated with a monoclonal anti-mouse IgE antibody or OVA (2 µg/well in 0.1 M NaHCO₃, pH 8.3), respectively, and blocked with 1% BSA in phosphate-buffered saline (PBS-BSA). Serum samples diluted in PBS-BSA were then incubated, followed by a biotinylated anti-mouse IgE antibody and an extravidin-horseradish peroxidase conjugate. The horseradish peroxidase substrate, TMB was added, and colouration was allowed to develop for 15–20 min. The reaction was stopped by addition of 0.5 M H₂SO₄ and absorbance intensity was read at 450 nm. Total IgE concentrations in samples were determined from a standard curve of mouse IgE. Sample levels of OVA-specific IgE were related to a pooled serum from OVA-sensitized animals (internal standard) and expressed as arbitrary units (AU).

Total and differential cell counts in bronchoalveolar lavage fluids

BAL fluids (BALF) were centrifuged (160 × *g* for 5 min at 4 °C) to pellet cells and erythrocytes were lysed by hypotonic shock. Cells were then resuspended in 500 µL ice-cold saline EDTA and total cell counts were determined using a haemocytometer (Neubauer's chamber; VWR, Strasbourg, France). Differential cell counts were assessed on cytologic prepara-

tions obtained by cytocentrifugation (Cytospin 3, Shandon Ltd, Runcorn, Cheshire, UK) of 200 µL of diluted BALF (250 000 cells/mL in ice-cold saline-EDTA). Slides were stained with Diff-Quik and determinations were performed by counting at least 400 cells for each preparation. Cells were identified as macrophages, neutrophils, eosinophils and lymphocytes, and expressed as absolute numbers from total cell counts.

Cytokine assays

IL-4 and IL-5 were quantified in BALF by capture ELISA kits according to instructions provided by the manufacturer (PharMingen).

Statistical analysis

Data are presented as means ± SEM. Statistical differences were analysed from raw data by analysis of variance followed by unpaired two-tailed Student's *t*-test. Data were considered significantly different when *P* < 0.05.

Results

Exposure to lipopolysaccharide at high doses during allergen sensitization prevents immunoglobulin E production and reduces airway inflammation

Levels of total and OVA-specific IgE were significantly increased in OVA-sensitized and challenged mice treated with saline, as compared with unsensitized animals (14.5- and

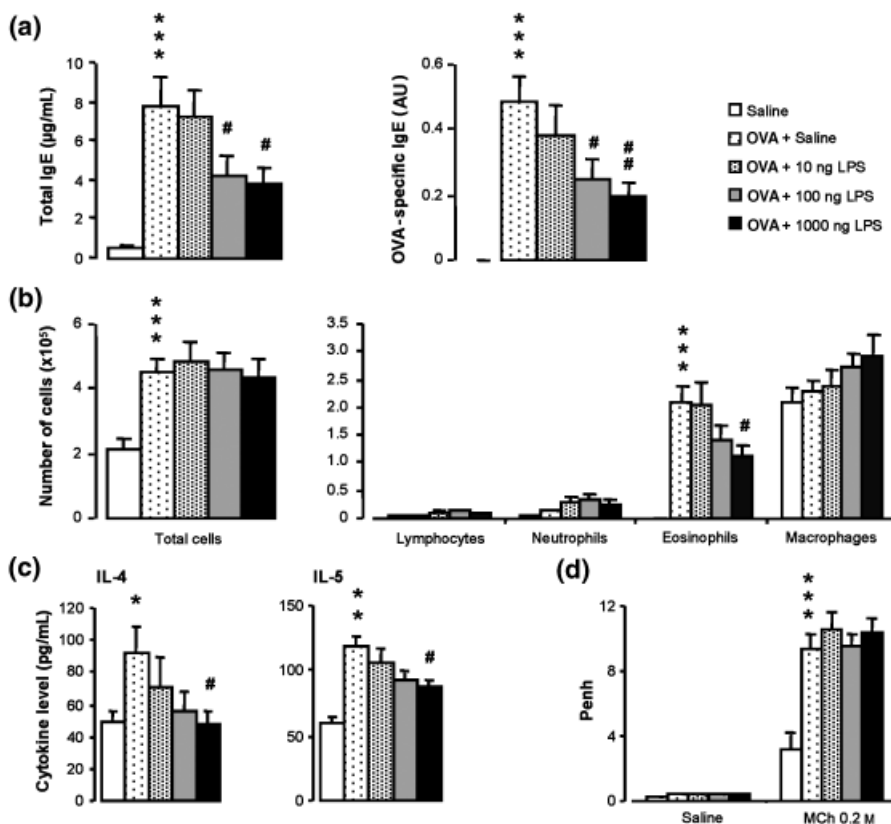


Fig. 2. Effect of exposure to lipopolysaccharide (LPS) during sensitization on allergen-induced responses. (a) Serum levels of total and ovalbumin (OVA)-specific IgE. (b) Total and differential cell counts in bronchoalveolar lavage fluid (BALF). (c) T-helper type 2 (Th2) cell-derived cytokine levels in BALF. (d) Airway reactivity to aerosolized saline and methacholine (MCh). See Fig. 1b, group A, for experimental protocol. Data are means ± SEM of *n* = 6–12 animals. Statistically significant differences at **P* < 0.05; ***P* < 0.01 and ****P* < 0.001, as compared with unsensitized mice (saline). Statistically significant differences at (#)*P* < 0.05 and (##)*P* < 0.01, as compared with OVA-sensitized and challenged mice exposed to saline (OVA+saline).

480-fold, respectively, $P < 0.001$) (Fig. 2a). These increased levels were dose-dependently reduced in OVA-sensitized and challenged mice treated with LPS at high doses (100 or 1000 ng) during allergen sensitization (Fig. 2a). For OVA-specific IgE, reduction reached 60.2% ($P < 0.01$) at 1000 ng LPS.

Airway eosinophilia induced by allergen sensitization and challenge was also dose-dependently inhibited after treatment with high doses of LPS during sensitization (Fig. 2b). Inhibition reached 46.2% at 1000 ng LPS ($P < 0.05$ as compared with OVA-sensitized and challenged mice treated with saline).

Levels of the Th2 cytokines, IL-4 and IL-5, that promote, respectively, IgE production and eosinophil infiltration were increased in BALF from OVA-sensitized and challenged mice as compared with unsensitized mice (1.9-fold increase for both cytokines; $P < 0.05$ and $P < 0.01$, respectively) (Fig. 2c). These increased levels were dose-dependently reduced when LPS was administered during sensitization (Fig. 2c). Reduction reached, respectively, 45.2% and 23.1% at 1000 ng LPS, as compared with saline-exposed mice ($P < 0.05$ for both cytokines).

In contrast, treatment with LPS (10, 100 or 1000 ng) during sensitization did not alter AHR triggered by allergen sensitization and challenge (Fig. 2d). Indeed, maximal Penh values in response to aerosolized MCh (0.2 M) were 10.3 ± 0.8 ($n = 11$) and 9.3 ± 0.9 ($n = 12$) in OVA-sensitized and challenged mice exposed to 1000 ng LPS and saline, respectively, as compared with 3.2 ± 0.9 ($n = 8$) in unsensitized mice.

Exposure to lipopolysaccharide during allergen challenge exacerbates airway inflammation, even at infra-clinical dose

Exposure of OVA-sensitized mice to LPS in a large dose panel (0.1–100 ng) during allergen challenge did not modify serum levels of both total and OVA-specific IgE whatever the dose of LPS used (Fig. 3a). In mice treated with 100 ng LPS, levels of total and OVA-specific IgE were $7.2 \pm 1.2 \mu\text{g/mL}$ and $0.58 \pm 0.08 \text{ AU}$ ($n = 9$), respectively, as compared with $7.6 \pm 1.0 \mu\text{g/mL}$ and $0.45 \pm 0.05 \text{ AU}$ in saline-exposed animals ($n = 9$).

However, treatment with LPS during allergen challenge resulted in a dose-dependent increase in total cell number in BALF, with a 1.8- ($P < 0.01$) and 2.8-fold ($P < 0.001$) increase at 1 and 100 ng LPS, respectively, and no significant changes at 0.1 ng LPS (Fig. 3b). Differential cell counts showed that this increase resulted from increased infiltration of eosinophils at 1 ng LPS (2.4-fold, $P < 0.001$), with no significant changes in the number of other cell types, particularly neutrophils, suggesting potentiation of allergen-induced eosinophil infiltration by LPS. In contrast, at 100 ng LPS, increase in total cell number resulted from increase in all cell types, namely eosinophils (2.8-fold, $P < 0.001$), lymphocytes (5.5-fold, $P < 0.001$), neutrophils (8.0-fold, $P < 0.001$), and macrophages (2.2-fold, $P < 0.001$). As controls, we assessed the effect of LPS alone (0.1, 1 or 100 ng) on airway inflammatory cell infiltration in naïve mice (Fig. 4a). LPS at 0.1 or 1 ng did not exhibit any local pro-inflammatory activity; number of total

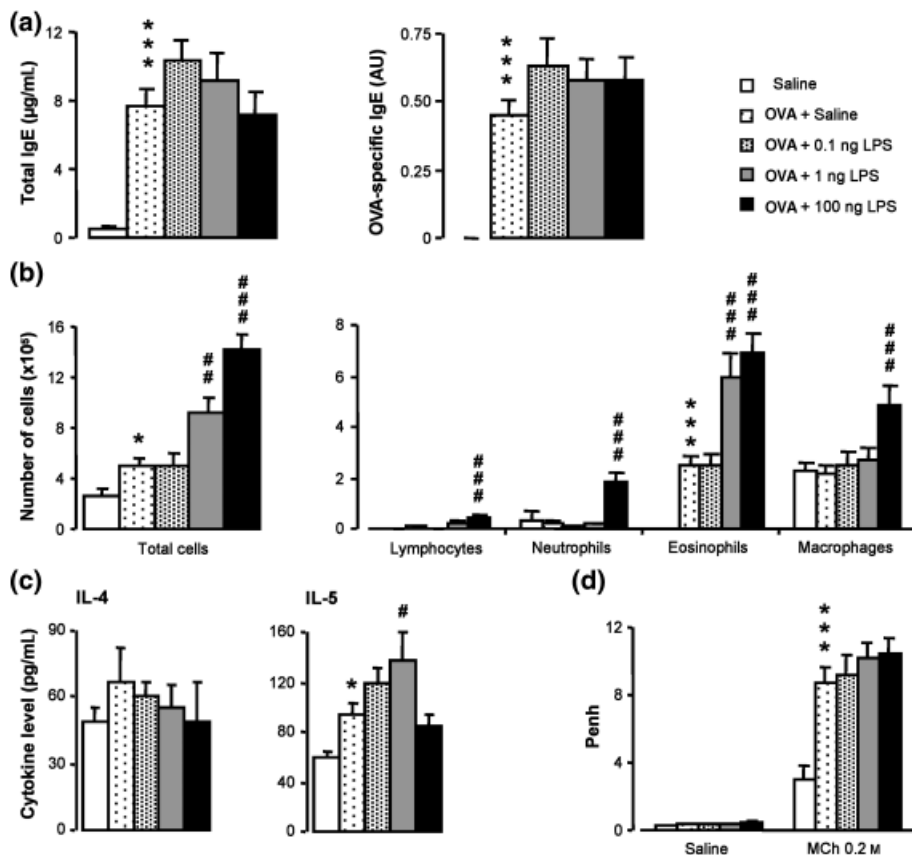


Fig. 3. Effect of exposure to lipopolysaccharide (LPS) during allergen challenge on allergen-induced responses. (a) Serum levels of total and ovalbumin (OVA)-specific IgE. (b) Total and differential cell counts in bronchoalveolar lavage fluid (BALF). (c) T-helper type 2 (Th2) cell-derived cytokine levels in BALF. (d) Airway reactivity to aerosolized saline and methacholine (MCh). See Fig. 1b, group B, for experimental protocol. Data are means $\pm$ SEM of $n = 6$ –12 animals. Statistically significant differences at * $P < 0.05$ and *** $P < 0.001$, as compared with unsensitized mice (saline). Statistically significant differences at (#) $P < 0.05$, (##) $P < 0.01$ and (###) $P < 0.001$, as compared with OVA-sensitized and challenged mice exposed to saline (OVA + saline).

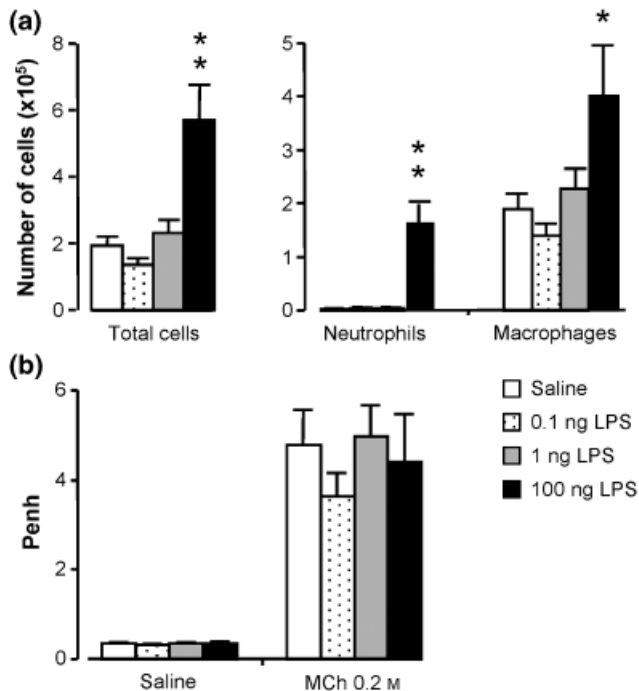


Fig. 4. Lipopolysaccharide (LPS)-induced airway inflammation and hyper-responsiveness in unsensitized mice. LPS (0.1, 1 or 100 ng) was administered by intranasal injection once daily for 4 consecutive days, and animals were assessed 18–24 h after the last instillation. (a) Total and differential cell counts in bronchoalveolar lavage fluid. (b) Enhanced pause (Penh) values in response to aerosolized saline and methacholine (MCh). Data are means $\pm$ SEM of $n = 6$ –12 animals. Statistically significant differences at * $P < 0.05$ and ** $P < 0.01$, as compared with mice exposed to saline.

cells and neutrophils of $232\,344 \pm 38\,399$ and 3796 ± 1075 at 1 ng LPS ($n = 12$), as compared with $192\,788 \pm 27\,587$ and 2693 ± 792 in mice instilled with saline alone ($n = 8$, NS). In contrast, LPS at 100 ng triggered an increase in total cell number (3.0-fold, $P < 0.01$), resulting from increased number of both neutrophils and macrophages (60.0-fold ($P < 0.01$) and 2.1-fold ($P < 0.05$), respectively, as compared with saline-exposed mice), but not eosinophils (not shown).

In parallel, levels of the eosinophil chemoattractant, IL-5 were significantly increased in BALF from mice exposed to 1 ng LPS during allergen challenge (1.5-fold, $P < 0.05$) (Fig. 3c). In contrast, levels of IL-4 were not significantly modified, whatever the dose of LPS used (Fig. 3c).

Allergen-induced AHR remained unchanged in sensitized mice exposed to LPS during allergen challenge, whatever the dose of LPS used (Fig. 3d). As controls, LPS at neither doses (0.1, 1 or 100 ng) induced any change in AHR by itself in naïve animals (Fig. 4b).

Discussion

In this study, we have assessed the effect of LPS given at a wide range of doses on allergen sensitization and challenge in the mouse. Doses of LPS included high doses, such as 100 and 1000 ng, as well as infra-clinical doses as low as 0.1 and 1 ng. Our data clearly demonstrate that exposure to LPS influences allergen-induced IgE production and airway inflammation in a time and dose-dependent manner.

We first showed that LPS at the highest doses prevented allergen-induced IgE production and airway eosinophilia when administered to mice during sensitization. Similar results were obtained by others in mice or rats exposed to LPS upon allergen sensitization [9, 13]. However, exposure used in these studies, i.e. 10 μ g LPS by i.n. instillation or 50 μ g/mL by aerosol were markedly higher than exposure used in the present work. As well, in these studies, no dose-response assessment was conducted. Our work extends these previous data by showing that protection conferred by LPS is dose-dependent. Indeed, whereas protection was achieved at 100 and 1000 ng LPS, low doses of LPS, as observed with 10 ng were ineffective. Interestingly, effective doses in the present study are at least 10–100 times lower than the one previously reported [9, 13, 14]. This suggests that protection conferred by endotoxins may occur for a wide range of exposure, and not only in the most contaminated settings. In agreement with this hypothesis, lower risk of atopic diseases or asthma has been reported in farmers' children [5–7], as well as in urban childrens living in highly contaminated homes [24, 25].

In an opposite manner, we found that LPS increased allergen-induced airway eosinophilia when co-administered locally during allergen challenge. Although endotoxin levels in household have been associated with increased risk of wheeze and clinical severity of asthma [10, 26, 27], experimental studies reported inhibition of allergen-induced inflammation by LPS administered locally during allergen challenge in sensitized animals [9, 13, 14]. These data were however obtained after administration of LPS at high doses, i.e. of 10 and 20 μ g. In the present study, we clearly demonstrate that exacerbation of airway inflammation induced by allergen occurs for lower levels of LPS. Interestingly, this included 100 ng LPS that exhibits a protective effect when given during sensitization, suggesting that endotoxins may trigger opposite effects according to time of exposure. As well, our data show that exacerbation of inflammation associated with allergic asthma may occur upon exposure to infra-clinical doses of endotoxins, as observed for 1 ng LPS. Indeed, this dose of LPS did not have any effect by itself in unsensitized animals and did not trigger neutrophilia in sensitized animals. Taking into account the role of airway inflammation in asthma, these data suggest that low levels of airborne endotoxins, especially in metropolitan household where contamination is generally weak, may influence the severity of respiratory allergic diseases.

Reduction in airway eosinophilia induced by administration of LPS during allergen sensitization was associated with a reduction in serum levels of specific IgE and in BALF levels of the Th2 cytokines, IL-4 and IL-5, which induces IgE production and promotes eosinophil infiltration, respectively. LPS triggers in dendritic cells the production of IL-12, which acts directly on CD4⁺ T cells to favour the generation of a Th1 rather than a Th2 immune response [28, 29]. Gerhold et al. [9] showed that systemic administration of anti-IL-12 monoclonal antibody fully restored allergen-induced IgE production and airway eosinophilia in mice exposed to LPS during sensitization, suggesting that LPS prevented IgE production and development of airway eosinophilia by decreasing the allergen-induced Th2 immune response in a IL-12-dependent manner. In agreement with this hypothesis, we found in the present study that decreased levels of IL-4

and IL-5 in BALF from mice exposed to LPS during sensitization were associated with increased levels of IL-12 (data not shown). Although administration of LPS during sensitization reduced levels of IgE in serum, exposure to LPS during allergen challenge did not affect them. Accordingly, mice exposed to LPS during allergen challenge exhibit no changes in the IgE-inducing cytokine, IL-4 in BALF. This lack of changes in IgE production is in agreement with findings by Tulic et al. [13] who showed that although rats exposed to LPS during sensitization exhibited decreased levels of IgE in serum, down-regulation of IgE production vanished when time left between primary sensitization and LPS exposure increased. By 10 days after primary sensitization, exposure to LPS did not affect IgE production anymore. The lack of effect of LPS on the immune response when given during allergen challenge may be explained by the fact that exposure to LPS occurred after expansion of Th2 clones and generation of IgE-producing B cells. Nevertheless, in the present study, mice exposed to LPS during allergen challenge exhibited increased airway eosinophilia. In animals treated with 1 ng LPS, this was associated with a local rise in the eosinophil chemoattractant, IL-5. By contrast, mice exposed to 100 ng LPS displayed increased airway eosinophilia in the absence of local changes in IL-5, suggesting that another mediator pathway than IL-5 may then trigger eosinophil infiltration. Allergen challenge in the presence of 1 ng LPS triggered eosinophilia only, whereas challenge in the presence of 100 ng LPS led to an increase in eosinophils, lymphocytes, macrophages and neutrophils. Whether this may explain the dissociation between IL-5 and eosinophilia in mice treated with 100 ng LPS remains to be determined.

In agreement with data obtained by others, we found a dissociation between airway eosinophilia and AHR in our model. Indeed, although allergen sensitization and challenge resulted in airway eosinophilia associated with AHR, neither reduced airway eosinophilia in animals exposed to LPS during allergen sensitization nor increased airway eosinophilia in animals exposed to LPS during allergen challenge resulted in any change in AHR. Watanabe et al. [30] recently showed that contamination of OVA with LPS resulted in suppression of allergen-induced AHR in the mouse. However, similar to us, Gerhold et al. [9] found no changes in AHR in allergen-sensitized and challenge mice exposed to LPS during sensitization, despite almost complete abolition of allergen-induced eosinophilia. As well, Rodriguez et al. [14] reported persistence of AHR in animals exhibiting suppressed airway eosinophilia and Th2 cytokine production after treatment with LPS. These two studies, like ours, were conducted in Th2 strains of mice, namely BALB/c or BP2. Whether the Th1 background of the strain used by Watanabe et al. (C57BL/6) plays a role in this discrepancy remains to be determined [31]. Dissociation between changes in airway eosinophilia and AHR has been largely documented in allergen sensitized and challenged animals. Using different strains of mice, Wilder et al. [32] clearly showed that AHR was not correlated with levels of airway eosinophilia in allergen-sensitized and challenged animals. Also, various therapeutic interventions have been reported to inhibit allergen-induced airway eosinophilia, without affecting AHR. In a recent study, Birrell et al. [33] found a clear dissociation between the dose of steroid needed to inhibit airway eosinophilia and that

required to affect AHR in mice. Dissociation between changes in airway eosinophilia and AHR has been also largely documented in asthmatic patients, as reviewed by Brusasco et al. [34]. Our data therefore reinforce the idea that other factors than eosinophils are important in the development of AHR.

In conclusion, through a careful dose-response determination, our study clearly demonstrates that exposure to endotoxins influences allergen-induced IgE production and airway eosinophilia in a time and dose-dependent manner. We first confirm that exposure to high levels of endotoxins during sensitization exhibits a protective effect, and further demonstrate that this effect is dose-dependent. In addition, our data report for the first time that endotoxins administered during allergen challenge exacerbate allergen-induced airway eosinophilia, even at infra-clinical doses. Taking into account the role of airway inflammation in asthma, these data suggest that low levels of airborne endotoxins may be an important environmental factor in the severity of the disease.

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