

Endotoxins prevent murine IgE production, T_H2 immune responses, and development of airway eosinophilia but not airway hyperreactivity

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Background: Contact with immunomodulatory factors, such as LPS, in early infancy is associated with decreased allergen sensitization.

Objective: We sought to study the effects of systemic or airway exposure with LPS on the development of allergen sensitization, eosinophilic airway inflammation, and increased in vivo airway reactivity (AR) in a mouse model.

Methods: BALB/c mice were systemically sensitized with ovalbumin (OVA) plus adjuvant on days 1 and 14 and challenged through the airways with allergen on days 34 to 36. We performed measurement of OVA-specific IgE serum levels, in vitro T_H2 cytokine production, differential cell counts in bronchoalveolar lavage fluids, and assessment of in vivo AR to inhaled methacholine by means of barometric whole-body plethysmography.

Results: Systemic LPS administration before OVA sensitization reduced OVA-specific IgE serum levels (426 ± 76 vs 880 ± 104 U/mL, $P < .01$), T_H2 cytokine production by splenic mononuclear cells (IL-4: 0.08 ± 0.01 vs 0.17 ± 0.01 ng/mL; IL-5: 1.98 ± 0.52 vs 4.11 ± 0.54 ng/mL; $P < .01$), and extent of airway eosinophilia (total cell counts: 93 vs 376×10^3 /mL; eosinophils: 23% vs 51% ; $P < .01$) compared with that in OVA-sensitized mice. Local LPS administration to sensitized mice before airway allergen challenges particularly induced IFN- γ production by peribronchial lymph node cells in vitro (1718 ± 315 vs 483 ± 103 ng/mL, $P < .01$) associated with reduced airway eosinophilia compared with that seen in OVA-sensitized mice. Development of increased AR was not affected by systemic or local LPS exposure. Inhibitory effects of LPS on allergen sensitization and eosinophilic airway inflammation were inhibited by administration of anti-IL-12 antibodies before LPS exposure.

Conclusion: These data indicate that local and systemic application of LPS modulates systemic and local T_H1/T_H2 immune responses in a distinct but similarly IL-12-dependent mode. (J Allergy Clin Immunol 2002;110:110-6.)

Key words: Mice, eosinophils, T_H1/T_H2 cells, cytokines, lipopolysaccharide

Abbreviations used

AI:	Airway inflammation
APC:	Antigen-presenting cell
AR:	Airway reactivity
BAL:	Bronchoalveolar lavage
DC:	Dendritic cell
MCh:	Methacholine
MNC:	Mononuclear cell
OVA:	Ovalbumin
PBLN:	Peribronchial lymph node
Penh:	Enhanced pause

The dramatic increase in prevalence rates of atopic disorders, such as bronchial asthma, might be related to environmental factors associated with the Western lifestyle, including high socioeconomic standards, smaller sibship size, and decreasing incidence of infections during early infancy.¹⁻³ This is supported by the observation that children living on a farm, under rather traditional circumstances, have a reduced risk of development of atopic disorders.⁴ This protective farm effect might be attributed to the intensive exposure to potentially immunomodulatory factors during early infancy, such as LPS, inhibiting the formation of allergic immune responses.⁵⁻⁷ Indeed, LPSs are found in high concentrations in house dust and in stables, mattresses, and raw milk on farms, and a direct correlation of LPS concentrations with diminished incidences of atopic disorders in infants living on these farms was observed.^{5,8-10} In a study on 61 infants at high risk for development of asthma, Gereda et al⁷ found that lower concentrations of house dust endotoxins were associated with allergen sensitization, whereas increased endotoxin levels correlated with increased proportions of (T_H1-like) IFN- γ -producing CD4 T cells. This might be the result of LPS-mediated activation of dendritic cells (DCs) to produce IL-12, the main regulatory cytokine initiating T_H1-type and suppressing T_H2-type immune responses.¹¹ On the other hand, it is well established that endotoxin exposure is associated with exacerbations in asthma severity and augments allergic airway inflammation (AI).^{12,13} Endotoxin exposure thus might affect allergen sensitization and development of asthma by means of distinct and counterbalancing mechanisms. Thus the aim of the current study was to better delineate the kinetics and mechanisms of LPS in the context of allergen-mediated immune responses. Immunomodulatory

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Received for publication December 19, 2001; revised April 16, 2002; accepted for publication April 18, 2002.

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0091-6749/2002 \$35.00 + 0 1/83/125831

doi:10.1067/mai.2002.125831

effects of systemic LPS exposure before first allergen contact and potential therapeutic effects of airway LPS exposure after established allergen sensitization but before airway allergen challenges were investigated for the first time in a murine model.

METHODS

Animals

Female BALB/c mice (BgVV, Berlin, Germany) aged 6 to 12 weeks and weighing 18 to 22 g were maintained on ovalbumin (OVA)-free diets and water ad libitum. All of the experimental procedures were approved by the animal ethics committee.

Experimental design

Systemic LPS exposure before OVA sensitization. BALB/c mice were exposed to a low, nonendotoxemic dose of LPS (0.5 mg/kg *Escherichia coli* O111:B4; Sigma, St Louis, Mo) in a volume of 100 μ L (reconstituted in PBS; Seromed Biochrom, Berlin, Germany) by means of intraperitoneal injections on days -5, -3, -1, and 12, respectively. On days 1 and 14, mice were systemically sensitized by means of intraperitoneal injections of 20 μ g of OVA (Grade VI, Sigma) emulsified in 2 mg of aluminum hydroxide (AlumImject; Pierce, Rockford, Ill) as adjuvant in a total volume of 200 μ L. Mice were placed in a Plexiglas chamber and exposed to aerosolized OVA (0.1 g reconstituted in 10 mL of PBS, Grade V, Sigma) for 20 minutes on days 34, 35, and 36 (intraperitoneal LPS/OVA mice). Control animals for systemic LPS exposure were sham exposed to PBS intraperitoneally instead of LPS intraperitoneally before OVA sensitization (intraperitoneal PBS/OVA mice). Control animals for OVA sensitization were exposed to systemic LPS but systemically sham sensitized with PBS instead of OVA (intraperitoneal LPS mice). Negative control animals were both sham treated with PBS instead of LPS intraperitoneally on days -5, -3, -1, and 12 and systemically sham sensitized with PBS instead of OVA on days 1 and 14 before airway allergen challenges (PBS mice).

Local LPS exposure before airway allergen challenges. On days 1 and 14, mice were systemically sensitized with OVA plus adjuvant, as described above. On days 27, 29, and 31, OVA-sensitized mice were exposed to LPS by means of intranasal instillations (10 μ g/50 μ L per mouse) before airway allergen challenges on days 34 to 36 (OVA/intranasal LPS mice). This LPS dose was shown in preliminary experiments to induce significant but transient (up to 7 days) increases in AI and AHR (data not shown). Control animals for local LPS exposure were systemically sensitized with OVA but sham exposed to PBS instead of LPS intranasally before airway allergen challenges (OVA/intranasal PBS mice). Control animals for OVA sensitization were sham sensitized with PBS intranasally before local LPS exposure (intranasal LPS mice).

Treatment with rat anti-mouse IL-12

To investigate whether LPS effects depend on the presence of IL-12, BALB/c mice were treated with anti-IL-12 mAb (500 μ g/150 μ L per mouse administered intraperitoneally, rat anti-mouse IgG3, clone C17.8; Deutsches Rheumaforschungszentrum, Berlin, Germany) 2 hours before each systemic (α -IL-12/intraperitoneal LPS/OVA) or local LPS administration (OVA/ α -IL-12/intranasal LPS mice). Naive animals received anti-IL-12 mAb intraperitoneally on days -5, -3, -1, and 12 before OVA or sham sensitization with PBS on days 1 and 14 (α -IL-12/OVA mice, α -IL-12/PBS mice) to investigate the effects of IL-12 depletion without LPS exposure. Control animals for anti-IL-12 treatment received polyclonal rat IgG instead of anti-IL-12 mAb (500 μ g/150 μ L per mouse intraperitoneally, Sigma).

Serum levels of total and OVA-specific Ig

On day 36, blood samples were taken out of tail veins, and levels of total IgE and OVA-specific IgE and IgG2a were measured by means of ELISA, as previously described.¹¹ Levels of OVA-specific antibodies were related to pooled standards generated in our laboratory and expressed as arbitrary units per milliliter.

In vitro production of cytokines

On day 39, mice of all groups were killed by means of cervical dislocation. For in vitro cytokine production, splenic mononuclear cells (MNCs) isolated by means of density gradient centrifugation (Lympholyte-M; Cedarlane Laboratories, Hornby, Ontario, Canada), and peribronchial lymph node (PBLN) cells were suspended in culture medium (RPMI 1640; Seromed, Biochrom) and supplemented with amphotericin B, penicillin, streptomycin (Seromed, Biochrom), L-glutamine (PAA Laboratories, Linz, Austria), and 10% FCS (PAA Laboratories). Cells (10⁶/200 μ L) were cultured in 96-well round-bottom plates at 37°C in a 5% CO₂ atmosphere with culture medium, concanavalin A (2.5 μ g/mL, Sigma), or OVA (50 μ g/mL, Grade VI). At optimal time points assessed in preliminary experiments (after 48 hours for IFN- γ and after 96 hours for IL-4, IL-5, and IL-10), supernatants were harvested, and levels of cytokines were assessed by means of ELISA, as previously described.¹⁴ Detection limits were 0.02 ng/mL for IL-4, 0.03 ng/mL for IL-5, and 0.08 ng/mL for IFN- γ . Levels of IL-10 were assessed with the Pharmingen optEia kit, according to the manufacturer's instructions (PharMingen, San Diego, Calif). Detection limit was 0.002 ng/mL.

Proliferation assay with ³H thymidine

Splenic MNCs (2 $\times$ 10⁵/200 μ L) were cultured for 96 hours under the same conditions as described above. Proliferative responses were determined by means of ³H thymidine incorporation (0.5 μ Ci/200 μ L; Amersham Buchler, Braunschweig, Germany) over the last 24 hours of culture.

Bronchoalveolar lavage

On day 39, lungs underwent lavage through a tracheal drain tube twice with 1 mL of cold PBS. In addition, cytospin slides were stained with Diff Quik (Dade Behring AG, Düringen, Switzerland), and bronchoalveolar lavage (BAL) cells were differentiated on the basis of morphological criteria by counting 200 cells under light microscopy.

In vivo airway reactivity

On day 38, in vivo lung function to inhaled methacholine (MCh; Sigma) was performed by means of whole-body plethysmography (EMKA Technologies, Paris, France, similar to the Buxco system), as previously reported.¹⁵ Briefly, each mouse was placed in a main chamber of the body plethysmograph. Aerosolized PBS (baseline) and MCh in increasing concentrations (6-50 mg/mL) were nebulized ultrasonically through an inlet of the main chamber for 3 minutes, respectively. Pressure differences between the main and a reference chamber caused by volume and resultant pressure changes during inspiration and expiration of the mouse were measured and averaged for 3 minutes after each nebulization. As an index of airway obstruction, enhanced pause (Penh) values were calculated, and airway reactivity (AR) was expressed as a fold increase of Penh values for each concentration of MCh compared with baseline Penh values. Concentrations of MCh inducing a 200% increase of corresponding baseline Penh values (MCh-Penh₂₀₀) were calculated for each animal.

Statistical analysis

Data of up to 6 independent experiments were analyzed. Results from control animals for systemic (intraperitoneal PBS/OVA mice, n = 10) and local (OVA/intranasal PBS mice, n = 5) LPS exposure

TABLE I. Serum levels of total and OVA-specific IgE and IgG2a

	Total IgE (ng/mL)	OVA-specific IgE (U/mL)	OVA-specific IgG2a (U/mL)
Groups of mice			
PBS (n = 9)	447 ± 110	90 ± 26	10 ± 0
α-IL-12/PBS (n = 3)	687 ± 83	397 ± 116*	91 ± 60
OVA (n = 15)	3378 ± 424†	880 ± 104†	1020 ± 261†
α-IL-12/OVA (n = 5)	6205 ± 2264	1722 ± 305‡	1502 ± 693
Intraperitoneal LPS (n = 5)	140 ± 18	62 ± 0	10 ± 0
Intraperitoneal LPS/OVA (n = 15)	1451 ± 229§	426 ± 76§	1577 ± 547
α-IL-12/intraperitoneal LPS/OVA (n = 10)	4647 ± 836	3491 ± 531§,	84,818 ± 34,123§,
Intranasal LPS (n = 12)	224 ± 28	62 ± 0	14 ± 3
OVA/intranasal LPS (n = 7)	4844 ± 995	1053 ± 185	1010 ± 315
OVA/α-IL-12/intranasal LPS (n = 8)	7103 ± 925§,	1816 ± 256§,	1672 ± 706

On day 36, blood samples were collected, and serum levels of Ig were measured by means of ELISA (Mann-Whitney *U* test: **P* < .05 and †*P* < .01 vs PBS; ‡*P* < .05 and §*P* < .01 vs OVA; ||*P* < .01 vs intraperitoneal LPS/OVA; ¶*P* < .05 vs OVA/intranasal LPS).

TABLE II. Allergen-specific responsiveness and cytokine production by splenic MNCs in vitro

	³ HThymidine incorporation (cpm)		OVA			ConA
	Medium	OVA	IL-4 (ng/mL)	IL-5 (ng/mL)	IL-10 (ng/mL)	IFN-γ (ng/mL)
Groups of mice						
PBS (n = 9)	72 ± 24	84 ± 22	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.00	0.96 ± 0.43
α-IL-12/PBS (n = 3)	96 ± 25	88 ± 6	0.11 ± 0.05	0.47 ± 0.13	0.75 ± 0.06	0.15 ± 0.02
OVA (n = 15)	389 ± 67†	2873 ± 636†	0.17 ± 0.01†	4.11 ± 0.54†	1.57 ± 0.32†	1.20 ± 0.32
α-IL-12/OVA (n = 4)	848 ± 501†	2375 ± 1271†	0.22 ± 0.00†	4.11 ± 0.00†	3.44 ± 0.00†	0.20 ± 0.06
Intraperitoneal LPS (n = 4)	297 ± 153	297 ± 119	0.02 ± 0.00	0.10 ± 0.02	0.07 ± 0.03	1.53 ± 0.57
Intraperitoneal LPS/OVA (n = 12)	2371 ± 865†	6138 ± 2260†	0.08 ± 0.01*,§	1.98 ± 0.52†,§	0.42 ± 0.09*,‡	0.91 ± 0.28
α-IL-12/intraperitoneal LPS/OVA (n = 10)	5159 ± 1624†,§	8778 ± 2081†	0.76 ± 0.25†,§,	6.78 ± 0.81†,	4.52 ± 1.97†	0.95 ± 0.23
Intranasal LPS (n = 5)	289 ± 87	507 ± 169	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.33 ± 0.07
OVA/intranasal LPS (n = 7)	361 ± 101*	3049 ± 699†	0.14 ± 0.03*	2.07 ± 0.65†	0.81 ± 0.26*	1.97 ± 0.21*
OVA/α-IL-12/intranasal LPS (n = 7)	1171 ± 395†,§,	3035 ± 938†	0.29 ± 0.12*	4.78 ± 1.32†	0.26 ± 0.83*,§,¶	1.05 ± 0.16#

On day 39, splenic MNCs were isolated and cultured with medium (96 hours), OVA (50 μg/mL, 96 hours), and concanavalin A (2.5 μg/mL, 48 hours) in vitro. Proliferative responses of 2 × 10⁵ cells/200 μL were determined by means of ³H thymidine incorporation. Cytokine production (10⁶ cells/200 μL) was measured in supernatants by means of ELISA (Mann-Whitney *U* test: **P* < .05 and †*P* < .01 vs PBS; ‡*P* < .05 and §*P* < .01 vs OVA; ||*P* < .01 vs intraperitoneal LPS/OVA; ¶*P* < .05 and #*P* < .01 vs OVA/intranasal LPS).

ConA, Concanavalin A.

were similar, and data were combined (OVA mice, n = 15). Values for all measurements are expressed as means ± SEM. Pairs of groups were compared with the Mann-Whitney *U* test. Significance was set at a *P* value of .05.

RESULTS

Systemic LPS inhibited total and OVA-specific IgE production

OVA-sensitized mice had strongly elevated levels of total and OVA-specific IgE and IgG2a compared with those seen in nonsensitized animals (PBS, intraperitoneal LPS, and intranasal LPS mice; Table I). Systemic administration of LPS before OVA sensitization reduced serum levels of total and OVA-specific IgE to more than half compared with that seen in OVA mice. Systemic LPS did not affect production of OVA-specific IgG2a. Pretreatment with anti-IL-12 mAb reversed the inhibitory effects of systemic LPS and led to increased serum levels of OVA-specific IgE (4-fold increase), as well as IgG2a, even compared with that seen in OVA mice (Table I). Local LPS administered before airway allergen chal-

lenges did not alter OVA-induced Ig production. In OVA/α-IL-12/intranasal LPS mice, serum levels of total and OVA-specific IgE were increased compared with those in OVA/intranasal LPS and OVA mice, whereas levels of OVA-specific IgG2a were similar to both groups (Table I).

LPS did not inhibit allergen-specific immune responsiveness

OVA sensitization led to a significant increase of proliferative responses by splenic MNCs after culture with OVA compared with that seen in corresponding medium-cultured control animals (Table II). This responsiveness was not inhibited by either systemic LPS administered before OVA sensitization or local LPS administered before airway allergen challenges (Table II). Compared with PBS mice, all groups of OVA-exposed mice showed an increase of ³H thymidine incorporation in splenic MNCs cultured only with medium, indicating an unspecific preactivation of these cells. This effect was mostly enhanced in animals receiving the combination of anti-IL-

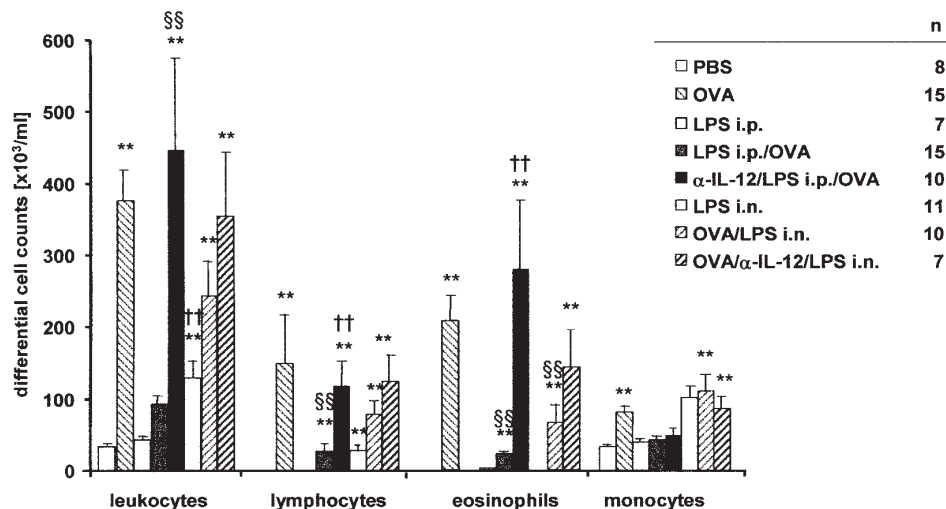


FIG 1. Allergen-induced AI. On day 39, BAL fluids were harvested to objectify airway eosinophilia caused by total and differentiated cell counts (Mann-Whitney *U* test: ***P* ≤ .01 vs PBS; §*P* ≤ .05 and §§*P* ≤ .01 vs OVA; ††*P* ≤ .01 vs intraperitoneal LPS/OVA). *i.p.*, Intraperitoneal; *i.n.*, intranasal.

12 mAb before LPS (α-IL-12/intraperitoneal LPS/OVA and OVA/α-IL-12/intranasal LPS mice, Table II).

LPS inhibited T_H2 cytokine production

OVA sensitization initiated strongly increased in vitro production of IL-4, IL-5, and IL-10 by splenic MNCs after culture with OVA compared with that seen in non-sensitized mice (PBS, intraperitoneal LPS, and intranasal LPS mice; Table II). Levels of IFN-γ, only detectable in response to mitogenic stimulation in all groups, were similar to those of PBS mice (Table II). This predominant T_H2 immune response was also reflected by high in vitro production of IL-4 and IL-5 by local PBLN cells (Table III). Treatment of mice with anti-IL-12 mAb before OVA sensitization did not significantly alter in vitro cytokine production by splenic MNCs, even though IL-10 production was slightly increased and levels of IFN-γ were slightly decreased compared with those seen in OVA mice (Table II).

Systemic LPS administered before OVA sensitization suppressed in vitro T_H2 cytokine production by splenic MNCs (Table II), as well as by PBLN cells (Table III), compared with that seen in OVA-sensitized mice. Unexpectedly, IFN-γ production by splenic MNCs and PBLN cells was very similar to those of nonsensitized and OVA-sensitized mice, respectively (Tables II and III). Antagonization of IL-12 before administration of systemic LPS and OVA sensitization completely abolished LPS effects on in vitro cytokine production, and the predominant T_H2 cytokine pattern resembled that of OVA-sensitized mice (Tables II and III). In summary, in our model systemic LPS suppressed both systemic and local T_H2 cytokine production in an IL-12-dependent pathway, without enhancing overall T_H1 immune responses.

In contrast, local LPS administration to sensitized mice before airway allergen challenges did not alter cytokine production by splenic MNCs but led to slightly

reduced levels of IL-4 and IL-5 and, particularly, enhanced levels of IFN-γ produced by PBLN cells in vitro compared with that seen in OVA mice (Tables II and III). In vivo antagonization of IL-12 before local LPS administration neutralized the effects of local LPS on in vitro T_H1 and T_H2 cytokine production by PBLN cells (Table III). Taken together, the data showed that local LPS administration before airway allergen challenges induced an IL-12-dependent shift from a predominant T_H2 cytokine pattern toward a predominant T_H1 cytokine pattern in the local compartment.

LPS inhibited development of allergen-induced AI

OVA-sensitized and OVA-challenged mice experienced allergen-induced AI, as compared with PBS-challenged mice, which was characterized by high numbers of mostly lymphocytes (150 ± 67/μL, 35%) and eosinophils (209 ± 34/μL, 50%) detected in BAL fluids (Fig 1). Anti-IL-12 antibody administered before OVA sensitization did not alter this pattern of AI, although total cell counts in BAL fluids were slightly decreased (Fig 1).

Systemic administration of LPS before sensitization nearly completely abolished development of allergen-induced airway eosinophilia. Total cell counts, especially lymphocytes (26 ± 8/μL, 20%) and eosinophils (23 ± 4/μL, 20%), were low, and cells now consisted mostly of monocytes (Fig 1). In vivo blockade of IL-12 before systemic LPS administration eliminated this inhibitory effect of LPS on the development of allergen-induced AI, and airway eosinophilia now was very similar to that of OVA-sensitized mice (Fig 1). Administration of anti-IL-12 antibodies before OVA sensitization without LPS treatment did not enhance allergen-mediated AI or significantly change cellular distribution of BAL fluid compared with that seen in OVA-sensitized animals.

Local LPS exposure of naive mice before airway aller-

TABLE III. In vitro cytokine production by PBLN cells

	OVA		ConA
	IL-4 (ng/mL)	IL-5 (ng/mL)	IFN- γ (ng/mL)
Groups of mice			
OVA (n = 6)	1.1 $\pm$ 0.3	14.9 $\pm$ 4.2	0.5 $\pm$ 0.1
Intraperitoneal LPS/OVA (n = 6)	0.5 $\pm$ 0.4	4.3 $\pm$ 0.8*	0.7 $\pm$ 0.3
α -IL-12/intraperitoneal LPS/OVA (n = 4)	1.2 $\pm$ 0.4	40.3 $\pm$ 8.1	0.9 $\pm$ 0.4
OVA/intranasal LPS (n = 4)	0.6 $\pm$ 0.3	8.8 $\pm$ 4.1	1.7 $\pm$ 0.3†
OVA/ α -IL-12/intranasal LPS (n = 3)	1.5 $\pm$ 0.4	34.4 $\pm$ 12.1	0.3 $\pm$ 0.1

On day 39, PBLN cells of 2 mice per group were pooled and cultured with concanavalin A and OVA in vitro, as described in Table II. Levels of cytokines in supernatants were measured by means of ELISA (Mann-Whitney *U* test: **P* < .05 and †*P* < .01 vs OVA).

ConA, Concanavalin A.

gen challenges induced a moderate AI with mostly lymphocytes (29 $\pm$ 6/ μ L, 22%) compared with that seen in PBS mice. Neutrophils and eosinophils were not detectable in this group (Fig 1). Local LPS administration of OVA-sensitized mice before airway allergen challenges slightly reduced total cell counts in BAL fluids. The majority of infiltrating cells were lymphocytes (78 $\pm$ 20/ μ L, 30%), whereas eosinophils were significantly reduced (67 $\pm$ 24/ μ L, 22%) compared with that seen in OVA mice. Neutrophils were not detectable (Fig 1). In vivo antagonization of IL-12 before local LPS administration counteracted the LPS effect and led to development of airway eosinophilia comparable with that of OVA mice (Fig 1).

LPS had no effect on development of increased in vivo AR

OVA sensitization (n = 15) induced increased in vivo AR to inhaled MCh, which was indicated by a significant decrease of MCh-Penh₂₀₀ values compared with that seen in nonsensitized PBS mice (n = 5; 10 $\pm$ 1 vs 27 $\pm$ 3, *P* < .01).

Systemic (n = 5) or airway (n = 7) LPS administration to nonsensitized animals did not induce development of increased in vivo AR assessed on day 38 of the protocol compared with that seen in nonsensitized control animals (24 $\pm$ 6 and 22 $\pm$ 4). Both systemic LPS administration before sensitization (n = 13) and local LPS exposure before airway allergen challenges (n = 10) did not inhibit development of increased in vivo AR, and increases of AR were similar to those seen in OVA-sensitized mice (14 $\pm$ 4 [*P* < .05] and 8 $\pm$ 2 [*P* < .01 vs PBS mice]).

DISCUSSION

A potential association between microbial substances, such as LPS, and decreased prevalences of atopic disorders has been suggested by several epidemiologic studies. We studied the effects of systemic or local LPS exposure on the induction and quality of allergen-mediated immune responses and AI in a murine model. Systemic administration of LPS before OVA sensitization of BALB/c mice significantly inhibited systemic and local T_H2 immune responses, which was rather surprising, without directly enhancing T_H1 immune responses. This

might be a consequence of the used mouse strain, BALB/c, which is known to be genetically determined to have high T_H2 responses.¹⁶ Rather unlikely as a possible mechanism for the observed downregulation of cytokine production after systemic LPS administration is so-called LPS tolerance, the functional deficiency of antigen-presenting cells (APCs) to present antigen after repeated systemic LPS administration,^{17,18} because in vitro responsiveness after activation with allergen was maintained. Local LPS administration of sensitized mice before allergen airway challenges induced a general shift from a predominant T_H2 immune response toward a predominant T_H1 immune response specifically in the local compartment, as demonstrated by decreased T_H2 and, at the same time, enhanced T_H1 cytokine production by PBLN cells, despite an already established systemic T_H2 response. This supports that formerly established T-cell responses can be reversed or further augmented depending on the type of APCs initiating secondary immune responses.^{19,20} DCs in the respiratory tract are specialized for initiating strong T_H2 immune reactions.²¹ In contrast, lung macrophages in OVA-sensitized mice are capable of antagonizing ongoing T_H2 immune responses by activating T_H1 cells.²² In our model local LPS treatment of OVA-sensitized mice before airway allergen challenges induced a predominant T_H1 immune response in the local compartment, presumably by promoting T_H1-inducing activity of APCs.

Associated with decreased T_H2 cytokine production, systemic and local administration with LPS inhibited development of allergen-mediated airway eosinophilia. This underlines the pivotal role of IL-4 and IL-5 for development of AI in our model^{23,24} and supports data from a rat model in which LPS exposure prevents allergen-mediated AI.²⁵ Airway administration of LPS to naive nonsensitized mice led initially to the development of a moderate neutrophilic AI that lasted up to 48 hours after the last LPS administration (total cell counts up to 160 $\times$ 10³/mL, neutrophils up to 40%, and lymphocytes up to 20%, unpublished data).²⁶ Five days after the last LPS administration and after repeated airway allergen challenges of sensitized mice, total cell counts in BAL fluids were still increased, and cells consisted mostly of lymphocytes, whereas numbers of neutrophils had now decreased. Concurrently, local LPS administration to sensitized mice

before airway allergen challenges resulted in a combination of LPS-induced infiltration with lymphocytes and LPS-mediated inhibition of eosinophilic infiltration.

Several in vitro studies demonstrate that LPS promotes DCs to produce IL-12,^{11,27} which is pivotal for the induction of T_H1 immune responses.²⁸ Treatment of allergen-sensitized mice with exogenous IL-12 suppresses T_H2 immune responses and inhibits the development of AI.^{29,30} In our model systemic administration of anti-IL-12 mAb before systemic or local LPS exposure fully restored allergen-mediated T_H2 immune responses and development of airway eosinophilia. Moreover, anti-IL-12 antibodies before systemic LPS and OVA sensitization even enhanced T_H2 immune responses compared with that seen in OVA-sensitized mice. This might be explained by means of unspecific and direct activation of APCs,^{31,32} B cells,³³ or both by LPS, leading, in the presence of IL-12 blocking antibodies, to enhanced T_H2 responses. In summary, the most likely and initial mechanism underlying the immunomodulatory capacity of LPS observed in our model is activation of IL-12 production by DCs.^{27,34}

Similar to other recent publications,^{25,35,36} development of T_H2 immune responses and eosinophilic AI on the one side and development of increased in vivo AR on the other side were dissociated in our model. In contrast, Tulic et al²⁵ showed, in a rat model of allergic AI, reduced in vivo AR after only a single dose of local LPS before OVA sensitization. Our results might be explained first by impaired, but not completely abolished, accumulation of eosinophils in the peribronchial regions of the airways found by means of immunohistochemistry of lung tissues (data not shown). This is supported by data showing that peribronchial eosinophilia found in mice transgenic for the expression of IL-5 in lung clara cells induces increased AR, even without allergen sensitization.³⁵ Second, lymphocytic infiltration of the airways might induce increased AR independent of eosinophils. This phenomenon might be caused by T_H2 cytokines, such as IL-9 and IL-13, leading to increased mucus production.^{36,37} Third, the induction of T_H1/proinflammatory cytokines by LPS might itself enhance development of increased AR. In accordance, T_H1 cells are found in animal models cooperating with T_H2 cells in establishing and even intensifying established increased AR.^{38,39} Furthermore, this is supported by the well-known fact that established asthmatic reactions in human and animal models are enhanced by subsequent exposure to LPS.⁴⁰

In conclusion, LPS prevented T_H2 immune responses and development of airway eosinophilia in an IL-12-dependent manner when administered before allergen sensitization. These immunomodulatory effects of LPS support data recently found by Gereda et al,⁷ indicating that chronic exposure to LPS in infants at high risk for development of asthma is associated with decreased allergen sensitization. Furthermore, airway administration of LPS suppressed local T_H2 cytokine production and AI, even after establishing systemic sensitization with allergen. The potential benefit of LPS for treatment of allergic

diseases is limited by the fact that LPS exposure before airway allergen challenges is unable to prevent development of increased AR and that LPS, as observed in various studies in human and animal models, rather enhances established asthmatic reactions. Still, treatment with LPS (or similarly acting immunomodulatory mediators) might provide a novel mode to prevent sensitization in children with high risk of allergic diseases.

We thank Petra Ellensohn, Margret Oberreit-Menesis, and Kathrin Borgwald for their excellent technical assistance.

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