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Oral Administration of Bacterial Lysates Attenuates Experimental Food Allergy

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Key Words
Food allergy · Bacterial lysates · Immune modulation · Primary prevention · Innate immunity

Abstract
Background: Modulating early immune response by application of bacteria and their by-products has been suggested as a preventive strategy against the development of allergic diseases. In light of this, the aim of the study was to test the effects of oral administration of bacterial lysates (BL) in a rat model of food allergy. ***Methods:*** BL or PBS were administered orally to neonatal Brown Norway rats up to an age of 42 days. Additionally, animals were sensitized 3 times (days 35, 40 and 45) intraperitoneally with ovalbumin (OVA). On days 60 and 61, rats were locally challenged with OVA by gavage feeding. ***Results:*** Detection of increased allergen-specific Ig serum levels and proliferative responses of spleen mononuclear cells confirmed systemic sensitization. In serum of animals that received BL in addition to OVA sensitization, the levels of allergen-specific IgE and IgG were significantly reduced compared to animals which were not exposed to BL. Allergen-stimulated lymphocytes from spleen and mesenteric lymph nodes of BL-treated animals showed a significantly elevated cytokine production of IL-10. To assess local functional changes of the intestinal barrier we measured the intestinal permeability, which was increased in OVA-sensitized and challenged animals compared to non-sensitized controls, yet significantly reduced in sensitized animals which received BL. ***Conclusion:*** These data suggest that local administration of BL (pathogen-associated molecular patterns) in the intestine exhibits immuno-modulating effects. Furthermore, pathophysiological features of food allergy, such as the loss of gut mucosal integrity, might be reduced by the treatment with BL.

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Introduction

The inverse correlation between diminished exposure to infectious agents early in life and increased prevalence rates of allergic diseases [1] has been revealed in many epidemiological studies in regard to respiratory allergies and sensitization to inhalant allergens [2, 3], whereas only one study has demonstrated a similar association for food (peanut) allergy [4].

Especially orofecal/foodborne infections (hepatitis A, *Toxoplasma gondii* and *Helicobacter pylori*) have been shown to act protective against atopic sensitization and allergic diseases, most likely through direct stimulation

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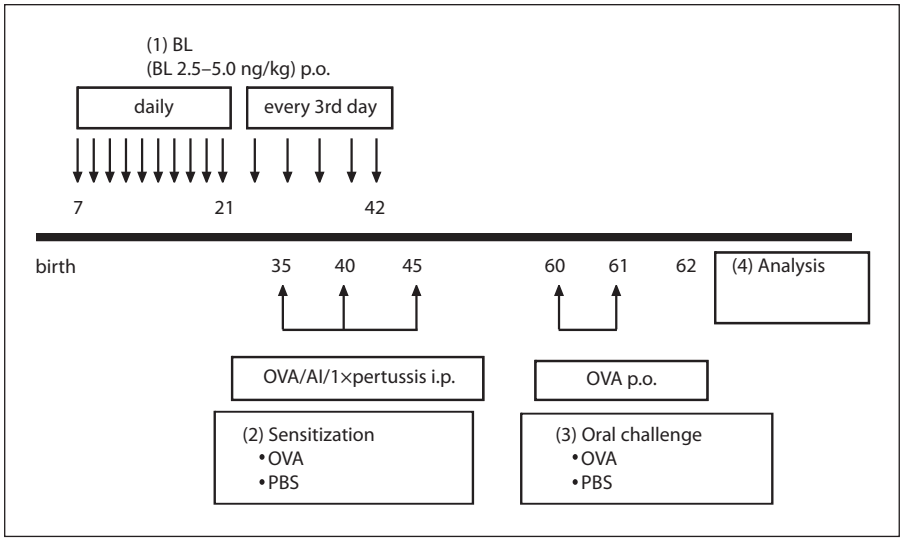

Fig. 1. Treatment protocol: BL (1.5–4.5 × 10^7 bacteria/ml = 2.5–5.0 ng/kg) or PBS were administered orally (p.o.), first once daily (days 7–21 postpartum), then every third day up to an age of 42 days. BN rats were sensitized to OVA intraperitoneally (i.p.) on days 35, 40 and 45. The negative control group was sham sensitized with PBS. For local challenges, animals were provoked by gavage feeding of OVA on days 60 and 61. Control animals received only PBS p.o., BN were analyzed 24 h after the last challenge, on day 62. AI = Aluminum hydroxide.

of the gut-associated lymphoid tissues, Peyer's patches and mesenteric lymph nodes [5]. Thus, it is consequent to mimic the immunomodulating effects of gastrointestinal infections by oral administration of similar products for the development of novel preventive concepts [6].

We therefore investigated the preventive effects of bacterial lysates from two strains of fecal bacteria in a rat model of food allergy. These bacterial preparations contain a variety of components, such as LPS, muramyldipeptide, lipoteichoic acid or bacterial DNA, all likely to modulate the immune system by eliciting innate immune responses [7]. Giese et al. [7] demonstrated that the suspensions from two different strains of living fecal bacteria *(Enterococcus faecalis, Escherichia coli)* induced a similar yet not completely identical gene expression profile as LPS, although one of the suspensions was almost free of LPS. They further showed that this profile was distinctly different from that of CpG oligonucleotides, since the latter did not induce significant amounts of IL-8 and MLP-2α, but in turn induced a much stronger IFN-γ inducible protein 10 signal, possibly due to the induction of type I interferons. In contrast, the autoclaved mixture of the two bacterial strains, which we used for our studies, contained approximately 1,000-fold less endotoxin than the LPS preparation employed in the control set by Giese et al. [7], suggesting that additional mechanisms of immune modulation may occur. In contrast to living bacterial suspensions, our combined autoclaved lysate does not induce type I interferon and interferon-dependent chemokines such as inducible protein 10 in PBMC [8]. We therefore hypothesized that this lysate may ameliorate allergic immune responses by suppressing Th2-type and inflammatory responses by inducing not Th1 but rather regulatory T cell responses.

Material and Methods

Animals

All experiments were approved by the animal research ethical institution (LAGetSi, Berlin, Germany). Pregnant Brown Norway rats (Federal Institute for Risk Assessment, Berlin, Germany) maintained on a peanut-, ovalbumin (OVA)- and soybean-free diet, arrived pathogen free and were maintained under controlled conditions.

Bacterial Lysates

Bacterial lysate [BL, Pro-Symbioflor®; sterile autolysate produced from equal amounts of (1) a nonpathogenic strain of *Streptococcus faecalis* (DSM 16440) and (2) a nonpathogenic strain of *E. coli* (DSM 17252) with an original concentration of 1.5–4.5 × 10^7 bacteria/ml of each strain; kindly provided by SymbioPharm, Herborn, Germany] at a dose of 2.5–5.0 ng/kg body weight or PBS was administered orally first daily on days 7–21 postpartum, then every third day up to the age of 42 days.

Sensitization and Oral Allergen Challenges

Animals were sensitized with 10 μg ovalbumin (OVA) and aluminum hydroxide by intraperitoneal injection on days 35, 40 and 45. In addition to the first allergen injection, animals received once pertussis whole-body vaccine 2 × 10^6 (PV; E. von Behring, Marburg, Germany) intraperitoneally. The negative control group was sham sensitized and challenged with PBS.

Sensitized animals were challenged by gavage feeding of 500 μg OVA (in 1 ml PBS) on days 60 and 61. Control animals received only 1 ml PBS by gavage feeding. Animals were analyzed 24 h after the last allergen challenge, on day 62 (fig. 1).

Data on the kinetics of our model including frequencies of sensitization and challenges, choice of allergen and adjuvant are presented in online supplementary figures S1, S2a, b and S3a–c (www.karger.com/doi/10.1159/000322352).

Assessment of Immunoglobulin

Total IgE and allergen-specific IgE and IgG serum antibody titers were measured by enzyme-linked immunosorbent assay (ELISA), as described [9]. For biotinylation of OVA the biotinylation kit of Sigma® (Deisenhofen, Germany) was used, the kit prepared according to the manufacturer's instructions.

Cell Culture

Spleen mononuclear cells (S-MNC) and mesenteric lymph node mononuclear cells (ML-MNC) were purified by density gradient centrifugation (Lympholyte Rat; Cedarline Laboratories, Hornby, Ont., Canada; 1,000 *g*, 20 min at room temperature), and suspended in RPMI 1640/10% FCS culture medium (Biochrom, Berlin, Germany) for proliferation assays and cytokine production.

Proliferative Responses

MNC (3×10^5/well) were incubated in 96-well U-bottom tissue-culture plates (Costar, Cambridge, Mass., USA) at 37°C and 5% CO_2 and stimulated for 96 h with mitogen (concanavalin A, 2.5 μg/ml; Sigma) or allergen (OVA 100 μg/ml; Sigma). 3[H]-thymidine (Amershan Buchler, Braunschweig, Germany) was added for the last 18 h of the cell culture (1 μCi/well), and thymidine uptake was measured in a liquid scintillation counter (Wallac, Väsby, Sweden). The proliferation rate was calculated as fold increase of 3[H]-thymidine incorporation by stimulated cells compared to nonstimulated cells.

Cytokine Production

S-MNC and ML-MNC (1×10^6/well) were incubated in 96-well U-bottom tissue-culture plates (Costar) at 37°C and 5% CO_2, and stimulated for 72 h with mitogen (concanavalin A, 2.5 μg/ml; Sigma) or OVA (100 μg/ml; Sigma). In vitro cytokine production in the supernatant was measured using the ELISA kit from Pharmingen as described by the manufacturer's instruction. Detection levels were 2 pg/ml for IL-4, 80 pg/ml for IL-10 and 100 pg/ml for IFN-γ.

Intestinal Permeability

The intestinal permeability was assessed using a sugar recovery test as previously described in detail [10, 11]. The test is based on the measurement of the urinary excretion of orally administered nonmetabolized sugar probe molecules. Lactulose and mannitol have been demonstrated to meet the criteria for usage as tracers [12]. Two hours after the second allergen challenge, animals received by oral route 2 ml PBS containing lactulose (10 mg) and mannitol (5 mg), followed 2 h later by 2 ml drinking water. The tracers recovered in the first 8 h in the urine were detected and quantified by high-performance liquid chromatography with pulsed electrochemical detection (Dionex, Idstein, Germany); chromatography module 250 × 40 mm Carbopac PA-1 column (Dionex); eluent 150 mmol NaOH; flow 1 ml/min [12]. Results were expressed as the percentage recovery of the ingested dose of the sugars. The ratio of recovered lactulose/mannitol (permeability index) served as a marker for intestinal permeability.

Statistical Analysis

Calculations were performed with the statistical software SPSS (SPSS Inc., Chicago, Ill., USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Graphs were generated in GraphPad Prism (version 4; GraphPad Software Inc., San Diego, Calif., USA) and R.

Results

Immunoglobulin Production after Allergen Sensitization

In order to analyze the sensitization status of Brown Norway (BN) rats, total and specific IgE serum levels were measured before and after sensitization (days 0 and 34) and after allergen challenges (day 62). On day 34, prior to sensitization, total IgE levels were in the middle of the different groups at 955 ± 890 ng/ml (single analysis). After OVA sensitization, an increase of total IgE by up to 75% (3,400 ± 2,000 ng/ml) was detected, showing the expected immune response. There was no difference between OVA-sensitized animals treated or not treated with BL (data not shown).

There were no detectable OVA-specific IgE antibodies in nonsensitized BN rats. In serum of OVA-sensitized and challenged BN rats, the OVA-specific IgE titer was significantly increased. Interestingly, BN rats, which additionally received BL together with allergen sensitization, showed only a mild increase in OVA-specific IgE compared to nonsensitized animals (fig. 2a).

The production of OVA-specific IgG showed similar results: nonsensitized BN rats did not show OVA-specific IgG production. OVA sensitization significantly increased specific IgG production, whereas OVA-sensitized and BL-treated BN rats produced only little amounts of OVA-specific IgG (fig. 2b).

Proliferative Response of Mononuclear Cells

In OVA-sensitized and challenged BN rats proliferative responses of S-MNC after in vitro stimulation with OVA were significantly enhanced compared to nonsensitized controls (fig. 3a). Sensitized animals treated with BL demonstrated significantly reduced allergen-specific recall responses in comparison to S-MNCs from sensitized, nontreated BN rats. Proliferation of MLN from BL-treated animals was reduced compared to untreated sensitized and challenged animals without reaching statistical significance (fig. 3b).

Cytokine Production by MNC upon Allergen Sensitization and Local Challenges

Changes in immune responses upon allergen sensitization and repeated local challenges were analyzed both on a systemic (spleen) and local level (mesenteric lymph nodes). First, S-MNC were evaluated for their in vitro capacity to produce different cytokines in response to stimulation with either mitogen or allergen. After allergen stimulation, cells from sensitized and challenged animals displayed (not significantly) increased production of IL-4 and IFN-γ in vitro, compared to S-MNC from PBS-treated animals (fig. 4). Interestingly, the IL-10 production by S-MNC from allergen-sensitized and challenged animals that had been treated with BL was significantly enhanced compared to S-MNC of non-BL-treated but sensitized and challenged BN rats. This observation was even more pronounced on the local level, as revealed by the analysis of ML-MNCs (fig. 5).

After mitogen stimulation (Con A), the IL-10 production was also enhanced in S-MNC and ML-MNC, where the cytokine-pattern was very similarly distributed as after allergen stimulation (fig. 5). Details of data on proliferative responses and cytokine production for all experimental groups are provided in tables 1–3.

Intestinal Permeability upon Local Allergen Challenge

In order to evaluate local functional alterations following oral allergen challenges of sensitized animals, we analyzed changes in intestinal permeability. Urinary excre-

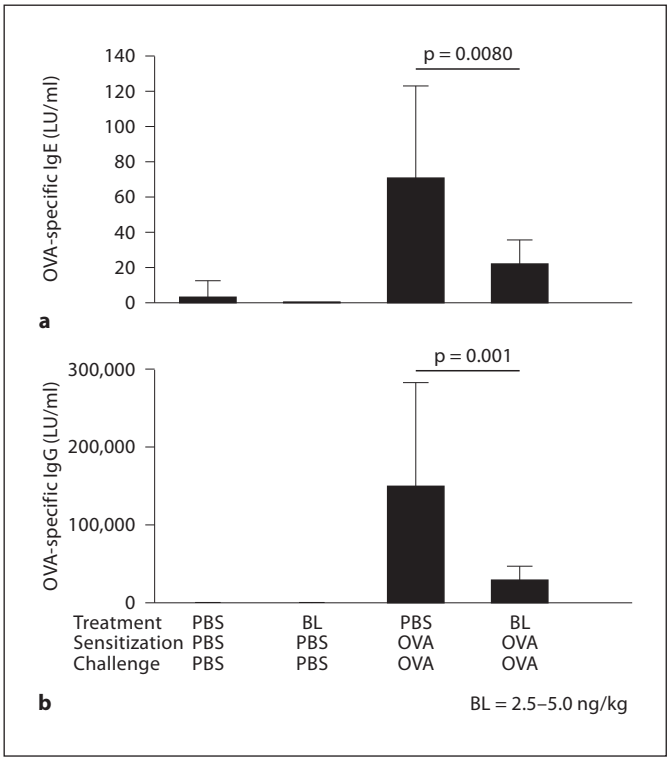

Fig. 2. Immunoglobulin titers in OVA-sensitized and OVA-challenged and/or BL-treated BN rats. BN rats were treated as described in figure 1. At day 34 (before sensitization) and on day 62, immunoglobulin levels were detected in blood samples. Serum levels of allergen-specific IgE (**a**) and allergen-specific IgG (**b**) were measured by ELISA. Values are given as means ± SEM (n = 12).

Table 1. Statistical properties and supplementary data of all performed experiments

	PBS/PBS/PBS	BL/PBS/PBS	BL/PBS/OVA	BL/OVA/OVA	PBS/OVA/OVA	PBS/PBS/OVA
Cytokine production, pg/ml						
IFN-γ-spleen-Con_A	53,500 ± 1,272.792	–	–	34,814.538 ± 17,397.292	24,116.667 ± 15,323.946	–
IFN-γ-spleen-OVA	n.d.	–	–	2,866.632 ± 2,984.593	1,511.536 ± 1,513.132	–
IL-10-MLN-Con_A	248.318 ± 86.662	322.995 ± 74.084	–	1,576.871 ± 1,105.042	743.054 ± 498.317	325.725 ± 319.223
IL-10-MLN-OVA	154.437 ± 42.741	163.15 ± 3.465	–	281.358 ± 154.844	138.005 ± 99.582	100 ± 0
IL-10-spleen-Con_A	1,608.487 ± 1,175.287	–	–	4,278.418 ± 2,376.895	2,317.436 ± 1,100.475	1,422.66 ± 422.426
IL-10-spleen-OVA	463.172 ± 164.908	100 ± 0	–	2,730.32 ± 1,783.546	1,629.091 ± 970.339	100 ± 0
IL-4-MLN-Con_A	n.d.	–	–	20.504 ± 14.946	7.827 ± 8.267	–
IL-4-spleen-Con_A	9.23 ± 6.888	–	–	88.705 ± 77.135	53.601 ± 59.618	–
IL-4-spleen-OVA	n.d.	–	–	52.006 ± 59.321	30.088 ± 42.152	–
Proliferation (proliferation index)						
MLN-Con_A	84.458 ± 3.627	95.883 ± 38.281	88.911 ± 11.384	105.225 ± 108.925	102.587 ± 70.16	–
MLN-OVA	1.015 ± 0.043	1.142 ± 0.322	1.497 ± 0.06	1.896 ± 0.903	3.447 ± 3.37	–
Spleen-Con_A	39.817 ± 27.355	49.881 ± 39.319	35.593 ± 29.68	50.847 ± 46.734	45.032 ± 30.451	62.777 ± 34.121
Spleen-OVA	2.01 ± 1.146	1.153 ± 0.113	1.881 ± 0.77	3.638 ± 1.833	5.748 ± 3.345	3.017 ± 1.167

Values are expressed as means ± standard deviation. n.d. = Not determined.

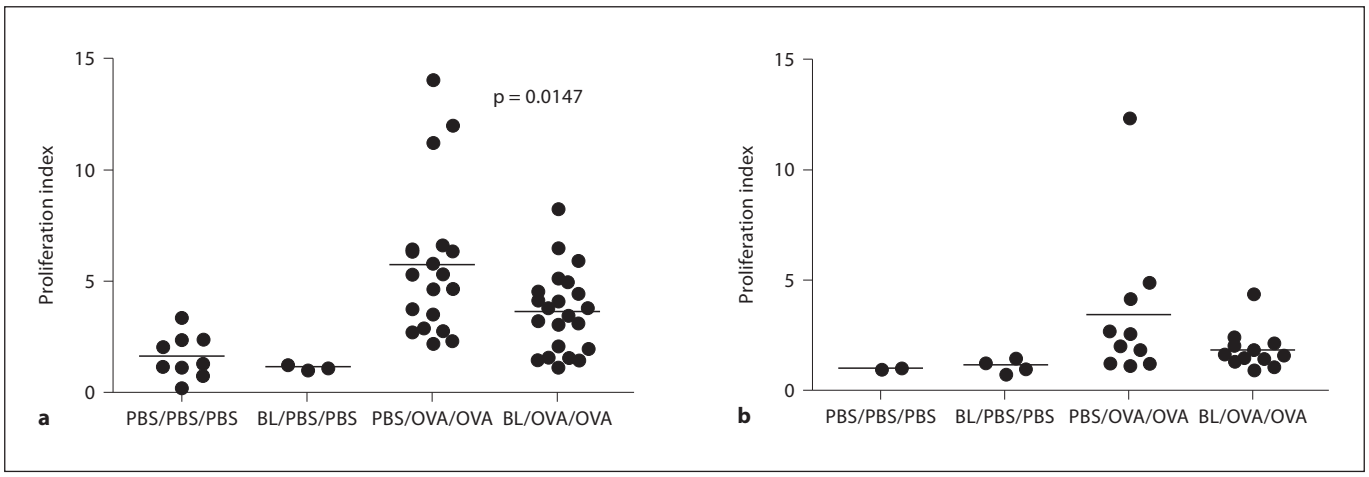

Fig. 3. Influence of OVA sensitization, OVA challenge and/or BL treatment on allergen-specific proliferation. BN rats were treated as described in figure 1. On day 62, S-MNC (**a**) and ML-MNC (**b**) were stimulated for 96 h with OVA (100 μg/ml) and 3[H]-thymidine was added for the last 18 h. The proliferation rate (proliferation index) was calculated as fold increase of 3[H]-thymidine incorporation by stimulated compared to nonstimulated cells.

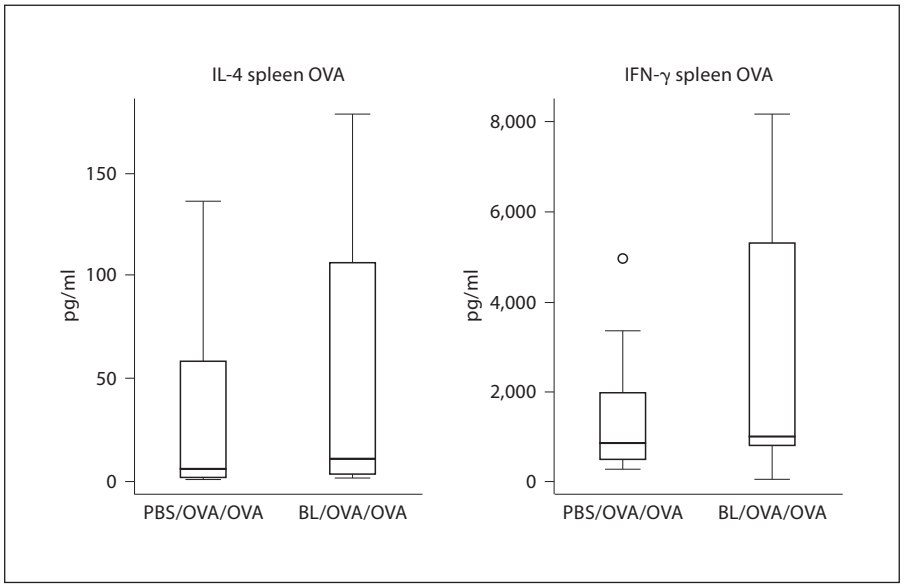

Fig. 4. Influence of OVA sensitization, airway challenges and/or BL treatment on allergen-specific cytokine production. BN rats were treated as described in figure 1. Animals were sacrificed and analyzed 24 h after the last allergen challenge, on day 62. S-MNC and ML-MNC were stimulated for 72 h with OVA (100 μg/ml). Cytokine levels of IL-4 and IFN-γ in the culture supernatant were measured by ELISA.

tion of lactulose and mannitol was evaluated after oral uptake. The lactulose/mannitol ratio (permeability index) served as a marker for intestinal permeability. Intestinal absorption of lactulose, a disaccharide containing galactose and fructose, occurs predominantly through a paracellular pathway via tight junctions, whereas mannitol is absorbed mainly through an intracellular pathway. Allergen sensitization and dual oral allergen provocation led to a significant increase in permeability, which was apparent in comparing OVA-sensitized and challenged BN rats versus those which were PBS treated (fig. 6). Treating OVA-sensitized and challenged BN rats with BL resulted in 20% reduction of intestinal permeability, as compared to OVA-sensitized and challenged animals not treated with BL. These data confirmed that the loss of gut mucosal integrity and elevated intestinal per-

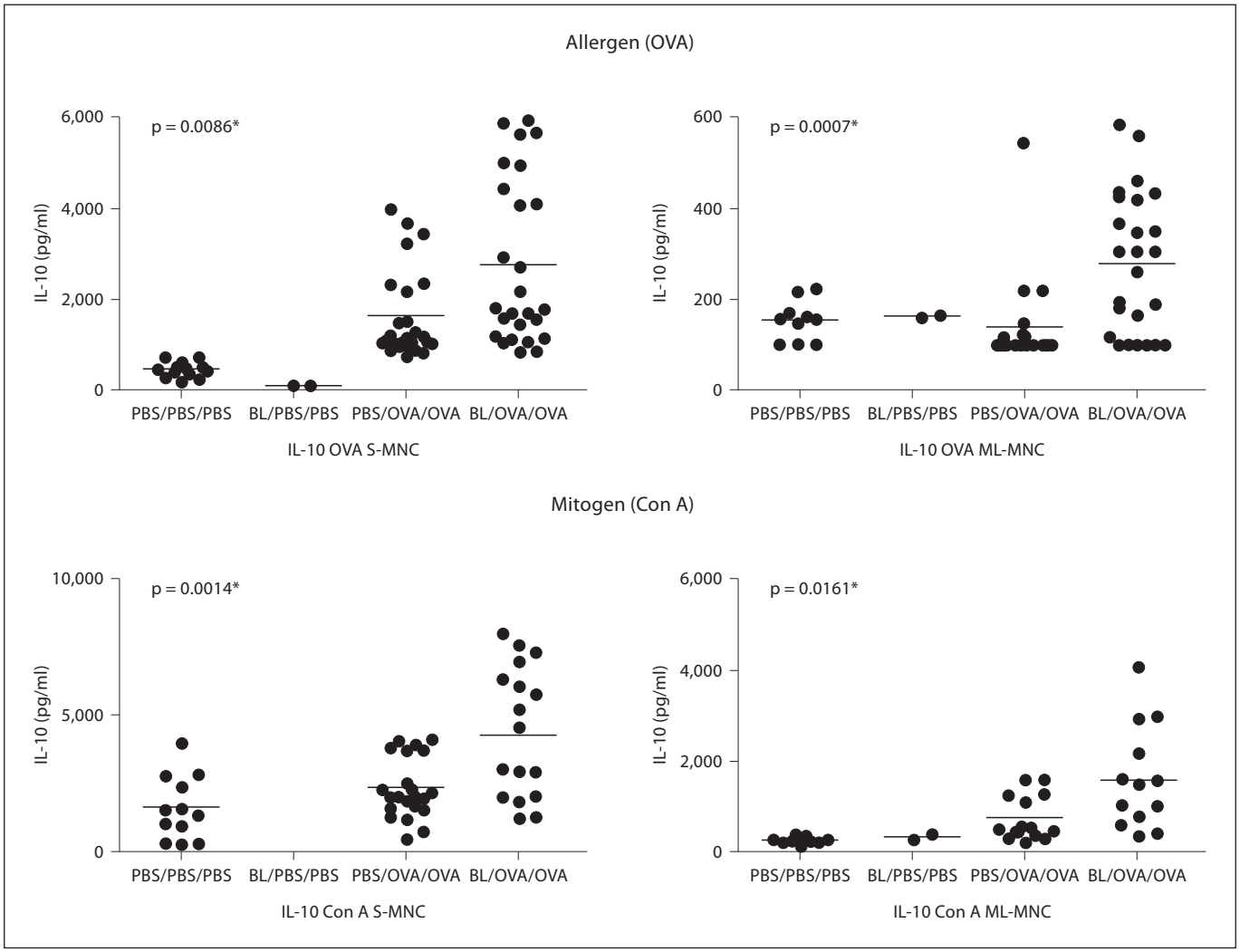

Fig. 5. Influence of OVA sensitization, airway challenges and/or BL treatment on mitogen-specific cytokine production. BN rats were treated as described in figures 1 and 4. S-MNC and ML-MNC were stimulated for 72 h with Con A (2.5 μg/ml). Cytokine levels of IL-10 in the culture supernatant were measured by ELISA. * Differences between PBS/OVA/OVA and BL/OVA/OVA.

meability was significantly ameliorated by local administration of BL.

Discussion

We show here for the first time that oral treatment of newborn rats with bacterial lysates, containing cell-membrane components from *E. coli* (Gram-negative) and *E. faecalis* (Gram-positive bacteria), significantly diminished allergic immune responses and symptoms in a rat model of food allergy. The inhibition of IgE was associated with significant protection against allergen-induced loss of gut mucosal integrity and elevated intestinal permeability. This is a cardinal feature of the disease in food-allergic patients and closely correlates with the frequency of gastrointestinal symptoms [13, 14]. Our data therefore suggest that this effect of bacterial components might serve as a new strategy for prevention of food allergies.

As a sign of systemic reduction of allergic immune responses, levels of allergen-specific serum IgE and IgG antibodies and allergen-specific proliferation of splenocytes were significantly reduced in sensitized, challenged and BL-treated BN rats as compared to nontreated BN rats.

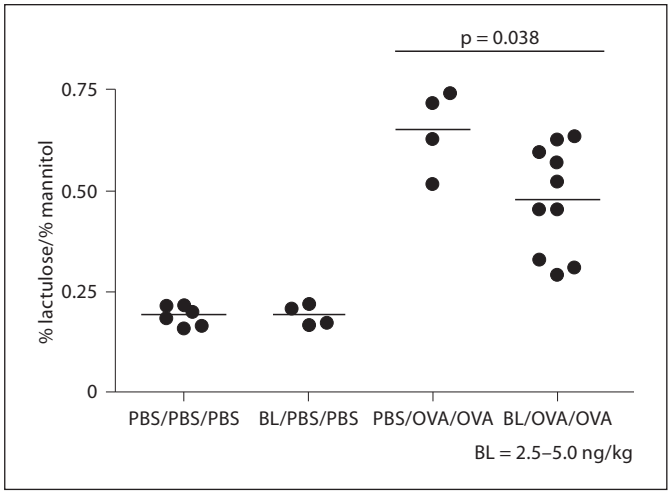

Fig. 6. Influence of OVA sensitization, OVA challenge and/or BL treatment on intestinal permeability. BN rats were treated as described in figure 1. Two hours after the second allergen challenge, animals received lactulose/mannitol in PBS orally. The tracers recovered in the first 8 h in the urine were detected by high-performance liquid chromatography. The ratio of recovered lactulose/mannitol (permeability index) served as marker for intestinal permeability.

Since there were no significant differences in total IgE production and in mitogen-induced cell proliferation between naive animals (data not shown), sensitized sham-treated animals and sensitized BL-treated animals, there is no evidence for general influence of BL on B or T cell functions, but rather for the inhibition of specific T cell stimulation in the course of allergic sensitization.

In our model we performed a 'selective colonization' strategy by stimulating the innate immune response early in life. One major component of BL is bacterial LPS in the outer membrane of Gram-negative bacteria. The effects of LPS on in vivo immune responses are described controversially and seem very time and dose sensitive. Single high-dose LPS exposure induced pro-inflammatory immune responses with increased production of TNF-α [15] resulting in acute neutrophilic airway inflammation and induction of airway hyperresponsiveness [16]. Systemic administration of LPS immediately before allergen sensitization of mice prevented Th2 immune and airway inflammatory responses in an IL-12-dependent mode [17]. Gerhold et al. [18] showed 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, most probably the consequence of LPS tolerance after repeated application of LPS. In accordance, human data confirmed that continuous environmental exposure of children to high concentrations of bacterial products in both farming and nonfarming households resulted in decreased LPS-induced in vitro cytokine production [19].

LPS trigger innate immune responses by binding to Toll-like receptor (TLR) 4. Other components from Gram-positive and Gram-negative bacteria act on dendritic cells and macrophages via activation of TLR2 [20] and TLR9, which specifically recognizes bacterial genomic DNA [20]. Several studies documented that activation of TLRs creates 'sensors for invading microbes' which act as key players in the initiation of Th1 immune responses by inducing secretion of pro-inflammatory cytokines, type I interferons and anti-inflammatory cytokines such as IL-10 [21].

Accordingly, we detected a strong elevation of IL-10 production in the local (mesenteric lymph nodes) as well as in the systemic (spleen) compartment after allergen stimulation of T cells from sensitized, challenged and BL-treated compared to sham-treated BN rats. In view of the properties of IL-10 to act as a regulatory cytokine and to drive the induction of allergen-specific tolerance [21], it is very likely that in the present model IL-10 was responsible for the reduced Ig production and reduced T cell stimulation. Our observation supports the idea that IL-10-producing immune cells with regulatory functions attenuate Th1 and Th2 responses and lead to a 'concept of immune suppression' [22]. This concept states that the effects of bacterial lysates are mainly based on the induction of regulatory T cells balancing and suppressing the Th2 dominance, but to a lesser extent on shifting the allergen-specific responses from a Th2 to a Th1 phenotype.

The enhancement of IL-10 production (fig. 5) was only detected in BL-treated and OVA-sensitized animals. It is therefore likely that the bacterial components influenced the quality of both the (systemic) allergic immune response and the (local) inflammatory response. Following this line, the induction of IL-10 production resulted from modulated immune responses to an allergen and thus depended on active allergen sensitization. This implies that the major benefit of BL would appear in preventive settings.

Biological significance of the treatment with bacterial lysates was confirmed by measurement of the intestinal permeability, demonstrating ameliorated levels in treated compared to untreated sensitized and challenged animals. This marker is directly associated with the disruption of the intestinal barrier [13], a pivotal event in the

Table 2. Statistical properties and supplementary data of all performed experiments

	BL/OVA/OVA	PBS/OVA/OVA	BL/OVA/OVA	PBS/OVA/OVA	p value	
	count	count	mean ± STD	mean ± STD	Wilcoxon	t test
Cytokine production, pg/ml						
IFN-γ-spleen-Con_A	13	12	34,814.54 ± 17,397.29	24,116.67 ± 15,323.95	0.0441*	0.1176
IFN-γ-spleen-OVA	19	11	2,866.63 ± 2,984.59	1,511.54 ± 1,513.13	0.3774	0.173
IL-10-MLN-Con_A	14	14	1,576.87 ± 1,105.04	743.05 ± 498.32	0.0258*	0.0161*
IL-10-MLN-OVA	25	21	281.36 ± 154.84	138.01 ± 99.58	0.0007*	0.0007*
IL-10-spleen-Con_A	18	22	4,278.42 ± 2,376.9	2,317.44 ± 1,100.48	0.0129*	0.0014*
IL-10-spleen-OVA	27	25	2,730.32 ± 1,783.55	1,629.09 ± 970.34	0.0067*	0.0086*
IL-4-MLN-Con_A	8	13	20.5 ± 14.95	7.83 ± 8.27	0.0886	0.0209*
IL-4-spleen-Con_A	14	24	88.71 ± 77.14	53.6 ± 59.62	0.3639	0.1251
IL-4-spleen-OVA	22	25	52.01 ± 59.32	30.09 ± 42.15	0.0861	0.1476
Proliferation (proliferation index)						
MLN-Con_A	15	14	105.23 ± 108.93	102.59 ± 70.16	0.4005	0.9393
MLN-OVA	12	10	1.9 ± 0.9	3.45 ± 3.37	0.2276	0.1401
Spleen-Con_A	32	27	50.85 ± 46.73	45.03 ± 30.45	0.7783	0.5815
Spleen-OVA	22	19	3.64 ± 1.83	5.75 ± 3.35	0.0226*	0.0147*
Intestinal permeability	10	4	0.48 ± 0.13	0.65 ± 0.1	0.0539	0.0389*

* $p < 0.05$.

Table 3. Statistical properties and supplementary data of all performed experiments

	PBS/PBS/PBS	BL/PBS/PBS	p value	
	mean ± STD	mean ± STD	Wilcoxon	t test
Cytokine production, pg/ml				
IFN-γ-spleen-Con A	53,500 ± 1,272.79	–	–	–
IFN-γ-spleen-OVA	n.d.	–	–	–
IL-10-MLN-Con A	248.32 ± 86.66	323 ± 74.08	0.2182	0.292
IL-10-MLN-OVA	154.44 ± 42.74	163.15 ± 3.47	0.4873	0.7861
IL-10-spleen-Con A	1,608.49 ± 1,175.29	–	–	–
IL-10-spleen-OVA	463.17 ± 164.91	100 ± 0		
IL-4-MLN-Con A	n.d.	–	–	–
IL-4-spleen-Con A	9.23 ± 6.89	–	–	–
IL-4-spleen-OVA	n.d.		–	–
Proliferation (proliferation index)				
MLN-Con A	84.46 ± 3.63	95.88 ± 38.28	0.7	0.6339
MLN-OVA	1.02 ± 0.04	1.14 ± 0.32	0.8	0.6281
Spleen-Con A	39.82 ± 27.36	49.88 ± 39.32	0.6202	0.5907
Spleen-OVA	2.01 ± 1.15	1.15 ± 0.11	0.2124	0.2333

Values are expressed as means ± standard deviation. n.d. = Not determined.

course of allergic gut inflammation. This allows allergens to cross the intestinal barrier and to stimulate the submucosal immune system, thus enhancing the production of inflammatory cytokines, mediators and most probably specific IgE antibodies [14, 23]. The importance of the intact skin/epithelial barrier has been highlighted in the last years by publications regarding the loss-of-function variants of the epidermal barrier protein, e.g. filaggrin, which display a predisposing factor for atopic eczema.

In summary, we demonstrated that early treatment with bacterial lysates suppressed allergen-specific immune responses in the local lymph nodes of the gut without showing any side effects. Notably, functional data of intestinal permeability demonstrated biological significance. These results highlight that stimulation of the innate immune system was able to prevent food allergy and warrant further intervention studies based on the hygiene hypothesis.

Acknowledgements

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Fig. 1: long-term protocoll

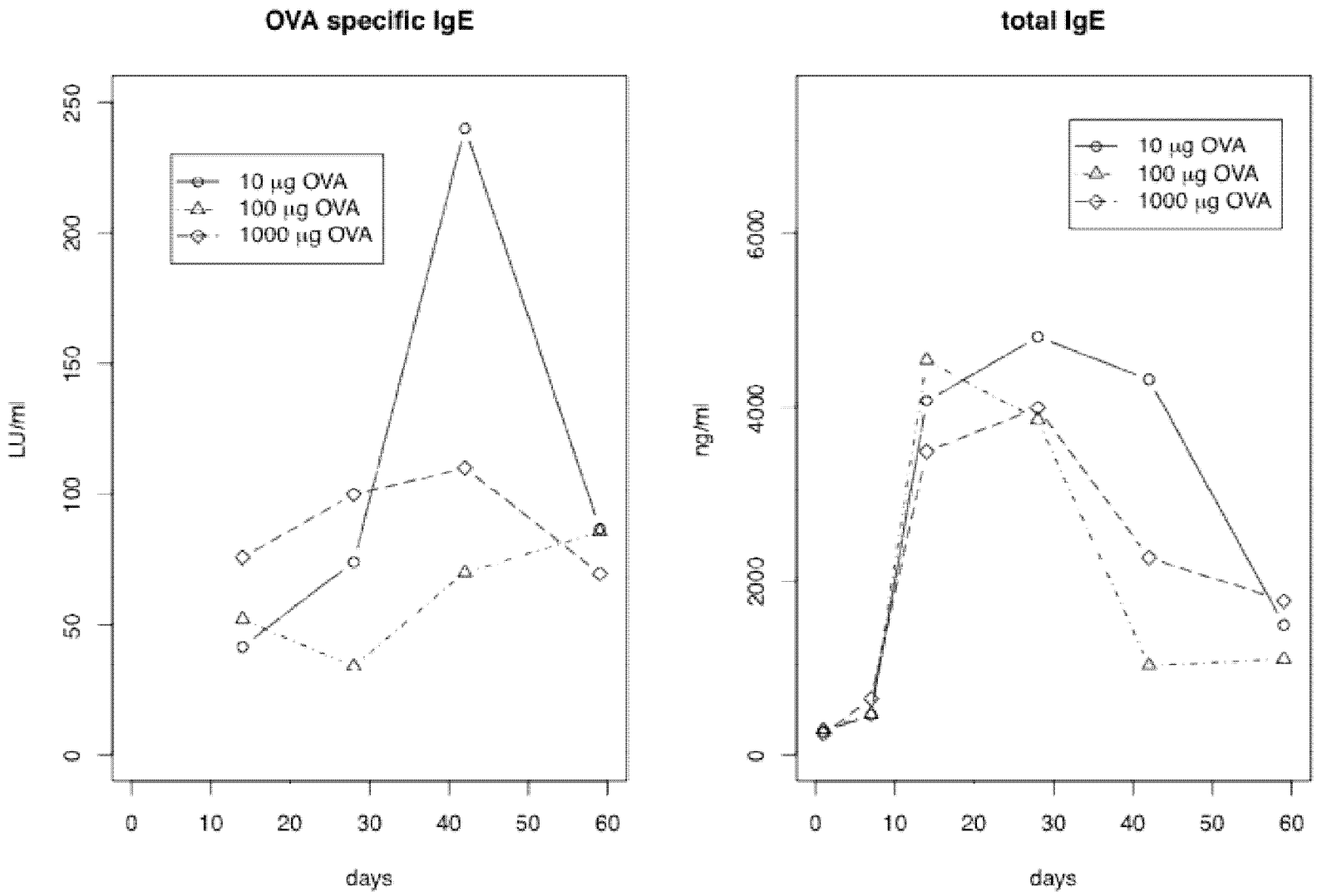


Fig. 2a: short-term protocol

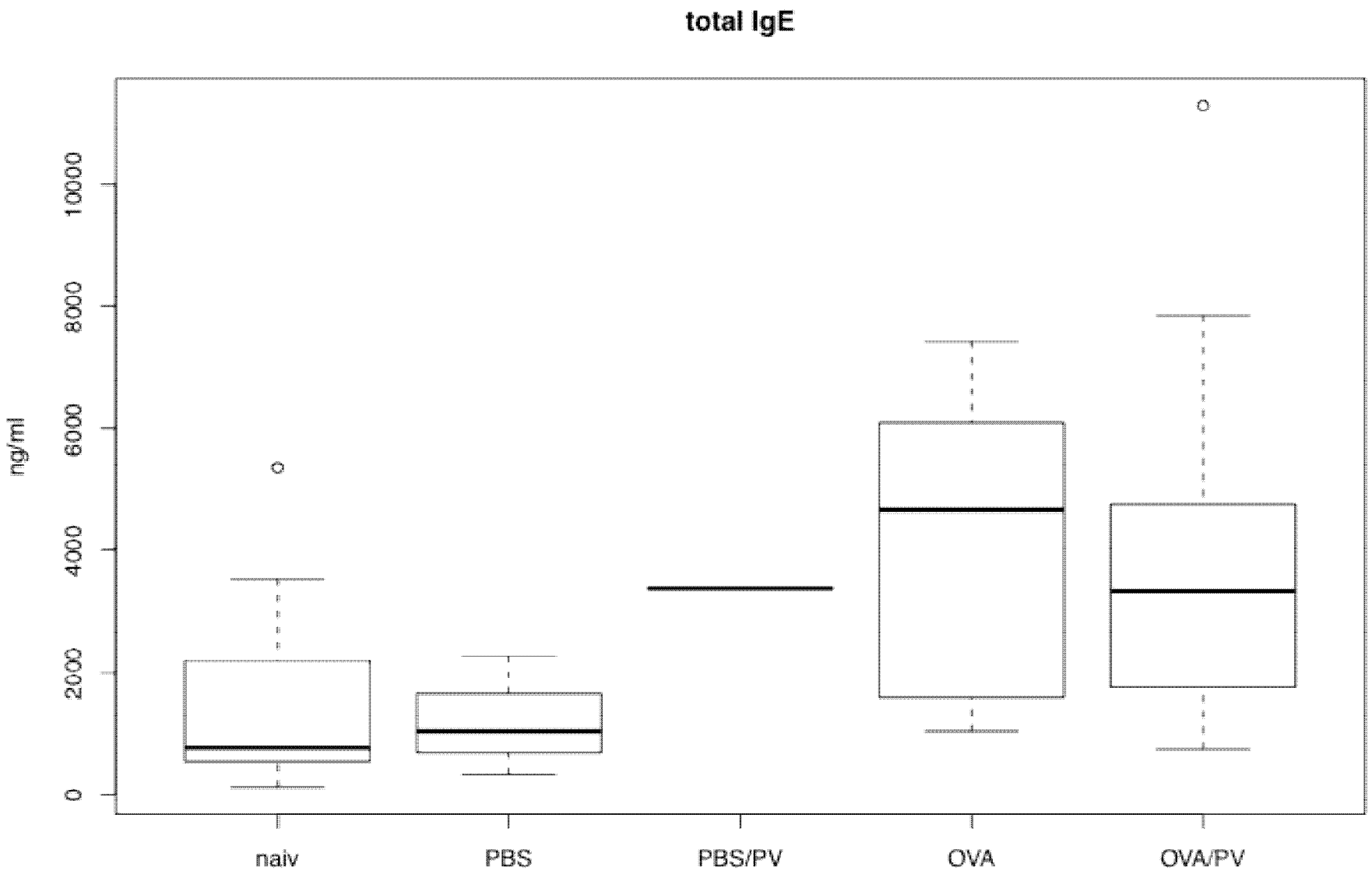


Fig. 2b: short-term protocol

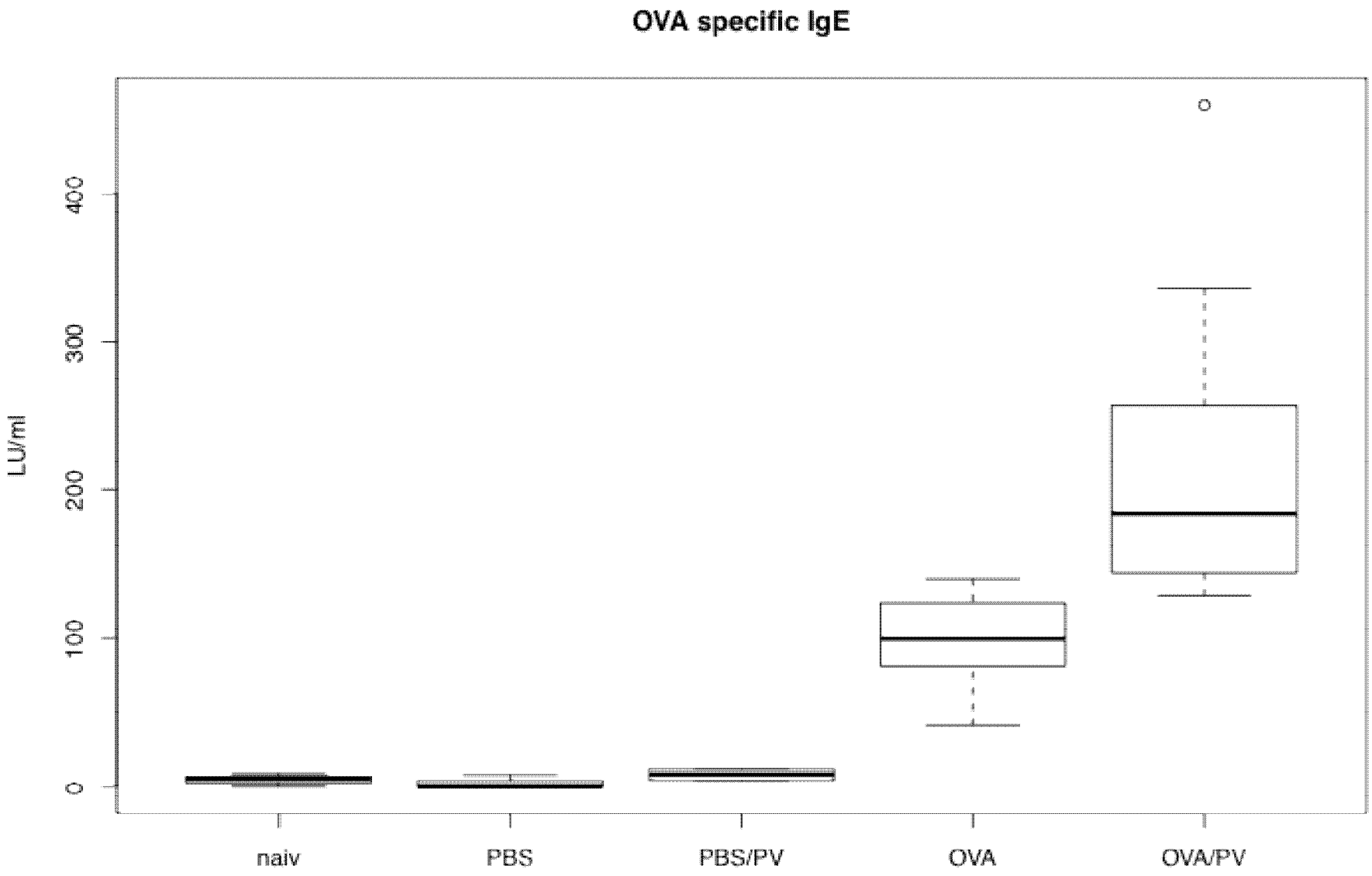
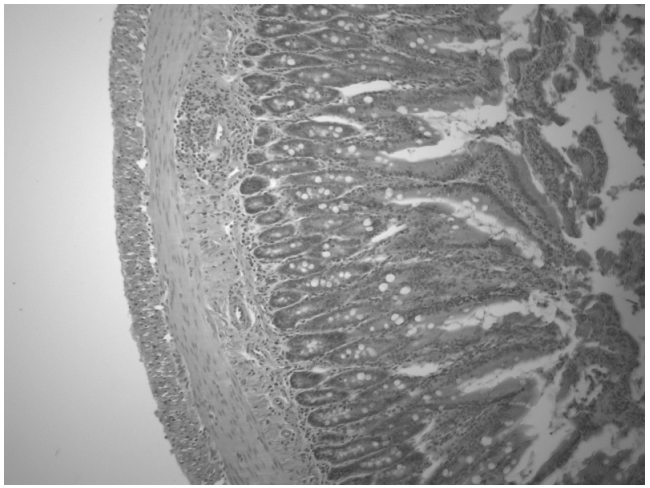
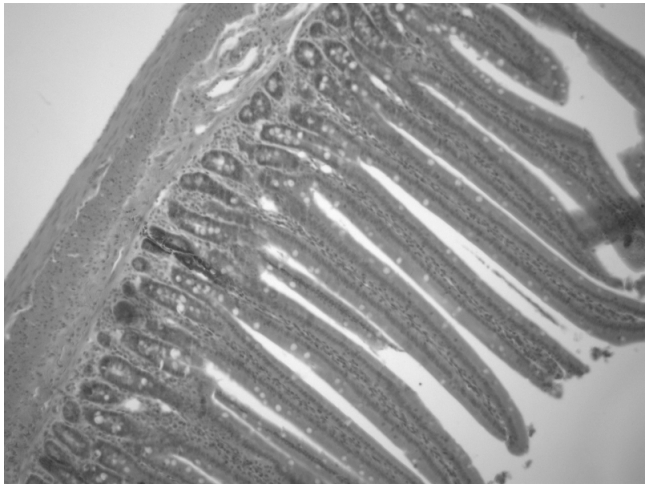


Figure 1: Immunoglobulin titers in OVA-sensitized BN rats: long-term protocol
Animals were sensitized by intraperitoneal injection of OVA (10 µg, 100 µg, or 1000 µg) once every second week for a total of 12 weeks (*long-term protocol*). The allergen of the first injection was absorbed to 2 mg aluminum hydroxide (AL) and *B. pertussis* whole body vaccine (PV) ($2x10^6$ PV, E. von Behring, Marburg, Germany). Blood samples were taken at indicated time points. Serum levels of allergen-specific IgE and total IgE were measured by ELISA.

Figure 2 a and b: Immunoglobulin titers in OVA-sensitized BN rats: short-term protocol
Animals were sensitized by intraperitoneal injection of 10 µg OVA on days 1, 5 and 10 (*short-term protocol*). To assess the effect of adjuvants on sensitization, the allergen of the first injection was absorbed to 2 mg aluminum hydroxide (AL) and/or *B. pertussis* whole body vaccine (PV) ($2x10^6$ PV, E. von Behring, Marburg, Germany). Blood samples were taken at day 24. Serum levels of total IgE (figure 2a) and allergen-specific IgE (figure 2b) were measured by ELISA.





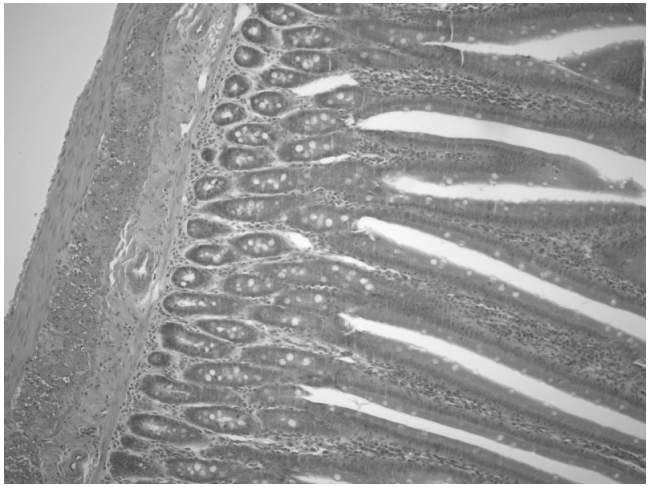


Fig. 3

Figure 3: Intestinal changes upon local allergen challenge

BN were sensitized by intraperitoneal injection of 10 µg OVA on days 1, 5 and 10 (*short-term protocol*). Allergen was absorbed to aluminium hydroxide (2 mg AL) and pertussis whole body vaccine ($2x10^6$ PV, E. von Behring, Marburg, Germany) at the first injection timepoint. Sensitized animals according to the short-term protocol were challenged locally by gavage feeding of 500 µg OVA (in 1 ml PBS) twice, on days 20 and 21 (*two challenges*) **(3a)**, or on ten consecutive days, beginning on day 20 (*repeated challenges*) **(3c)**. The negative control groups were sham-sensitized and challenged with PBS according to the same protocols **(3b)**. All animals were sacrificed and analyzed 24 h after the last allergen challenge. Intestinal tissue was fixed in 4% formalin, dehydrated, mounted in paraffin, sectioned, and stained with hematoxylin-eosin (HE) or periodic acid-Schiff (PAS) reaction by using a standard protocol.