

Exposure to endotoxin and allergen in early life and its effect on allergen sensitization in mice

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Background: Exposure to endotoxins, allergens, or both in early life might regulate the development of tolerance to allergens later in life.

Objective: We investigated whether continuous exposure of infant mice to aerosolized endotoxin, allergen, or both inhibits subsequent allergen-induced immune and inflammatory responses.

Methods: Infant BALB/c mice were pre-exposed to aerosolized endotoxin, ovalbumin (OVA), or both (3 times a week for the first 4 weeks of life) before systemic sensitization (days 1-14) and repeated airway challenge (days 28-30) with OVA.

Results: Compared with that seen in negative control animals, systemic sensitization and airway allergen challenges induced high serum levels of allergen-specific IgE (0.7 ± 0.09 vs 0.02 ± 0.01 OD units), predominant T_H2 -type cytokine production (IL-5 by splenic mononuclear cells in vitro, 1.2 ± 0.2 vs 0.04 ± 0.06 ng/mL), airway inflammation (bronchoalveolar lavage fluid leukocytes, 125 ± 15 vs $64 \pm 7/\mu\text{L}$; eosinophils, 28 ± 5 vs $1 \pm 0/\mu\text{L}$) and development of in vivo airway hyperreactivity (maximal enhanced pause, 11 ± 1.9 vs 4 ± 0.2). Pre-exposure with LPS before sensitization increased production of specific IgG2a (67 ± 10 vs 32 ± 5 U/mL) but failed to prevent T_H2 -mediated immune responses. Pre-exposure with OVA or with OVA plus LPS completely suppressed allergen sensitization, airway inflammation, and development of in vivo airway hyperreactivity; values were similar to those of negative control animals. Inhibition was due to allergen-specific T-cell anergy indicated by omitted allergen-specific T_H2 and T_H1 immune responses. In addition, combined exposure to endotoxin and allergen induced a general shift toward an unresponsive T_H1 immune response.

Conclusion: Exposure with endotoxins before allergen sensitization is not able to induce unresponsiveness but might decrease the susceptibility for sensitization to a variety of common allergens. (J Allergy Clin Immunol 2003;112:389-96.)

Key words: Asthma, cytokines, lipopolysaccharide, mice, ovalbumin

Abbreviations used

AR:	Airway reactivity
BAL:	Bronchoalveolar lavage
ConA:	Concanavalin A
DC:	Dendritic cell
MCh:	Methacholine
MNC:	Mononuclear cell
OVA:	Ovalbumin
PBLN:	Peribronchial lymph node

Atopic diseases, such as bronchial asthma or atopic dermatitis, are steadily becoming more prevalent.^{1,2} There is growing support for the hygiene hypothesis, with several studies demonstrating that children who grow up on farms are less likely to have allergic diseases.³ These children are exposed to immunomodulatory components, such as LPS, fungi, organic dusts, and animal proteins,^{4,5} at higher concentrations than in non-farming environments.⁶ One or the other of these environmental factors, such as LPS, might enhance T_H1 immune reactions, thus inhibiting development of proallergic T_H2 immune responses.⁷⁻⁹ In a murine model of bronchial asthma, systemic treatment with LPS immediately before allergen sensitization was shown to inhibit T_H2 immune responses and allergen-mediated airway inflammation through increased production of IL-12, a central T_H1 cytokine.¹⁰

Interestingly, children living in frequent contact with farm animals are not only exposed to increased concentrations of bacterial components, such as LPS, but also to high concentrations of various aeroallergens, yet they have atopic disorders to a lesser degree than peers living in urban conditions. This argues against the assumption that allergen avoidance is a perfect means for primary allergy prevention and highlights the paradox that early exposure to allergens has been shown to be associated on the one hand with increased sensitization rates¹¹ and on the other hand with decreased prevalence rates for allergic rhinitis and asthma.¹²

To further delineate the effects of exposure to bacterial components, allergens, or both in early life on the development of allergen-induced immune responses, we used a murine model of allergen sensitization to investigate the effects of continuous exposure to aerosolized LPS, allergen, or both early in life on T_H2 immune responses and development of airway inflammation and in vivo airway reactivity (AR) after subsequent allergen

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TABLE I. Effects of LPS pre-exposure on LPS-mediated immune responses

Pre-exposure	n	IgE (ng/mL)	IgG1 (μg/mL)	IgG2a (μg/mL)	Proliferative responses (cpm)	IFN-γ (pg/mL)
PBS	11	268 ± 72	225 ± 31	32 ± 5	753 ± 185	1064 ± 454
LPS	8	72 ± 3*	131 ± 24*	31 ± 2	222 ± 8*	529 ± 226*

Serum levels of total Ig and proliferative responses and IFN-γ production by splenic MNCs cultured with LPS *in vitro* were measured in sham-sensitized mice pre-exposed to PBS or LPS.

**P* < .05 versus negative control animals.

sensitization and airway challenges. The results of this study might improve understanding of the relationship between the environment and allergic disease.

METHODS

Experimental protocol

All experimental procedures were approved by the animal ethics committee. Pregnant BALB/c mice were purchased from Harlan Winkelmann. Offspring were placed in a Plexiglas chamber (20 × 20 × 15 cm³) and exposed for 10 minutes, 3 times per week, from birth (day 28) to day -3 to one of the following aerosolized solutions (nebulizer by Medical Assistance System): (1) LPS (1 μg/10 mL, *Escherichia coli* O111:B4, Sigma); (2) ovalbumin (OVA; Grade V, 100 mg/10 mL, Sigma); or (3) a combination of LPS and OVA at the same concentrations. OVA did not contain significant levels of LPS, as indicated by low IL-1 production by OVA-stimulated blood mononuclear cells (MNCs) from healthy subjects compared with IL-1 levels after stimulation with definite LPS concentrations (Ingo Spreitzer, Paul-Ehrlich-Institut). Controls for effects of pre-exposure were sham exposed to aerosolized PBS. Between day 0 and day 14, mice were systemically sensitized by means of 6 intraperitoneal injections of OVA (10 μg/100 μL per mouse, Grade VI, Sigma). Control animals for effects of sensitization were sham sensitized with PBS (100 μL per mouse administered intraperitoneally). Negative control animals were sham exposed and sham sensitized with PBS. On days 28, 29, and 30, mice of all groups were exposed to aerosolized OVA (100 mg/10 mL, Grade V) for 20 minutes daily.

Serum levels of total and OVA-specific Ig

On day 32, serum levels of total and OVA-specific Ig were measured by means of ELISA, as previously described.¹³ Levels of OVA-specific IgE were expressed as OD units, and levels of OVA-specific IgG1 and IgG2a were related to pooled standards generated in our laboratory and expressed as arbitrary units per milliliter.

In vitro cytokine production

On day 32, splenic MNCs and peribronchial lymph node (PBLN) cells were isolated and cultured with concanavalin A (ConA; 2.5 μg/mL, Sigma) or OVA (50 μg/mL).⁹ Levels of cytokines were assessed in cell culture supernatants by means of ELISA, as previously described.^{10,13} Detection limits were 63 pg/mL for IL-2, 16 pg/mL for IL-4, 32 pg/mL for IL-5, and 78 pg/mL for IFN-γ. Levels of IL-10 and TGF-β were assessed by using Pharmingen optEia kits (PharMingen), according to the manufacturer's instructions.

Proliferation assay with tritiated thymidine

On day 32, proliferative responses of splenic MNCs cultured with medium, ConA, OVA, or LPS (5 μg/mL) were determined by means of tritiated thymidine incorporation (0.5 μCi/200 μL, Amersham Buchler), as previously reported.¹⁰

Bronchoalveolar lavage

On day 32, lungs were lavaged twice with 0.8 mL of PBS. In the supernatants of the first aliquots, levels of IL-5 and IL-12 were measured by ELISA. Cells of both aliquots were pooled, and cytospin slides were stained with Diff Quik (Dade Behring AG, CH). Cells were differentiated because of morphologic criteria by counting 200 cells using light microscopy.

Immunohistochemistry of lungs

Localization of eosinophils in acetone-fixed lung sections were visualized by means of mixed immunohistochemistry with a mAb against mouse major basic protein (kind gift of J. Lee, Scottsdale, Ariz), as previously described.^{13,14} Slides were studied by means of fluorescence microscopy.

In vivo AR

On day 31, *in vivo* lung function (Sigma) was performed with whole-body plethysmography (EMKA Technologies, F, similar to Buxco system), as previously reported.¹⁵ Animals were exposed to aerosolized PBS as a baseline and then to methacholine (MCh) in increasing concentrations (6-50 mg/mL). AR was expressed as increase of enhanced pause (Penh) values for each concentration of MCh relative to baseline Penh values. Concentrations of MCh inducing a 200% increase of corresponding baseline Penh values (MCh-PC₂₀₀Penh) were calculated in each case.

Statistical analysis

Data of animals treated in the same way in up to 4 independent experiments were pooled. Values for all measurements are expressed as means ± SEM. Pairs of groups were compared by using the Mann-Whitney *U* test. Statistical significance was set at a *P* value of less than .05. *P* values of less than .01 are statistically highly significant.

RESULTS

Effects of aerosolized LPS exposure on LPS-mediated immune responses

In sham-sensitized mice pre-exposed to LPS, serum levels of total IgE and IgG1, but not levels of total IgG2a, were significantly decreased compared with those of negative control animals. The decrease is associated with diminished proliferative responses and IFN-γ production by splenic MNCs after culture with LPS (Table I) but not with ConA (data not shown). In addition, these animals did not have significant serum levels of TNF-α (below detection limits) or acute neutrophilic airway inflammation or airway hyperreactivity (data not shown) compared with those of negative control animals.

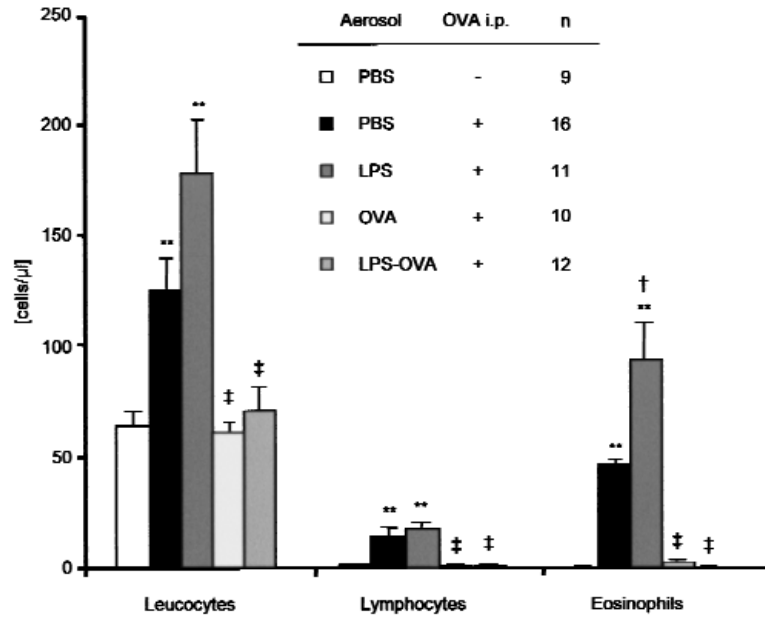


FIG 1. Effects of pre-exposure with LPS, OVA, or both on the development of allergen-induced eosinophilic airway inflammation. Cells of BAL fluid recovered from mice pre-exposed to PBS, LPS, OVA, or LPS plus OVA followed by sensitization with OVA were differentiated for investigation of allergen-induced airway inflammation. * $P < .05$, ** $P < .01$ versus negative control animals; † $P < .05$, ‡ $P < .01$ versus OVA-sensitized mice.

TABLE II. Effects of pre-exposure with LPS, OVA, or both on Ig production

Pre-exposure	OVA (ip)	n	OVA specific			Total		
			IgE (OD units)	IgG1 (U/mL)	IgG2a (U/mL)	IgE (ng/mL)	IgG1 (μg/mL)	IgG2a (μg/mL)
PBS	—	11	0.02 ± 0.01	7 ± 4	14 ± 0	127 ± 30	225 ± 31	32 ± 5
PBS	+	16	0.7 ± 0.09†	4176 ± 85†	41 ± 10*	2677 ± 625†	1259 ± 279†	67 ± 10†
LPS	+	12	0.94 ± 0.17†	7719 ± 2998†	39 ± 7†	3364 ± 677†	2281 ± 382†‡	120 ± 34†
OVA	+	10	0.01 ± 0.01§	15 ± 5§	14 ± 0‡	98 ± 9§	1057 ± 269*	93 ± 14†
LPS + OVA	+	10	0.01 ± 0.00§	18 ± 5§	14 ± 0‡	128 ± 35§	552 ± 112*‡	153 ± 21†§

Serum levels of OVA-specific and total Ig were quantified in mice pre-exposed to aerosolized PBS, LPS, OVA, or LPS plus OVA before sham sensitization with PBS or sensitization with OVA.

ip, Intraperitoneal.

* $P < .05$, † $P < .01$ versus negative control animals.

‡ $P < .05$, § $P < .01$ versus OVA-sensitized mice.

Effects of aerosolized LPS exposure, OVA exposure, or both on allergen-induced sensitization and inflammatory responses

Effects on Ig production. In OVA-sensitized mice, OVA-specific IgE, IgG1, and IgG2a levels were significantly increased compared with those of negative control animals accompanied by highly increased serum levels of total IgE and IgG1 (Table II). LPS pre-exposure did not inhibit production of OVA-specific and total Ig and did enhance serum levels of total IgG1 compared with that of OVA-sensitized mice (Table II). Pre-exposure with OVA abolished production of OVA-specific Ig, as well as total IgE (Table II), but did not alter levels of total IgG1 or IgG2a (Table II) compared with those of OVA-sensitized mice. In contrast, pre-exposure with LPS plus OVA suppressed production of OVA-specific and total

IgE nearly completely and reduced levels of total IgG1 significantly, whereas production of OVA-specific and total IgG2a was strongly enhanced compared with that seen in OVA-sensitized mice (Table II).

Effects on airway inflammation. Bronchoalveolar lavage (BAL) fluids of negative control animals contained few cells (Fig 1) differentiated mainly as monocytes (not shown), and no eosinophils were detectable in lung tissues (data not shown). Cellular composition of BAL fluids of mice pre-exposed to OVA ($n = 6$) or LPS plus OVA ($n = 6$) before sham sensitization with PBS was similar to that of negative control animals (data not shown). OVA-sensitized mice had allergen-induced airway inflammation, as characterized by influx of cells into the airways, mostly lymphocytes and eosinophils (Fig 1). Eosinophils accumulated predominantly in peribronchial and perivascular regions of lung tissues (Fig 2).

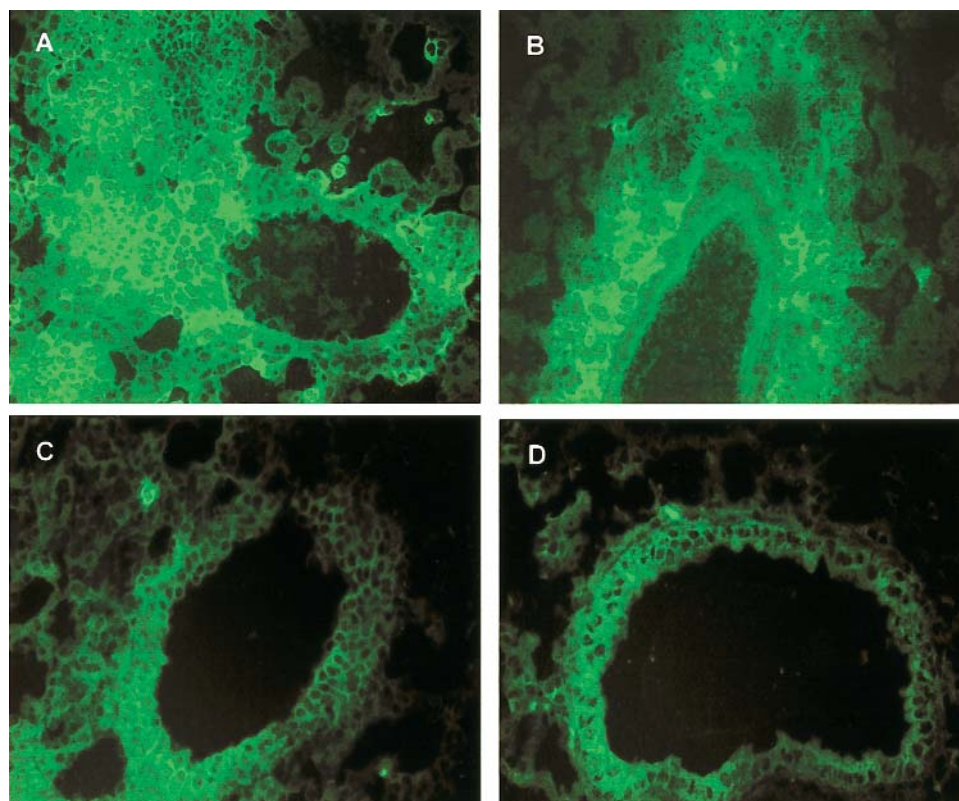


FIG 2. Effects of pre-exposure with LPS, OVA, or both on accumulation of eosinophils in lung tissues. In lung sections of mice pre-exposed to aerosolized PBS (**A**), LPS (**B**), OVA (**C**), or LPS plus OVA (**D**) followed by sensitization with OVA, distributions of eosinophils were investigated by means of immunohistochemistry with a mAb against major basic protein (original magnification 20 $\times$).

LPS pre-exposure failed to prevent development of airway inflammation (Figs 1 and 2) but rather enhanced recruitment of eosinophils into the airways compared with that seen in OVA-sensitized mice (Fig 1). On the contrary, pre-exposure with OVA or with LPS plus OVA almost completely prevented allergen-induced development of eosinophilic airway inflammation. Cellular patterns of BAL fluids were similar to those of negative control animals (Fig 1), and only very few eosinophils were scattered over the peripheral and peribronchial regions of lung tissues (Fig 2).

Effects on in vivo AR. Sham-sensitized mice pre-exposed to OVA ($n = 6$) or LPS plus OVA ($n = 3$) did not have increased in vivo AR to increasing doses of MCh compared with that seen in negative control animals (data not shown). In contrast, OVA-sensitized mice had increased in vivo AR compared with that of negative control animals (Fig 3): maximum Penh values were significantly enhanced ($\text{Penh}_{\text{max}} = 11 \pm 1.9$ vs 4 ± 0.2 , $P < .01$), and concentrations of MCh inducing a 200-fold increase of baseline Penh values were strongly decreased ($\text{MCh-PC}_{200}\text{Penh} = 6 \pm 1.5$ vs 15 ± 3.7 mg/mL, $P < .05$). LPS pre-exposure did not inhibit development of increased in vivo AR. In contrast, both pre-exposure with OVA and with LPS plus OVA in early life resulted in significantly decreased in vivo AR (OVA: $\text{Penh}_{\text{max}} = 4.9 \pm 1.2$, $P < .01$, $\text{MCh-PC}_{200}\text{Penh} = 20 \pm 5.8$ mg/mL, $P < .05$; LPS

plus OVA: $\text{Penh}_{\text{max}} = 4.5 \pm 0.5$ and $\text{MCh-PC}_{200}\text{Penh} = 15 \pm 2.3$ mg/mL, $P < .01$) compared with that of OVA-sensitized mice (Fig 3).

Effects of aerosolized LPS exposure, OVA exposure, or both on allergen-specific immune responses

Effects on allergen-specific proliferative responsiveness. For OVA-sensitized mice, proliferative responses of splenic MNCs to OVA in vitro were significantly increased compared with those seen in negative control animals (Table III). LPS pre-exposure did not alter allergen-specific immune responses compared with that of OVA-sensitized mice (Table III). In contrast, pre-exposure with OVA or LPS plus OVA completely suppressed allergen-specific immune responses of splenic MNCs compared with that of OVA-sensitized mice (Table III).

Effects on in vitro cytokine production. In OVA-sensitized mice production of IL-2 and T_H2 cytokines by splenic MNCs cultured with OVA in vitro were significantly enhanced compared with those of negative control animals (Table III). In all groups of OVA-sensitized animals, in vitro production of IFN- γ by splenic MNCs in response to OVA was less than the limit of detection (data not shown). LPS pre-exposure did not significantly modify in vitro cytokine production compared with that of OVA-sensitized

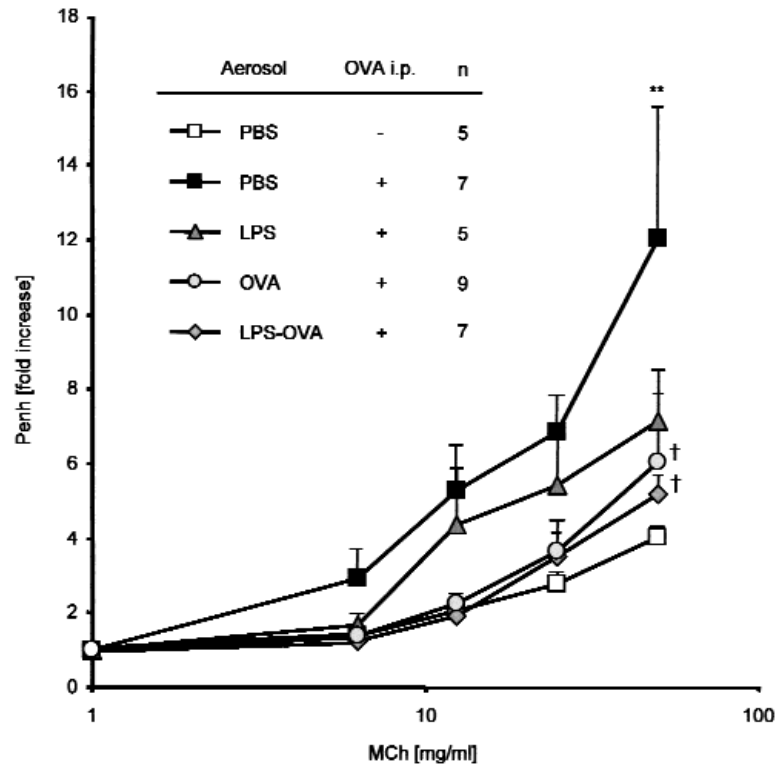


FIG 3. Effects of pre-exposure with LPS, OVA, or both on the development of increased in vivo AR. In mice pre-exposed to aerosolized PBS, LPS, OVA, or LPS plus OVA followed by sensitization with OVA, in vivo AR in response to increasing doses of aerosolized Mch was calculated by means of whole-body plethysmography. Data are expressed as increases of Penh values for each concentration of MCh relative to baseline Penh values. * $P < .05$ versus negative control animals, † $P < .05$ versus OVA-sensitized mice.

TABLE III. Effects of pre-exposure with LPS, OVA, or both on allergen-specific immune responses in vitro

Pre-exposure	OVA (ip)	n	Proliferative responses (cpm)	IL-2 (pg/mL)	IL-4 (pg/mL)	IL-5 (ng/mL)	IL-10 (pg/mL)
PBS	-	7	38 ± 9	63 ± 0	31 ± 0	0.04 ± 0.06	25 ± 6
PBS	+	8	323 ± 81*	183 ± 45†	133 ± 18†	2.9 ± 0.6†	1202 ± 194†
LPS	+	14	319 ± 139	160 ± 46†	91 ± 15†	4.1 ± 0.7†	1488 ± 236†
OVA	+	10	28 ± 6‡	63 ± 0‡	32 ± 1‡	0.03 ± 0‡	16 ± 0‡
LPS + OVA	+	11	54 ± 8‡	63 ± 0‡	31 ± 0‡	0.03 ± 0‡	26 ± 0‡

Proliferative responses and cytokine production by splenic MNCs cultured with OVA in vitro were measured in mice pre-exposed to PBS, LPS, OVA, or LPS plus OVA followed by sensitization with OVA.

ip, Intraperitoneal.

* $P < .05$, † $P < .01$ versus negative control animals.

‡ $P < .01$ versus OVA-sensitized mice.

mice (Table III). In contrast, both pre-exposure with OVA and with LPS plus OVA almost completely suppressed in vitro production of T_H1 and T_H2 cytokines compared with that of OVA-sensitized mice (Table III). In addition, both pre-exposure with OVA and LPS plus OVA did not induce significant in vitro production of TGF- β by splenic MNCs cultured with OVA (data not shown).

In OVA-sensitized mice local draining PBLNs were enlarged, and PBLN cell numbers significantly increased compared with those of negative control animals ($7.05 \pm 0.48 \times 10^6/\text{mL}$ [$n = 6$] vs $0.84 \pm 0.48 \times 10^6/\text{mL}$ [$n = 5$],

$P < .01$). LPS pre-exposure did not diminish allergen-induced infiltration of cells into PBLNs ($8.54 \pm 1.92 \times 10^6/\text{mL}$ [$n = 6$], $P < .01$ vs negative control animals). In contrast, pre-exposure with both OVA and LPS plus OVA inhibited increase of PBLN cell counts (OVA pre-exposure: $1.27 \pm 0.18 \times 10^6/\text{mL}$ [$n = 3$], $P < .05$; LPS plus OVA pre-exposure: $2.06 \pm 0.79 \times 10^6/\text{mL}$ [$n = 5$], $P < .01$ vs OVA-sensitized mice). The cytokine pattern produced by PBLN cells in response to OVA in vitro was very similar to that of splenic MNCs in each treatment group (data not shown).

TABLE IV. Effects of pre-exposure with LPS, OVA, or both on unspecific immune responses in vitro

Pre-exposure	OVA (ip)	n	IL-5 (pg/mL)	IFN- γ (pg/mL)
PBS	–	11	190 $\pm$ 27	1234 $\pm$ 235
PBS	+	16	386 $\pm$ 58*	646 $\pm$ 119*
LPS	+	12	446 $\pm$ 52†	773 $\pm$ 213*
OVA	+	10	307 $\pm$ 48*	1361 $\pm$ 257
LPS + OVA	+	10	216 $\pm$ 22‡	1174 $\pm$ 383‡

Cytokine production by splenic MNCs cultured with ConA in vitro were measured in mice pre-exposed to PBS, LPS, OVA, or LPS plus OVA before sham sensitization with PBS or sensitization with OVA.

ip, Intraperitoneal.

* $P < .05$, † $P < .01$ versus negative control animals.

‡ $P < .05$ versus OVA-sensitized mice.

Effects on cytokine levels in BAL fluids. Similar to decreased T_H2 cytokine production by splenic MNCs and PBLN cells in response to OVA in vitro, pre-exposure with OVA or LPS plus OVA almost completely suppressed levels of IL-5 in BAL fluids compared with those seen in OVA-sensitized mice. Pre-exposure with LPS or OVA did not alter levels of IL-12 in BAL fluids compared with those of OVA-sensitized mice. In contrast, pre-exposure with LPS plus OVA strongly enhanced levels of IL-12 compared with those of OVA-sensitized mice (1262 $\pm$ 412 vs 407 $\pm$ 102 pg/mL, $P < .05$).

Effects of aerosolized LPS exposure, OVA exposure, or both on unspecific immune responses

As shown in Table IV, OVA sensitization induced a general shift in T_H1/T_H2 immune responses indicated by significantly enhanced in vitro T_H2 (IL-5) and decreased in vitro T_H1 (IFN- γ cytokine production by splenic MNCs unspecifically cultured with ConA compared with that in negative control animals. This predominant in vitro T_H2 production in response to mitogen was not altered by LPS or OVA pre-exposure compared with that seen in OVA-sensitized mice. In contrast, pre-exposure with LPS plus OVA induced a shift from a predominant T_H2 toward a predominant T_H1 immune response on mitogenic stimulation indicated by significantly decreased IL-5 and enhanced IFN- γ production by splenic MNCs cultured with ConA compared with that seen in OVA-sensitized mice.

DISCUSSION

Compelling data from several epidemiologic studies show that growing up in farming or rural environments with continuous exposure to high concentrations of endotoxins, allergens, or both is protective against allergen sensitization and asthma without defining a precise mechanism.^{6,9} To further delineate the effects of environmental factors, such as exposure to LPS, allergen, or both in early life, on subsequent allergen-induced immunopathology, we used a mouse model of allergen sensitization and airway allergen challenges. This model features some of the patho-

physiologic changes also observed in human bronchial asthma: production of increased specific and total IgE serum levels, vast and predominantly eosinophilic airway inflammation, and development of in vivo airway hyperactivity dependent on increased production of T_H2 cytokines, such as IL-4 and IL-5, by allergen-specific CD4⁺ T cells.^{16,17} In the current study infant mice were continuously exposed to aerosolized LPS, allergen, or both, mimicking natural contact with these environmental factors. The results demonstrated that (1) allergen-mediated T_H2 immune responses were inhibited by repeated exposures of infant mice to aerosolized allergen early in life, and (2) continuous exposure with endotoxin-LPS did not prevent, but even enhanced, T_H2 -directed immune reactions on subsequent allergen sensitization unless the specific allergen was provided simultaneously with LPS.

The effects of LPS on in vivo immune responses are somewhat controversial. In animal models using single high-dose LPS exposure, pro-inflammatory immune responses are indicated by increased production of TNF- α ,^{18,19} resulting in acute neutrophilic airway inflammation and development of AHR.²⁰⁻²² In a rat model of allergen sensitization, Tulic et al²³ showed that a single dose of aerosolized LPS after allergen challenges exacerbated previously established eosinophilic airway inflammation by predominant accumulation of neutrophils. In contrast, aerosolized LPS given 1 day before sensitization was shown to inhibit OVA-specific IgE production and inflammatory responses, but it enhanced OVA-specific IgG production. Systemic administration of LPS immediately before allergen sensitization of mice prevented T_H2 immune and airway inflammatory responses in an IL-12-dependent mode.¹⁰ The results of the present study, in contrast, show that repeated low-dose LPS exposure of naive infant mice led to decreased in vitro proliferative responses and diminished IFN- γ production by MNCs after stimulation with LPS, without increasing Ig serum levels. This might be explained by induction of LPS tolerance after repeated application of LPS. In accordance, recently published data showed that continuous environmental exposure of children to high concentrations of LPS in both farming and nonfarming households resulted in decreased LPS-induced in vitro cytokine production.⁹

Unexpectedly, in the present study repeated exposure of infant mice with LPS before sensitization actually enhanced T_H2 -mediated total IgG1 serum levels and allergen-induced eosinophilic airway inflammatory responses. Several in vitro and in vivo studies have shown that LPS promotes human and mouse dendritic cells (DCs) to produce IL-12 through activation of Toll-like receptor 4.^{21,24,25} IL-12 is the main regulatory T_H1 cytokine that promotes naive T cells to differentiate into IFN- γ -producing T_H1 cells.^{10,26,27} However, Langenkamp et al²⁸ have recently shown that LPS-induced IL-12 production by DCs is only transient and that exhausted DCs then preferentially induce T_H2 or nonpolarized T-cell responses. Therefore enhanced T_H2 immune responses after aerosolized LPS exposure in our

model might be the consequence of activation of lung DCs, which then augment T_H2 polarization in response to subsequent allergen sensitization and airway challenge as a secondary stimulus. This demonstrates how complex the relationship between endotoxin and allergen-mediated immune responses and diseases might be and suggests that other factors in addition to endotoxin are likely to be relevant for reduced risk to farming families of development of allergic diseases.

In contrast to exposure of infant mice to LPS, repeated exposure to allergen before systemic sensitization, alone or in combination with aerosolized LPS, suppressed allergen-specific T- and B-cell immune responses. In vitro T_H2 cytokine production by splenic MNCs and PBLN cells cultured with OVA was decreased, and there was no in vivo production of allergen-specific Ig. This state of unresponsiveness of lymphocytes to a particular antigen is defined as immunologic tolerance and is preferentially induced by means of antigen presentation through the respiratory mucosa route.²⁹ Respiratory tolerance after exposure of mice to aerosolized allergen was first described by Holt et al³⁰ as a function of allergen-specific immune deviation, with suppression of T_H2 -induced IgE and IgG1 but enhanced T_H1 -induced IgG2a production. As a possible mechanism for this kind of tolerance, induction of IFN- γ -secreting regulatory $\gamma\delta$ T cells was suggested.³¹ Later, antigen-specific T-cell anergy induced by means of intranasal administration of the immunodominant peptide of the main house dust mite allergen Der p 1 was described by Hoyne et al³² as another mechanism of respiratory tolerance in adult mice. In accordance with (1) the lack of allergen-specific proliferative responses, (2) the suppression of both T_H2 (IL-4 and IL-5) and T_H1 (IL-2) cytokine production by splenic MNCs on stimulation with allergen, and (3) the lack of increased production of immunoregulatory cytokines (IL-10 and TGF- β) T-cell anergy seems the most likely mechanism for the induction of peripheral tolerance in our model. As previously shown by Hoyne et al,³² specific T-cell anergy results in suppression of the production of all allergen-specific Ig subclasses in vivo. Therefore in our model restraint of B-cell activity confirmed by suppressed Ig production in vivo might be a consequence of T-cell anergy induced by OVA exposure before OVA sensitization.

It has been shown that T-cell anergy resulted in impaired allergen-induced airway inflammation and airway hyperreactivity.³³ In our present study T-cell anergy was accompanied by complete suppression of allergen-induced airway inflammation and totally normalized in vivo AR compared with that seen in OVA-sensitized mice. T_H2 cytokines in BAL fluids after airway allergen challenges were not detected in animals pre-exposed to OVA or LPS plus OVA, supporting the importance of T_H2 cytokine-producing, allergen-reactive CD4⁺ T cells for initiating allergen-induced inflammatory responses.¹⁷ Early and repeated exposure to specific allergen through the inhalative route is effective for the prevention of subsequent allergen-induced sensitization in this model.

As a major difference to OVA exposure, the combined exposure of infant mice to LPS plus OVA before OVA sensitization did not just induce tolerance but moreover shifted the T_H1/T_H2 balance toward unspecific T_H1 immune responses. Accordingly, in this group of mice, allergen challenges of sensitized animals resulted in enhanced IL-12 levels in BAL fluids. The absence of strong allergen-induced T_H2 responses in animals tolerized by means of simultaneous exposure with aerosolized OVA plus LPS might allow a general T_H1 dominance because of LPS-induced activation of pulmonary DCs.^{20,24,25} Therefore compared with solitary allergen exposure, immune-modulating properties of combined OVA-LPS exposure might be more effective in preventing the development of allergic sensitization and diseases. Moreover, the combination might not only inhibit allergen sensitization to a specific allergen but might also decrease the susceptibility for sensitization to a variety of allergens by initiating not only specific T-cell anergy but also inducing a general shift from predominant T_H2 immune responses toward T_H1 immune responses. Furthermore, the present study raises the possibility that the effects of LPS on the immune system might differ depending on the kinetics of simultaneous allergen exposure. Clinical studies now have to investigate whether the concept of mucosa exposure of infants with low doses of allergen, with or without the addition of endotoxin, might be effective in preventing or treating allergic diseases.

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