

Lipopolysaccharides Modulate Allergen-Specific Immune Regulation in a Murine Model of Mucosal Tolerance Induction

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Key Words

Asthma · Mouse model · Mucosal tolerance ·
Lipopolysaccharides · ‘Farming effect’

Abstract

Background: Farming has been widely reported to be associated with decreased risk of developing atopic disorders, but underlying immunomodulatory mechanisms are still not fully defined. We delineated T-cell functions after induction of mucosal tolerance in the context of intranasally delivered organic dust compounds, lipopolysaccharides (LPS).

Methods: BALB/c mice were pretreated intranasally with ovalbumin (OVA) with or without LPS (*Escherichia coli*) three times (days –21, –14, –7) prior to systemic OVA sensitization (days 1 and 14) and airway allergen challenges (days 28–30). CD4⁺ spleen T cells from pretreated and sensitized donors were characterized for cytokine function, and transferred into naive recipients prior to subsequent OVA sensitization and challenges. **Results:** Intranasal OVA pretreatment suppressed Th2-mediated immune and inflammatory responses and enhanced frequency of regulatory T cells in OVA-sensitized and -challenged mice. Addition of LPS to OVA, but not LPS alone, inhibited development of allergen-induced sensitization and eosinophilic airway infiltration, and markedly enhanced allergen-specific IgG1 serum levels and frequencies of IL-10- and IFN- γ -producing CD4⁺ T cells. Transfer of

CD4⁺ spleen T cells from OVA-pretreated animals protected naive recipients against subsequent allergen sensitization and airway disease, whereas transfer from LPS/OVA-pretreated animals only protected against allergen sensitization. **Conclusion:** Microbial LPS modulated mucosal tolerance by inducing allergen-specific IgG1 production and distinct effector CD4⁺ T cells with a mixed regulatory/Th1 phenotype. Organic dust components such as LPS might therefore be important immune modulators in naturally occurring or preventive allergen-specific tolerance induction.

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Introduction

Numerous studies have described the ‘farm effect’ as a protective result of rural lifestyle that lowers the risk of developing atopic disorders. However, to date, the underlying mechanisms – the key players, their potential immunomodulatory properties and their interplay in preventing allergic diseases – remain unclear [1–3]. Complex and somewhat controversial effects of different compounds of organic dusts have been reported regarding the development of allergen sensitization or allergic asthma, and multiple factors such as timing, dosing, routes of exposure, as well as individual genetic variations in Toll-like receptors (TLRs) of microorganism-associated mo-

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lecular patterns (MAMPs) were found to be decisive for these specific effects [recently reviewed in 4]. Moreover, exposure not only to organic dust compounds, but also the simultaneous exposure to allergens may play a crucial role in defining the subsequent immune response of a host. This may explain epidemiological findings that show, on the one hand, that the risk for allergic sensitization increases with intensity of allergen exposure early in life [reviewed in 5], but on the other hand that allergen exposure via the airways may induce allergen-specific mucosal tolerance, as demonstrated in non-atopic humans and in experimental animal models [5–8]. The individual immune response therefore might be the consequence of allergen exposure and parallel encounter of the host with MAMPs such as lipopolysaccharides (LPS) activating the innate immune system via TLR-bearing antigen-presenting cells and thus skewing the immune system towards predominant Th1-immune responses [newly reviewed in 9]. Accordingly, we and others described in murine models that LPS application to pregnant mice suppressed allergen-mediated Th2-immune and inflammatory responses in the offspring [10, 11], apparently the result of increased expression of TLR-4 and the Th1-inducing differentiation factor T-bet in lung tissues of the LPS-exposed offspring [10]. This may also explain the very recent observation that contact of pregnant women to stables and farm animals was associated with increased expressions of LPS receptors, TLR-2, TLR-4 and CD14, and reduced sensitization rates in their children [12].

In order to further delineate the interplay between MAMPs and allergen in this context, we studied the modulation of mucosal tolerance induction with allergen by additional exposure to the ‘model dust compound’ LPS. We show here that the addition of LPS to allergen induced the production of certain isotypes of allergen-specific antibodies and a distinct T-cell subpopulation that was able to transfer suppressive effects against allergic immune responses to naive recipients. These data improve our immunological understanding of the ‘farm effect’ and may lead to novel strategies for prevention of allergy and asthma.

Material and Methods

Experimental Protocols

Animals. Female BALB/c mice aged 6–8 weeks (Harlan Winkelmann, Borcheln, Germany) were kept under pathogen-free conditions on ovalbumin (OVA)-free diets. The regional animal ethics committee approved all experimental procedures.

Pretreatment. Slightly anesthetized (ketamine hydrochloride/Rompun 2%, 100 µl i.p., Curasan AG, Kleinostheim, Germany; Bayer, Leverkusen, Germany) BALB/c-mice were pretreated intranasally with LPS (10 µg/50 µl, *Escherichia coli* O111:B4, Sigma; the chosen LPS dose was used previously [13]), or with OVA (Grade VI, 20 µg/50 µl) with or without additional LPS three times on days –21, –14 and –7, respectively. In our model, LPS contamination of the commercially available OVA did not significantly alter the effect of OVA pretreatment as a current study with LPS-free OVA compared to LPS-contaminated OVA showed (up to now unpublished data). To confirm allergen specificity of pretreatment effects some mice were pretreated with latex extract (kindly provided by H. Breiteneder, University of Vienna, Austria) with or without LPS instead of OVA. Controls for effects of pretreatment were placebo-exposed to PBS (50 µl i.n.).

Systemic Sensitization and Airway Allergen Challenge. Mice were systemically sensitized by intraperitoneal injections of OVA (Grade VI, Sigma, St. Louis, Mo.) emulsified in aluminum hydroxide as adjuvant (20 µg OVA in 2 mg Alum in 200 µl PBS/mouse/injection, Alum, Pierce, Rockford, Ill., USA) on days 1 and 14. Alum itself did not induce a detectable Th2-immune response (data not shown). Negative controls were placebo-sensitized with PBS (200 µl/mouse/injection, i.p., Biochrom, Berlin, Germany). On days 28, 29 and 30, all mice were challenged with aerosolized OVA (100 mg/10 ml PBS, Grade V, Sigma) for 20 min.

Transfer Experiments (see fig. 1). Donors were pretreated with OVA, LPS plus OVA, or PBS, and subsequently sensitized with OVA as described above. On day 28, CD3+CD4+ and CD3+CD8+ spleen cells were isolated by magnet absorbent cell sorting (>95% purity, MACS, BD Biosciences, Heidelberg, Germany) and intravenously transferred into naive recipients (3×10^6 cells/mouse). One day later, recipients were sensitized and airway challenged with allergen as described above (fig. 1).

Immunoglobulin (Ig) Serum Levels

On day 32, serum levels of total and OVA-specific Ig were detected by ELISA as previously described [14].

Bronchoalveolar Lavage (BAL)

On day 32, lungs were lavaged twice with PBS. Cells were counted and differentiated by Diff Quick (Dade Behring AG, Düringen, Switzerland) to examine the extent of allergen-mediated airway inflammation; levels of IL-5, IL-12 and TNF-α were assessed by means of ELISA with the Pharmingen optEia kit (Pharmingen, San Jose, Calif., USA).

In vivo Airway Reactivity

On day 31, in vivo airway reactivity was measured by means of whole-body plethysmography (EMKA Technologies, Paris, France) in response to methacholine (MCh, 6–50 mg/ml) as previously reported [15]. MCh concentrations resulting in a 200% increase of matching baseline Penh values (MCh-PC₂₀₀Penh) were analyzed for every individual animal.

Proliferation Assay with Tritiated Thymidine

On day 32, proliferative responses of spleen mononuclear cells (MNCs) cultured with medium (in vitro negative control, data not shown), concanavalin A (2.5 µg/ml, Sigma, St. Louis, Mo., USA; in vitro positive control, data not shown) or OVA (50 µg/ml)

Fig. 1. Protocol of transfer experiments. Donors were pretreated with OVA, LPS plus OVA, or PBS on days -21, -14, and -7, and subsequently sensitized with OVA on days 1 and 14. On day 28, CD3+CD4+ and CD3+CD8+ spleen cells were intravenously transferred into naive recipients. One day later, recipients were sensitized and airway challenged with allergen.

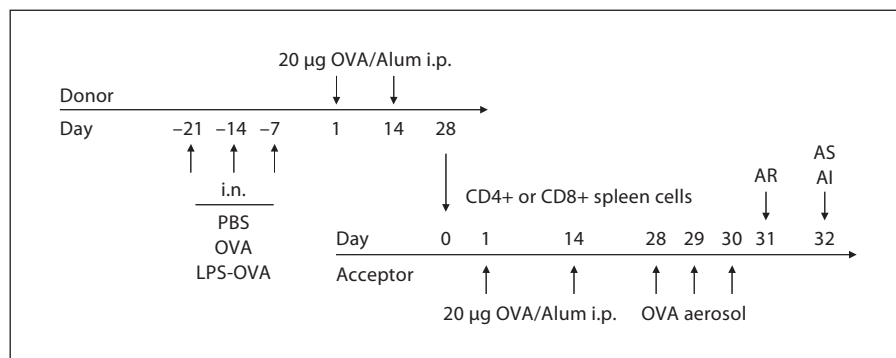
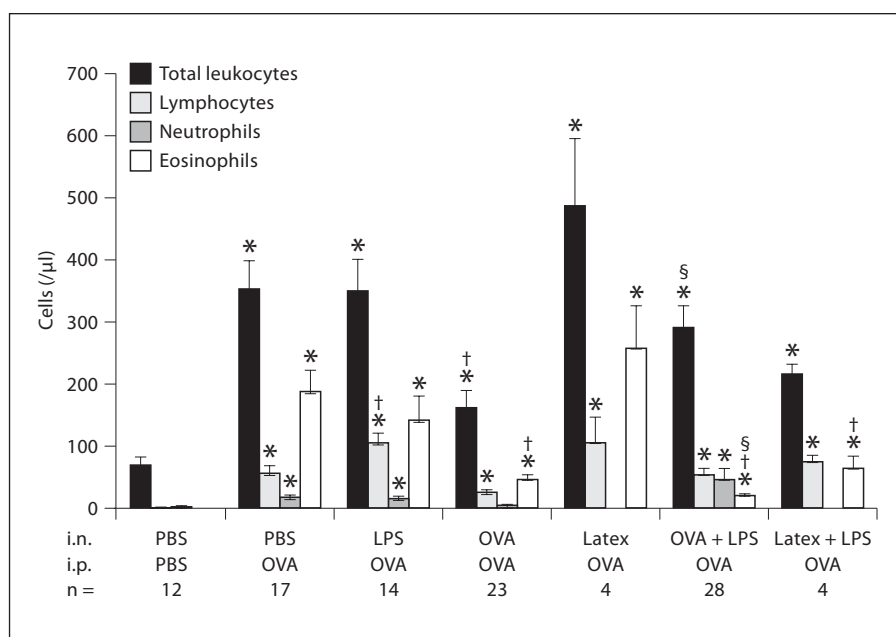


Fig. 2. Effects of pretreatment with OVA and LPS on allergen-mediated eosinophilic airway inflammation. Extent of eosinophilic airway inflammation was measured after intranasal pretreatment with OVA with or without LPS prior to OVA sensitization and repeated airway allergen challenges. * $p < 0.05$ vs. placebo-pretreated placebo-sensitized negative controls; † $p < 0.05$ vs. placebo-pretreated OVA-sensitized positive controls; § $p < 0.05$ vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).



were determined by means of the tritiated thymidine incorporation assay (Amersham Buchler, Braunschweig, Germany) as previously reported [6].

In vitro Cytokine Production

On day 32, spleen MNCs were cultured with OVA, and levels of IL-4, IL-5 and IFN- γ in culture supernatants were assessed by means of ELISA with the Pharmingen optEia kit (Pharmingen).

Flow Cytometric Analysis of Peribronchial Lymph Node (PBLN) Cells

PBLN cells were pooled from 2 animals of the same group – a group contained up to 10 animals – stained with anti-CD3, anti-CD4 and anti-CD8 plus anti-CD62L, anti-CD69, anti-CD25, or anti-CD45RB mAb (kindly provided by R.A. Kroczeck, Robert Koch Institute, Berlin, Germany), and analyzed on FACSCalibur using CELLQuest software (Becton Dickinson, Mountain View, Calif., USA). Intracellular cytokine production by PBLN cells was measured according to the manufacturer's instructions (Pharmingen). IFN- γ /IL-4 and IL-4/IL-10 ratios of respective

CD4+ (CD8-) T cells are indicated. We showed representative data of PBLN cells of one of three performed independent experiments.

Statistical Analysis

Treatment groups include all animals of three independent experiments that were treated in the same way. Values for all measurements are expressed as mean $\pm$ SEM. Pairs of treatment groups were compared by two-tailed Mann-Whitney U test. Statistical significance was set at $p < 0.05$.

Results

Intranasal Pretreatment with LPS Induced

OVA-Specific IgG1 and IgG2a in OVA-Tolerized Mice

Pretreatment with LPS prior to OVA sensitization did not affect allergen sensitization (table 1), eosinophilic airway inflammation (fig. 2) or in vivo airway hyperre-

Table 1. Effects of pretreatment with OVA and LPS on allergen-mediated sensitization

i.n.	OVA i.p.	n	OVA-specific		
			IgE U/ml	IgG1 $\times 10^3$ U/ml	IgG2a $\times 10^3$ U/ml
PBS	–	12	77 $\pm$ 17	0.1 $\pm$ 0	5 $\times 10^{-3} \pm 0$
PBS	+	17	3,401 $\pm$ 603*	18 $\pm$ 4.8*	2.1 $\pm$ 0.7*
LPS	+	14	3,243 $\pm$ 714*	57 $\pm$ 20*	12 $\pm$ 3.5*, †
OVA	+	23	557 $\pm$ 115*, †	32 $\pm$ 11*	1.3 $\pm$ 0.4*
Latex	+	4	2,031 $\pm$ 191*	25 $\pm$ 6.6*	2.4 $\pm$ 0.5*
OVA+LPS	+	28	283 $\pm$ 44*, †, §	208 $\pm$ 38*, †, §	93 $\pm$ 44*, †, §
Latex+LPS	+	4	2,322 $\pm$ 396*	25 $\pm$ 8*	26 $\pm$ 4.6*, †

Serum levels of OVA-specific Ig were examined after intranasal pretreatment with OVA with or without LPS prior to OVA sensitization and repeated airway allergen challenges.

* $p < 0.05$ vs. placebo-pretreated placebo-sensitized negative controls. † $p < 0.05$ vs. placebo-pretreated OVA-sensitized positive controls. § $p < 0.05$ vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).

Table 2. Effects of pretreatment with OVA and LPS on in vivo airway reactivity

i.n.	OVA i.p.	n	MCh-PC ₂₀₀ Penh
PBS	–	12	42 $\pm$ 7.6
PBS	+	17	11 $\pm$ 2.7*
LPS	+	14	17 $\pm$ 5.2*
OVA	+	23	29 $\pm$ 4.5†
Latex	+	4	9.4 $\pm$ 1.7*
OVA+LPS	+	28	14 $\pm$ 3.1*, §
Latex+LPS	+	4	23 $\pm$ 9

Extent of in vivo airway reactivity were measured after intranasal pretreatment with OVA with or without LPS prior to OVA sensitization and repeated airway allergen challenges.

* $p < 0.05$ vs. placebo-pretreated placebo-sensitized negative controls. † $p < 0.05$ vs. placebo-pretreated OVA-sensitized positive controls. § $p < 0.05$ vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).

Table 3. Effects of pretreatment with OVA and LPS on in vitro Th2-/Th1-cytokine production

i.n.	OVA i.p.	n	Spleen: OVA in vitro			
			³ H-thymidine incorporation fold increase	IL-4 pg/ml	IL-5 ng/ml	IFN- γ pg/ml
PBS	–	12	1.5 $\pm$ 0.1	16 $\pm$ 0	0.8 $\pm$ 0.3	73 $\pm$ 16
PBS	+	17	11 $\pm$ 3*	93 $\pm$ 15*	3.5 $\pm$ 1*	63 $\pm$ 7
LPS	+	14	11 $\pm$ 4*	105 $\pm$ 12*	2.2 $\pm$ 0.6*	78 $\pm$ 0
OVA	+	23	2.7 $\pm$ 0.4*, †	28 $\pm$ 5†	0.9 $\pm$ 0.2*, †	68 $\pm$ 6
OVA+LPS	+	28	2.8 $\pm$ 0.5*, †	43 $\pm$ 5*, †	0.3 $\pm$ 0.04*, †, §	118 $\pm$ 14†, §

Cellular responsiveness and productions of Th2 and Th1 cytokines by spleen MNCs cultured with OVA in vitro were measured by means of ELISA.

* $p < 0.05$ vs. placebo-pretreated placebo-sensitized negative controls. † $p < 0.05$ vs. placebo-pretreated OVA-sensitized positive controls. § $p < 0.05$ vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).

activity (table 2), but slightly increased levels of OVA-specific IgG2a, compared to OVA-sensitized positive controls (table 1). In contrast, pretreatment with OVA prior to OVA sensitization induced allergen-specific mucosal tolerance as indicated by suppressed OVA-specific IgE serum levels (table 1), allergen-mediated airway inflammation (fig. 2) and in vivo airway hyperreactivity (table 2), compared to control mice. Addition of LPS to OVA for pretreatment not only similarly suppressed OVA-specific IgE production (table 1) and eosinophilic airway inflammation (fig. 2), but also strongly enhanced OVA-specific IgG1 and IgG2a serum levels (table 1), compared to OVA-sensitized or OVA-tolerized controls.

Most likely due to a slight increase in number of neutrophils in the BAL fluids (47 $\pm$ 19 vs. 17 $\pm$ 4 cells/ μ l), allergen-induced airway hyperreactivity in the group of LPS plus OVA-pretreated mice was not diminished, compared to OVA-sensitized positive controls (table 2). Allergen specificity of the tolerance induction was shown by pretreatment with a control allergen, latex, instead of OVA that did not affect Ig serum levels (table 1), airway inflammation (fig. 2) or airway reactivity (table 2) after OVA sensitization and airway challenges, compared to placebo pretreatment. Addition of LPS to pretreatment with the control allergen latex prior to OVA sensitization only slightly increased OVA-specific IgG2a serum levels,

compared to OVA sensitization that was most likely induced by LPS (table 1).

Intranasal Pretreatment with LPS Induced Allergen-Specific Th1-Cytokine Production in OVA-Tolerized Mice

In OVA-tolerized mice, recall responses of spleen MNCs cultured in vitro with OVA were significantly suppressed with low proliferation and decreased production of Th2 cytokines, IL-4 and IL-5, compared to placebo-pretreated OVA-sensitized controls (table 3). LPS pretreatment prior to OVA sensitization caused no significant alterations of in vitro T-cell responses to OVA compared to OVA-sensitized controls (table 3). In contrast, in mice pretreated with LPS in addition to OVA, T-cell proliferation and Th2-cytokine production by spleen MNC after in vitro stimulation with allergen were not only suppressed, but IFN- γ production was significantly enhanced, compared to OVA-sensitized and to OVA-tolerized animals (table 3). These results suggested that LPS modulated allergen-specific tolerance induction.

Intranasal Pretreatment with LPS Induced Activated Effector CD4+ T Cells in OVA-Tolerized Mice

To further delineate this effect of LPS on the development of mucosal tolerance, we analyzed phenotypes of T cells from the local draining lymph nodes, PBLN cells, in response to airway allergen challenges in OVA-sensitized, in OVA-tolerized, and in mice pretreated with LPS in addition to OVA. FACS staining of PBLN cells revealed that a number of CD4+ T cells were slightly, but not significantly, enhanced in OVA-tolerized mice ($40 \pm 1\%$ of lymphocytes) or mice pretreated with OVA plus LPS ($40 \pm 1.6\%$ of lymphocytes), compared to OVA-sensitized positive controls ($34 \pm 0.1\%$ of lymphocytes). In OVA-tolerized mice, subsets of CD62L⁻ (fig. 3a) and CD45Rb^{low} memory CD4+ T cells (fig. 3b) were enhanced, and frequency of CD4+ T cells with a regulatory phenotype CD25+Foxp3+ were increased, compared to OVA-sensitized mice (fig. 3c). In accordance with the assumption of increased immune regulation, we found in OVA-tolerized mice higher frequencies of IL-10-producing CD4+ T cells, and therefore a decreased IL-4/IL-10 ratio, compared to OVA-sensitized positive control mice (fig. 3f). Frequencies of IL-4- and IFN- γ -producing CD4+ T cells were very similar to those in OVA-sensitized controls (fig. 3g).

Tolerance induction in the presence of LPS produced a different subset of regulatory cells: CD4+ T cells from this group showed high expression of CD69 (fig. 3d) and

CD25 (fig. 3e), whereas the frequency of IL-4-producing T cells was strongly suppressed and the ones of IFN- γ - and IL-10-producing T cells were enhanced, compared to placebo-pretreated OVA-sensitized mice. Thus, the IL-4/IL-10 ratio of CD4+ PBLN cells was also slightly diminished (fig. 3f), but the IFN- γ /IL-4 ratio was importantly increased (fig. 3g), compared to OVA-sensitized positive control mice. This suggested that the addition of LPS to OVA for pretreatment prior to sensitization induced not only the production of the regulatory cytokine IL-10, but also shifted the predominant Th2- towards a predominant Th1-immune response.

In all treatment groups that were sensitized, CD8+ PBLN cells were stained mostly positive for IFN- γ production (data not shown), and we did not observe a significant shift in the phenotype or cytokine production of CD8+ T cells after tolerance induction, regardless of the type of pretreatment (data not shown). Thus, particularly CD4+ lymphocytes were shifted towards a regulatory phenotype and showed a distinct cytokine secretion pattern correlating to the simultaneous absence or presence of LPS together with allergen in the induction phase of mucosal tolerance.

Immunomodulatory Effects of LPS Were Partly Transferable by CD4+ T Cells

In order to detect the immunomodulatory properties of CD4+ T cells after mucosal tolerance induction with allergen plus LPS, we carried out adoptive transfer experiments. Naive mice received CD4+ or CD8+ spleen T cells from either OVA-sensitized, OVA-tolerized, or OVA-sensitized mice that had been pretreated with LPS in addition to OVA. Recipient mice were subsequently sensitized and airway challenged with OVA to monitor the effects of the transferred T cells on allergic sensitization and airway responses.

CD8+ T cells from OVA-tolerized or OVA-sensitized mice previously pretreated with LPS and OVA did not transfer significant protective effects against subsequent allergen sensitization and airway challenges into naive recipients (data not shown).

In recipients of CD4+ T cells from OVA-tolerized mice or OVA-sensitized mice pretreated with OVA plus LPS, serum levels of OVA-specific IgE were diminished following OVA sensitization, compared to recipients of CD4+ T cells from OVA-sensitized positive controls (fig. 4a). Only in the group receiving CD4+ T cells from OVA- plus LPS-pretreated mice, we observed markedly enhanced levels of OVA-specific IgG2a, compared to recipients of CD4+ T cells from OVA-sensitized positive controls (fig. 4b).

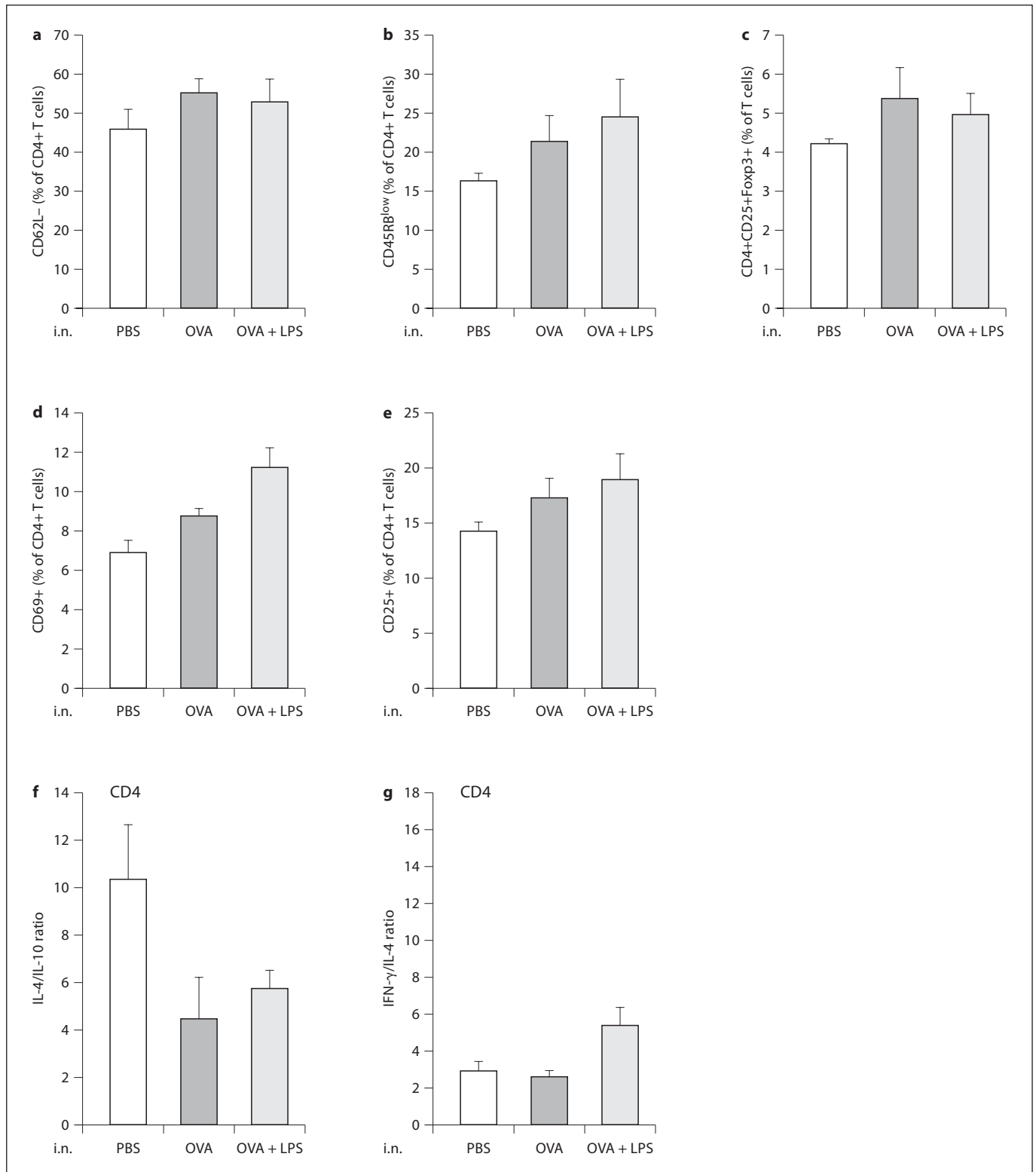
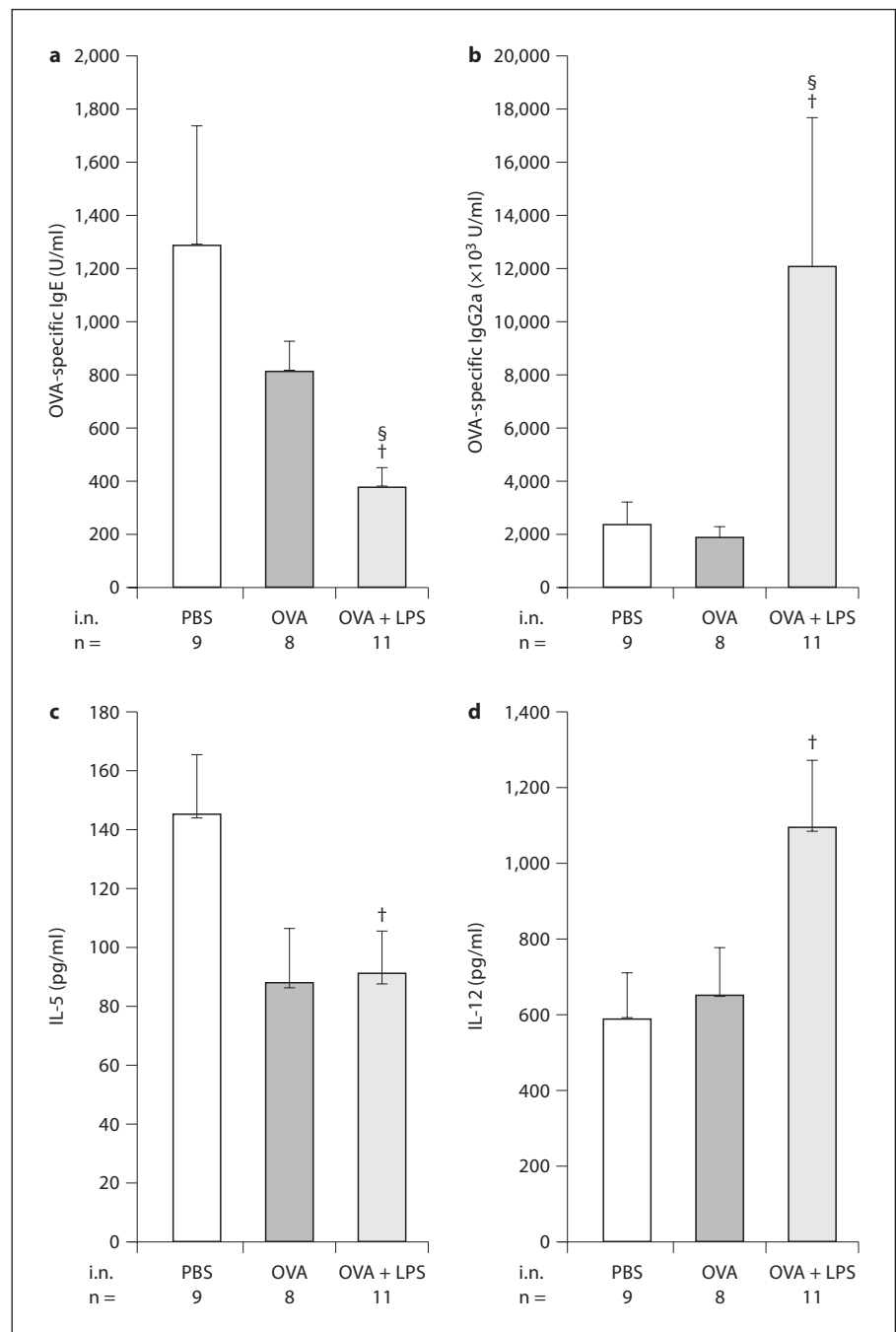


Fig. 3. Effects of pretreatment with LPS and OVA on T-cell repertoires. BALB/c mice were intranasally pretreated with OVA with or without LPS prior to systemic OVA sensitization and repeated airway allergen challenges. PBLN cells from 2 mice of the same

treatment group were pooled. Surface molecules, Foxp3, and intracellular cytokine production were examined by flow cytometric analyses to characterize activated and regulatory PBLN T cells. One of three representative experiments is shown.

Fig. 4. Effects of CD4⁺ T cells from LPS- and OVA-pretreated OVA-sensitized mice on subsequent OVA sensitization and Th1/Th2 cytokines in recipient mice. Serum levels of OVA-specific Ig, and levels of Th2 and Th1 cytokines in BAL fluids were analyzed in recipients after transfer of CD4⁺ spleen T cells from OVA-sensitized mice previously pretreated with OVA with or without LPS. * $p < 0.05$ vs. placebo-pretreated placebo-sensitized negative controls; [†] $p < 0.05$ vs. placebo-pretreated OVA-sensitized positive controls; [§] $p < 0.05$ vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).



This modulation of allergic sensitization was associated with significantly altered airway responses in the recipients, depending on the mode of mucosal tolerance induction of the donor mice: The transfer of CD4⁺ T cells from mice that had been pretreated only with a specific allergen suppressed allergen-mediated eosinophilic airway inflammation (table 4) and decreased levels of IL-5

in BAL fluids (fig. 4c) in subsequently OVA-sensitized and -challenged recipients. In contrast, the transfer of CD4⁺ T cells from OVA-sensitized mice previously pretreated with LPS plus OVA markedly increased the cellular influx into the airways (table 4) with enhanced absolute numbers of total leukocytes, lymphocytes and neutrophils, whereas relative numbers of eosinophils

Table 4. Effects of CD4+ T cells from LPS- and OVA-pretreated OVA-sensitized mice on subsequent OVA-induced airway inflammation in OVA-sensitized recipient mice

Donors i.n.	Recipients n	BAL				AR MCh-PC ₂₀₀ Penh
		total leuko cells/ μ l	Ly	Eo	Neut	
PBS	9	128 $\pm$ 15	25 $\pm$ 4	64 $\pm$ 10	4 $\pm$ 2	7.5 $\pm$ 1.2
OVA	8	83 $\pm$ 14 [†]	13 $\pm$ 5	26 $\pm$ 12 [†]	3 $\pm$ 2	22 $\pm$ 5.5 [†]
OVA + LPS	11	279 $\pm$ 56 [§]	67 $\pm$ 16 [§]	97 $\pm$ 29 [§]	39 $\pm$ 11 ^{†, §}	10 $\pm$ 1.3

Extent of eosinophilic airway inflammation and in vivo airway reactivity were analyzed in recipients after transfer of CD4+ spleen T cells from OVA-sensitized mice previously pretreated with OVA with or without LPS.

[†] p < 0.05 vs. placebo-pretreated OVA-sensitized positive controls. [§] p < 0.05 vs. OVA-pretreated OVA-sensitized mice (= OVA-tolerized mice).

were significantly decreased (29 $\pm$ 4 vs. 49 $\pm$ 4%, p $\leq$ 0.05) in later OVA-sensitized and -challenged recipients. Levels of IL-5 in BAL fluids from this group were also significantly decreased, but levels of IL-12 strongly increased, compared to recipients of CD4+ T cells obtained from OVA-sensitized mice (fig. 4d). BAL fluid levels of the pro-inflammatory cytokine TNF- α , which is possibly induced by LPS, were similar in all groups of OVA-sensitized recipients (data not shown).

In accordance with the effects on airway inflammation, CD4+ T cells from OVA-tolerized mice, but not from mice pretreated with OVA plus LPS, suppressed allergen-mediated in vivo airway reactivity in OVA-sensitized and -challenged recipients, compared to recipients of CD4+ T cells obtained from OVA-sensitized positive controls (table 4).

Discussion

Immunomodulatory mechanisms of microbial compounds on immune reactions and tolerance induction against allergens are still not known. Thus, we examined LPS effects on allergen-induced mucosal tolerance in a mouse model. In accordance to our previously published data in newborn mice [6], intranasal pretreatment with the model allergen OVA-induced mucosal tolerance and prevented subsequent allergic sensitization and eosinophilic airway inflammation. Protective effects were allergen-specific, as intranasal pretreatment with an unrelated allergen, latex, instead of OVA did not inhibit development of OVA-induced allergic immune reactions. Pretreatment with added LPS to OVA, but not the use of

LPS alone, stimulated highly elevated serum levels of OVA-specific IgG1 in placebo-sensitized as well as in OVA-tolerized animals. Allergen-specific IgG antibodies in high doses may have the property to act as blocking antibodies and to prevent IgE-mediated anaphylactic and inflammatory reactions through the IgG-Fc γ RIII macrophage pathway, as recently shown by Strait et al. [16]. Thus, in our model, LPS might have modulated the development of allergen-specific mucosal tolerance by inducing a shift in antibody production from allergen-specific IgE toward allergen-specific blocking IgG subclasses, a process that may furthermore protect against allergic sensitization.

In OVA-tolerized mice, increased frequencies of CD4+ memory T cells expressing low levels of CD45RB and CD62L [17], but high levels of the regulatory marker CD25+ and the transcription factor Foxp3 [18], were present. These CD4+ T cells produced increased amounts of the regulatory cytokine IL-10. IL-10 is well known to diminish systemic T-cell responsiveness and Th2-cytokine production [19], thus inducing tolerance to environmental antigens, as we have shown here. Importantly, transfer of CD4+ T cells from OVA-tolerized animals prevented subsequent allergen sensitization and allergic airway disease in naive recipients. Therefore, in the present model allergen-specific mucosal tolerance induction was a function of regulatory CD4+ T cells producing high levels of IL-10 [20].

Modulating mucosal tolerance by adding LPS to OVA induced subsets of highly activated CD69+ and CD25+ CD4+ effector T cells, which are known to be very efficient in immune regulation of foreign antigens [17]. In accordance, pretreatment with LPS in addition to OVA

enhanced the number of IFN- γ -producing CD4⁺ T cells that are known to aggravate Th1-immune responses and antagonize Th2-immune responses [21].

In humans and in animal models, LPS showed somewhat controversial and not yet fully explained effects on allergen-mediated immune and inflammatory responses, depending on kinetics, doses and route of exposure [reviewed by Liu in 22]. But, in conformity to our present data, Eisenbarth et al. [23] demonstrated that LPS doses of about 100 μ g/100 μ g OVA induced Th1-immune responses in a mouse model of OVA sensitization. In accordance, pretreatment with LPS as an activator of the innate immune system in addition to OVA as a specific allergen generated at least two distinct CD4⁺ effector T-cell populations, the regulatory IL-10-producing T cells and the Th1-type IFN- γ -producing T cells. Appropriately, transfer of naive recipients with CD4⁺ T cells obtained from OVA-sensitized mice previously pretreated with OVA plus LPS prevented allergen-induced sensitization, and resulted in a strong systemic and local Th1-immune response following subsequent allergen sensitization. These data fully agree with data found by Suttmüller et al. [24], who demonstrated very recently that LPS did not directly influence proliferation and function of allergen-specific regulatory T cells.

A significant cell infiltration of the airways, particularly with neutrophils, developed in mice pretreated with OVA plus LPS, which was not observed in LPS-pretreated OVA-sensitized and -challenged mice. Similarly, a significant neutrophilic airway inflammation was also observed in OVA-sensitized and -challenged recipients of CD4⁺ T cells from OVA plus LPS-pretreated OVA-sensitized donor mice. These data strongly indicated that the neutrophilic infiltration was almost certainly mediated by CD4⁺ T cells and therefore more likely a result of the adaptive T-cell-mediated immune response to OVA plus LPS, and not a result of the early innate immune response to LPS [25]. Accordingly, TNF- α , which was found to be enhanced in the acute neutrophilic airway inflammation during the initial innate immune response to LPS [26], was not enhanced in BAL fluids of the respective recipients. In this group we found high levels of IL-12 in BAL fluids, which were described to contribute particularly to neutrophilic airway inflammation [25]. As a plausible consequence of increased IL-12 production, levels of IL-5 in BAL fluids were reduced [21]. IL-12 was also shown to reduce eosinophilic airway inflammation [25]. But since Randolph et al. [27] had described that passive transfer of OVA-specific Th1 cells resulted in increased airway inflammation, the substantial recruitment of cells into the

airways and the relative, but not absolute diminution of airway eosinophilia in OVA-sensitized and -challenged recipients of CD4⁺ T cells from OVA plus LPS-pretreated OVA-sensitized donor mice might be a result of induction of Th1 cells by LPS and OVA pretreatment.

Even though Th1-immune responses are well known to inhibit development of allergen-mediated increased airway reactivity [21], Cui et al. [28] showed very recently that Th1 cells mediated increased airway reactivity particularly independent of neutrophils. Furthermore, neutrophils are also well known to provoke increased airway reactivity [29]. Thus, the increased airway reactivity in mice pretreated with OVA plus LPS might be a result of two components, the induced Th1-cytokine production and the development of neutrophilic airway inflammation.

In conclusion, various organic dust components, among them LPS, should be considered as a network of diverse factors mediating modulating effects on allergen-induced sensitization and inflammatory responses. Other components besides LPS and allergens, such as muropeptides from Gram-negative bacteria [30], might also take part in this alliance. In our present mouse model, immunomodulatory mechanisms of LPS and OVA might be based on the induction of blocking IgG antibodies and on the generation of distinct and highly sufficient subpopulations of CD4⁺ effector T cells. Whether this population might be useful for future therapeutic strategies must be decided after careful evaluation of possible side effects in future experiments.

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