

Contrasting Immunological Effects of Two Disparate Dusts – Preliminary Observations

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Key Words

Dendritic cell • Jagged-1 • OX40L • Atopy • Dust • Bacteria • Th2 • Th1 • *Corynebacterium* • *Streptococcus*

Abstract

Background: Modern lifestyle and urbanization have been associated with a raised risk for atopic diseases whereas early and long-term exposure to a farm environment confers protection against atopic sensitization. Immunomodulatory potential and microbiological characteristics of settled airborne dust from an urban house and a barn were examined.

Methods: Pulmonary inflammation was induced in mice by repeated intranasal administration of dusts. Monocyte-derived human dendritic cells (moDCs) were exposed to dusts followed by coculture with purified naïve T cells. Cytokine/chemokine mRNA and protein levels were analyzed by real-time polymerase chain reaction, enzyme-linked immunosorbent assay and flow cytometry. The dusts were analyzed by cloning and sequencing of 16S rRNA genes (290 sequences) for DNA, lipids, endotoxin and β -glucan, by live-dead staining, viable counting, isolation and identification of pure

cultures (n = 76). **Results:** Repeated exposure to house dust elicited pulmonary eosinophilia in mice whereas exposure to barn dust elicited neutrophilic and lymphocytic airway inflammation. Stimulation of moDCs with urban house dust elicited expression of Th2-promoting OX40L and Jagged-1 costimulatory molecules. Dendritic cells (DCs) exposed to house dust directed naïve T cells towards Th2 responses. Exposure of DCs to barn dust elicited the development of Th1-dominated immune responses. Urban house dust contained bacterial debris almost exclusively of human commensal species (*corynebacteria*, *streptococci*) whereas barn dust comprised mainly intact, viable bacteria of high diversity and no commensal species. **Conclusion:** Contact to debris originating from human commensal bacteria in urban house dust elicited a Th2-type response whereas barn dust with high bacterial diversity directed the cells towards a Th1 response.

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1018–2438/09/1491–0081\$26.00/0

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Introduction

The prevalence of atopic diseases such as asthma, rhinitis and atopic dermatitis has reached epidemic proportions in many modern countries [1]. A causative agent responsible for this increase has not been identified, although modern lifestyle and urbanization have consistently been associated with a raised risk for atopic diseases [2]. On the contrary, evidence indicates that early and long-term exposure to a farm environment with a high microbial content induces a protective effect against asthma and atopic sensitization.

Lower frequencies of asthma and atopic sensitization were observed in young children exposed to a farm environment and consuming farm milk as compared to non-exposed controls [3, 4]. Similarly, the prevalence of atopy and atopic diseases among both adults [5] and children [6] was significantly lower in Russian Karelia as compared with Finnish Karelia. Despite the close geographical proximity and similarities in geoclimatic conditions, exposure of the inhabitants of Russian Karelia to environmental microbes (e.g. drinking water) was dramatically higher compared to inhabitants of Finnish Karelia [7].

A consensus exists that immune response to environmental and food allergens is characterized by enhanced expression of Th2 cytokines, e.g. interleukin 4 (IL-4), leading to elevated production of IgE antibodies and tissue eosinophilia, which are hallmarks of atopic diseases [8]. Recent findings also highlight the central role of dendritic cells (DCs) in the sensitization process [9]. Exposure to certain microbial factors educates immature DCs to mature either to Th1- or Th2-promoting DCs [9]. Moreover, regulatory T cell function seems to be deficient in allergic patients, which might be due to reduced microbial burden to DCs [10]. Although much attention has indeed been paid to the role of the regulatory network in the development of atopy and atopic disease during the last decade, the role of Th1/Th2 imbalance in this context cannot be ignored either.

This preliminary study was undertaken to examine the immunomodulatory potential of two contrasting dusts and characterize their microbiological composition.

Materials and Methods

Sampling of Settled Dusts

Two disparate dust samples, house dust and barn dust, were sampled from buildings that had been in uninterrupted use as a human urban dwelling and a farm-attached barn, respectively, for at least 30 years, with no known moisture damage or construction

or renovation activity. The dusts accumulated over approximately 10 years were sampled manually from surfaces 1.5–2.5 m above floor level into Petri dishes and stored at room temperature.

In vivo Experiments

Animals

Female BALB/c mice, 6–8 weeks of age, were obtained from M&B Taconic (Ry, Denmark). All the experiments used were approved by the Social and Health Care Department at the State Provincial Office of Southern Finland.

Experimental Procedure

The dusts were administered intranasally twice a week for 3 weeks under light isoflurane anesthesia. The dusts were given in two different concentrations (10 and 100 µg per instillation) for each dust; the control group received PBS only. Eight mice were treated each time. Airway responsiveness was measured 48 h after the 7th intranasal instillation after which the mice were sacrificed and blood and lung samples were collected as previously described [11].

Real-Time Quantitative Polymerase Chain Reaction

RNA extraction, cDNA synthesis and real-time quantitative polymerase chain reaction (RT-PCR) were performed as described earlier [12].

Measurement of Cytokines and Chemokines in Bronchoalveolar Lavage Fluid

Cytokine levels in bronchoalveolar lavage fluid were measured as previously described [11].

Statistical Analysis

Data were analyzed with GraphPadPrism Software. Single-group comparisons were performed by the Mann-Whitney test. $p < 0.05$ was considered statistically significant.

In vitro Experiments

Dendritic Cells

DCs cells were derived from human monocytes (moDCs). Briefly, overnight buffy coats were obtained from the Finnish Red Cross (Helsinki, Finland). Peripheral blood leukocytes were isolated using gradient centrifugation (350 g, 25 min) with Ficoll-Paque Plus (GE Healthcare), washed twice with PBS and suspended in RPMI-1640 with penicillin-streptomycin (PEST, Sigma), L-glutamine (Invitrogen) and 2% fetal bovine serum (FBS; Invitrogen). Monocytes were allowed to adhere for 30 min and were washed 3 times with PBS. Differentiation was induced in RPMI-1640, 1% vol/vol PEST, 1% vol/vol L-glutamine, 10% vol/vol FBS containing 40 ng/ml recombinant GM-CSF and 20 ng/ml IL-4 (Invitrogen). Fresh medium was added on days 3 and 5. Settled dusts were homogenized in PBS with an Ultra-Turrax T8 (IKA Labortechnik, Germany). Immature DCs were exposed to 30 and 300 µg/ml dusts for 6 and 24 h. RNA was extracted using Eurozol (EuroClone, Italy). An RT-PCR assay was performed as described earlier [12].

Interaction between Autologous DCs and T Cells

DCs were obtained as above, except that buffy coats donated the same day were used and an additional gradient centrifugation (350 g, 25 min) with PBS-diluted Ficoll-Paque Plus (1.065 g/ml)

was done after the first gradient centrifugation and 2 PBS washes. The lower fraction from the additional gradient centrifugation was frozen at -70°C in RPMI-1640 (1% PEST, 1% L-glutamine, 15% DMSO and 50% FBS all vol/vol). DCs were exposed to 30 and 300 $\mu\text{g/ml}$ dusts for 24 h. Frozen leukocytes were melted slowly in RPMI-1640 and 50% FBS. Naïve T cells were isolated using magnetically labeled antibodies for CD4+CD45RA+ cells (Miltenyi Biotec, Germany). DCs were washed with PBS and cocultured with naïve T cells in round-bottom 96-well plates (10,000 DCs:100,000 T cells) in 100 μl RPMI-1640 (1% PEST, 1% L-glutamine, 5% human AB sera). 100 μl medium (IL-2, 20 U/ml final concentration) was added after 36 h. Half of the media were exchanged on days 5 and 8.

Flow Cytometry

T cells were stimulated on day 9 with 0.025 $\mu\text{g/ml}$ phorbol 12-myristate 13-acetate (PMA; Sigma) and 1 $\mu\text{g/ml}$ ionomycin (Sigma), for 6 h. 10 $\mu\text{g/ml}$ brefeldin A (Sigma) was added for the last 3 h. Intracellular staining was performed using the Cell Perm & Fix kit (Caltag Laboratories, Burlingame, Calif., USA). Antibodies and appropriate isotype controls were from eBioscience (San Diego, Calif., USA). Activated T cell blasts were gated for analysis of cytokine expression. The average percentage of IFN- γ and IL-4 cells from two independent experiments with both concentrations (30 and 300 $\mu\text{g/ml}$) was calculated.

Microbiological Analyses

Determination of Limulus Activity

Biologically active endotoxin and β -D-glucan in the dusts were measured using the standard kinetic turbidimetric limulus amebocyte lysate assay (Scan Dia Laboratory Service, Copenhagen, Denmark).

Determination of Bacterial-Signature Fatty Acids

The 3-hydroxy fatty acids in the settled dusts were quantified by gas liquid chromatography as methyl esters as described for pure cultures elsewhere [13]. Bacterial-signature fatty acids were calibrated by adding 10, 5 and 1 mg of biomass of *Bacillus pumilus* OS-141 (branched-chain C_{12-19} iso and anteiso acids), *Mycobacterium murale* MA-113 (tuberculostearic acid), *Sphingomonas aurantiaca* MA-101b (C_{14} 2-hydroxy acid) and *Escherichia coli* H-673 (C_{14} 3-hydroxy acid) to 200 mg of dust before methylation.

Enumeration of the Dust Microbial Contents

The dusts were suspended in tryptic soy agar; after overnight incubation at 6°C , they were live-dead stained with propidium iodide and calcein-acetyl methyl (Molecular Probes, Leiden, The Netherlands). For transmission electron microscopy, the specimens were fixed in 2.5% glutaraldehyde, stained and imaged as described elsewhere [14]. Bacterial cells were counted by epifluorescence microscopy (viability staining, 30 fields, $\times 40$ objective) or from transmission electron micrographs (200 fields, magnification $\times 10,000$ or 20,000).

Heterotrophic viable cells were counted [15] on R2A agar after aerobic incubation for 7–28 days at 16 – 22°C .

DNA Isolation and Determination of DNA Concentration

DNA was isolated from 30–50 mg of dust and DNA concentration was measured as described elsewhere [16].

Real-Time Quantitative Polymerase Chain Reaction

RT-PCR, using the primers pE and pF' targeted for the 16S rRNA gene of the *Eubacteria* domain, was carried out as described elsewhere [17].

DNA Cloning and Sequence Analysis

Three to 40 ng of DNA isolated from dust was PCR amplified using the primers pA and pH' [18], targeted for the 16S rRNA gene of *Eubacteria* and DyNAzyme II Taq polymerase enzyme (Finnzymes, Espoo, Finland). The 16S rRNA gene of the bacterial isolates from the dust cultures were PCR amplified using the primer pairs pA/pH', pA/pE', pA/pF' and pD/pH' [18].

DNA cloning and sequencing was carried out with robotized instruments at the DNA Laboratory of the University of Helsinki Institute of Biotechnology. The PCR products were cloned into *E. coli* using the PCR Cloning Kit (Qiagen, Valencia, Calif., USA), plasmid DNA was extracted using MultiScreen 96PLASMID Plates (Millipore, Billerica, Mass., USA); inserts were reamplified with vector primers and purified with MultiScreen 384PCR plates. Sequencing was done using the pD' primer [18] for the clones and the pA, pC, pD, pD', pE, pF, pF' and pH' primers [18] for the isolates, BigDye Terminator cycle sequencing kit and ABI 3730 sequencer (Applied Biosystems, Foster City, Calif., USA).

The sequences of the clones ($n = 290$, length 321–532 nt) and of the cultured isolates ($n = 86$, length 336–1,551 nt) were deposited in the European Molecular Biology Laboratory sequence database (accession No.: clones AM900041–AM900331, isolates AM237342–AM237400 and AM900771–AM900797). The sequences were analyzed using the RDP Classifier [19], Library Compare and Sequence Match tools [20] at the Ribosomal Database Project II website (<http://rdp.cme.msu.edu/>) and Basic Local Alignment Search [21] at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic analysis was performed using the DNAPARS program [22] of the Bioedit software package [23].

Biodiversity Analysis

Shannon-Wiener's and Simpson's indices and the evenness measure based on the Shannon-Wiener index were calculated as described elsewhere [24]. The Renkonen percentage similarity measure and the Sorensen coefficient were calculated for genus incidence data [24].

Results

Pulmonary Eosinophilia Was Significantly Stronger in Mice Exposed to Urban Dust Compared to Mice Exposed to Barn Dust

Airway exposure to house dust induced significantly more eosinophils in the bronchoalveolar lavage fluid compared to barn dust (fig. 1a). On the contrary, the numbers of neutrophils and lymphocytes were significantly higher in mice exposed to barn dust. Supporting the cellular distribution, mRNA levels for the chemokines attracting these cells were elevated in lung tissue. Expression of the eosinophil-attracting chemokine CCL11 (eo-

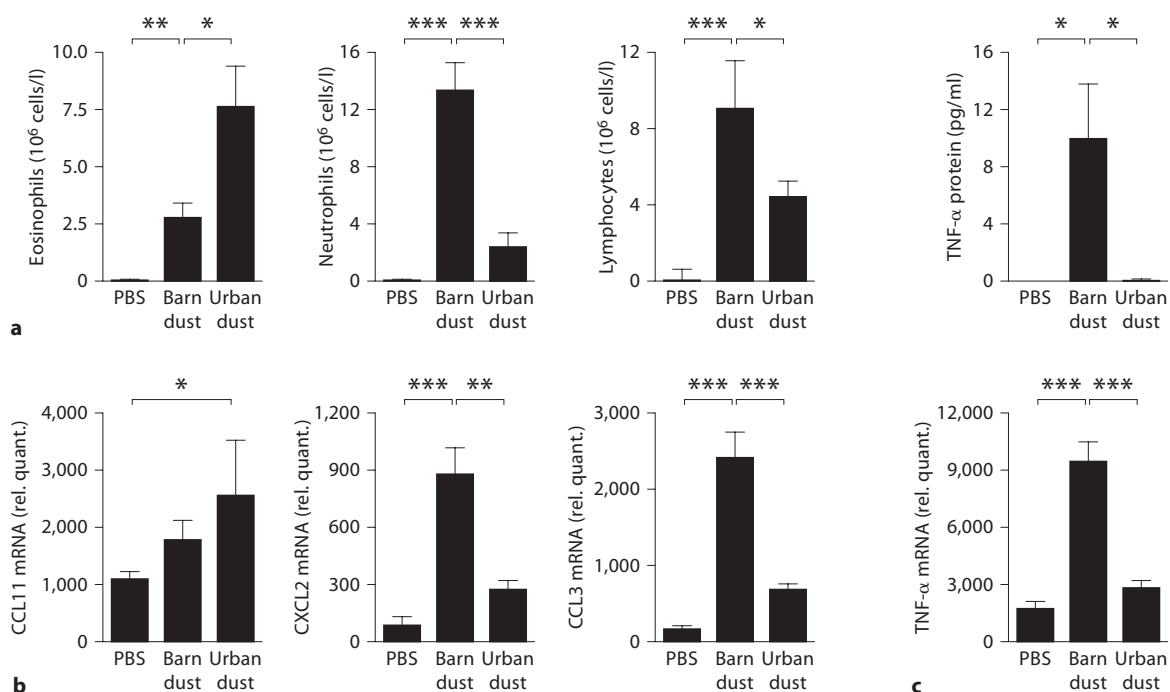


Fig. 1. In vivo experiments. BALB/c mice were given either urban house or barn dust intranasally twice a week for 2 weeks; the animals were then sacrificed and the following parameters measured. **a** Cell distribution in bronchoalveolar lavage fluid was counted using the May-Grünwald-Giemsa stain. **b** Relative mRNA levels of chemokines in lung tissues. Each chemokine is shown below the cell population it attracts. **c** TNF- α protein and mRNA levels.

taxin) mRNA was higher in the mice exposed to house dust whereas exposure to barn dust caused higher transcription of neutrophil-attracting chemokine CXCL2 and lymphocyte-attracting chemokine CCL3 (fig. 1b). Pro-inflammatory TNF- α in the lungs was also drastically higher after exposure of the mice to barn dust, both at the mRNA and the protein level (fig. 1c).

DCs Exposed to House Dust Induced the Generation of Th2 Cells Whereas Cells Exposed to Barn Dust Induced the Generation of Th1 Cells

Transcription of several cytokines and costimulatory molecules in human moDCs was measured by quantitative RT-PCR (fig. 2a). Exposure to house dust elicited higher levels of Th2-promoting costimulatory molecules OX40L [25] and Jagged-1 [26] mRNA compared to exposure to barn dust. In contrast, exposure to barn dust strongly induced expression of the Th1-promoting notch ligand Delta-4 as well as proinflammatory cytokines IL-

23 (functional IL-23 consists of a heterodimer of the IL-12p40 subunit and the p19 subunit) and TNF- α . Only marginal expression of these cytokines was seen after exposure to house dust (fig. 2a).

Dust-exposed moDCs were cocultured with magnetically isolated naïve T cells. Intracellular cytokine expression of interferon- γ (IFN- γ) and IL-4 were measured by flow cytometry after PMA stimulation in activated T cells (fig. 2b). The moDCs exposed to house dust skewed naïve T cells into Th2 cells whereas moDCs exposed to barn dust elicited the generation of Th1-polarized cells. The moDCs exposed to house dust generated significantly more IL-4-secreting T cells compared to moDCs exposed to barn dust (fig. 2b). In contrast, the moDCs exposed to barn dust generated significantly more IFN- γ -secreting T cells compared to the moDCs exposed to house dust (fig. 2b).

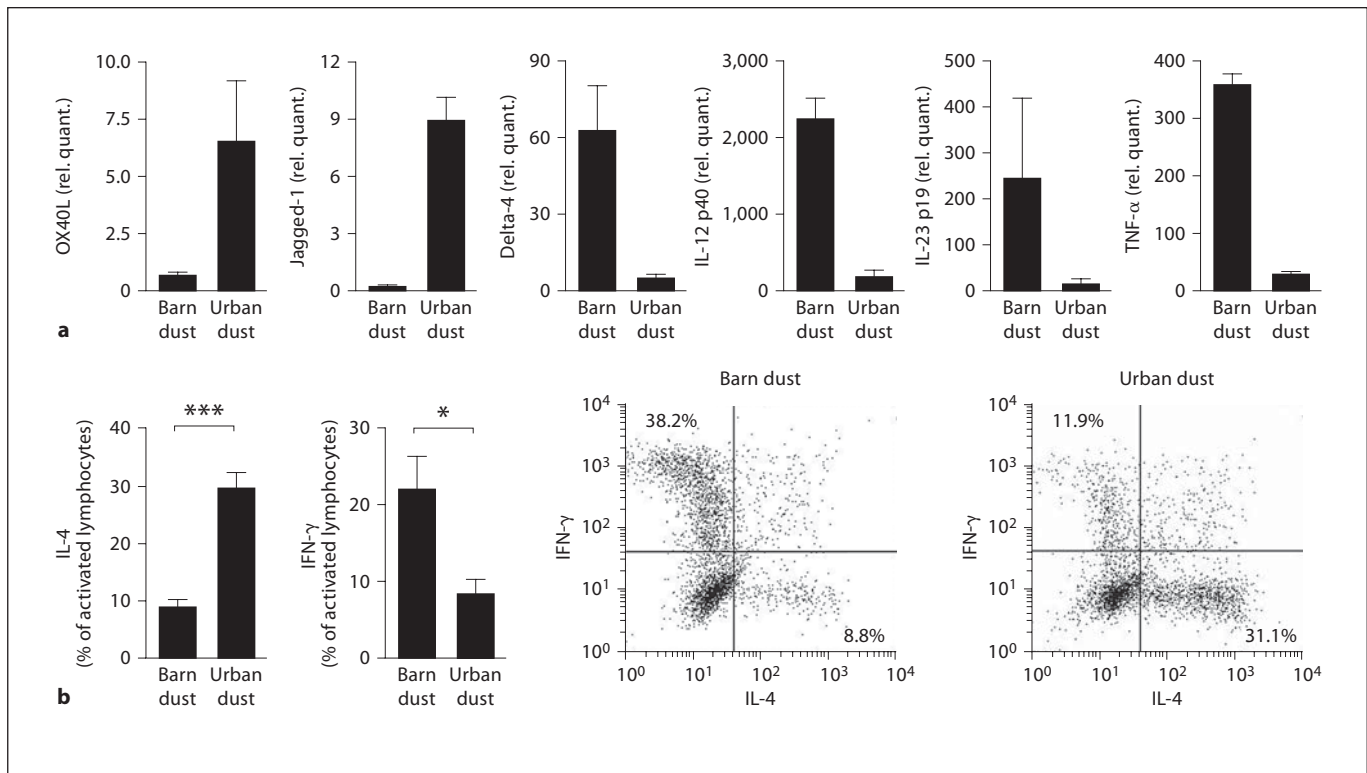


Fig. 2. In vitro experiments. **a** Relative mRNA levels in moDCs after 24 h of incubation with 300 µg/ml of either dust. **b** Th cell intracellular cytokines were analyzed by flow cytometry. MoDCs were exposed to dusts for 24 h and then coincubated with naïve T cells for 9 days. T cells were activated using PMA and ionomycin. To the left, mean percentages of IL-4- and IFN-γ-expressing cells; to the right, representative flow cytometry scatter plots.

The Dusts Differed Dramatically in the Viability, Composition and Diversity of Their Bacterial Communities

Table 1 describes the two dusts that provoked profoundly different immunological responses. Both contained similar amounts of the 16S rRNA gene copies, indicating similar contents of bacteria. However, microscopic analysis of urban house dust by live-dead staining showed that only a minor part of the bacterial DNA represented morphologically intact cells ($\leq 1\%$, $5 \times 10^6 \times \text{g}^{-1}$) of the proportion expected based on the measured copy number of the 16S rRNA genes (equivalent to $>10^8$ bacterial cells $\times \text{g}^{-1}$). This is in contrast to barn dust, where the microscopic analysis matched that of 16S rRNA gene number well, and staining showed that this dust mostly contained live bacteria. The tenfold excess of DNA of barn dust therefore originated from organisms other than bacteria.

The endotoxin and β -D-glucan contents in the dusts were similar by the limulus assay (table 1). Barn dust but

not urban house dust contained large amounts of 2- and 3-hydroxy fatty acids, indicative of sphingolipid- or endotoxin-containing Gram-negative bacteria. If all 3-hydroxy fatty acids in barn dust represented lipopolysaccharide, then only 0.01% of it was present in limulus-active form at the time of dust collection.

Bacterial Content of the Dusts Analyzed by 16S rRNA Gene Sequencing

Figure 3 shows that urban house dust was dominated by corynebacteria ($n = 34$), streptococci ($n = 33$), staphylococci ($n = 9$) and *Finnegoldia* spp. ($n = 9$), representing 78% of the clone library. The most frequent clones were *Corynebacterium tuberculo-stearicum* ($n = 26$), *Streptococcus gordonae* ($n = 15$) and *Finnegoldia magna* ($n = 9$). In barn dust there were no dominant species and none of the streptococcal, staphylococcal or corynebacterial species found in house dust were present (fig. 3, table 2). Instead, 20 species of *Staphylococcus*, *Bacillus*, *Planococcus* and *Jeotgalicoccus* were found and 11 different genera of

Table 1. Bacterial cell constituents found in indoor dusts collected from a barn and an urban house

	Barn dust	Urban house dust
DNA		
Total, $\mu\text{g} \times \text{g}^{-1}$	62	5.8
Bacterial, 16S rRNA gene copies $\times \text{g}^{-1}$	1×10^9 ^a	5×10^8 ^a
Microscopic count ^b		
Total, cells $\times \text{g}^{-1}$	5×10^8	5×10^6
Live, cells $\times \text{g}^{-1}$	2×10^8	$<10^6$
Viable count, CFU $\times \text{g}^{-1}$ ^c	4×10^5	1×10^4
Limulus-active substances		
Endotoxin, $\mu\text{g} \times \text{g}^{-1}$	0.45	0.33
β -D-Glucan, $\mu\text{g} \times \text{g}^{-1}$	0.57	0.73
Ester-bound fatty acids ^a , $\text{mg} \times \text{g}^{-1}$		
Total	1.9	1.5
Bacterial	0.18	0.60
Branched C ₁₁ –C ₁₉ ^d	0.088	0.060
3-Hydroxy ^e	0.015	<0.0005
2-Hydroxy C _{14:0} ^f	0.0068	<0.0005
Other ^g	0.073	<0.0005

^a With 3 16S rRNA gene copies/genome, cell number = copy number/3.

^b Counted by epifluorescence microscopy from live-dead stained preparations. The preparations were checked by transmission electron microscopy to verify that the fluorescent particles were mainly microbial cells.

^c CFU of aerobic heterotrophic bacteria.

^d Signature fatty acids for Gram-positive bacteria: C_{11:0}, C_{13:0}, C_{15:0}, C_{17:0}, C_{19:0} iso and anteiso branched chain acids.

^e Signature fatty acids for lipopolysaccharide-containing Gram-negative bacteria: C_{10:0}-3-OH, C_{12:0}-3-OH and C_{14:0}-3-OH acids.

^f Signature fatty acids for sphingolipid containing bacteria: C_{14:0}-2-OH acids.

^g Other bacterial fatty acids: C_{17:1} ω 8c, C_{17:0} cyclo, C_{10:0}-2-OH, C_{18:0}-2-OH, C_{18:1} ω 7c acids.

Gram-negative bacteria, none of which were found in urban house dust (fig. 3).

When the dusts were cultured on plates (fig. 4), the Gram-positive bacteria isolated from urban house dust were mostly bacilli (*Bacillus*, *Paenibacillus*) and those isolated from barn dust were mainly actinobacteria (7 genera). Besides Gram-positive bacteria, house dust grew mainly deinococci, none of which were found in barn dust. Instead, barn dust grew 7 different genera of Gram-negative bacteria, which explains the large content of 2- and 3-hydroxy fatty acids in the dust (table 1).

Bacterial diversity was dramatically higher in barn dust compared to house dust (Shannon-Wiener's and Simpson's indices) for both the cultured and the cloned

Table 2. Species of *Streptococcus*, *Staphylococcus*, *Fingoldia* and *Corynebacterium* in the 16S rRNA gene clones obtained from DNA contained in dust

Species	Clones	
	urban house	barn
<i>Streptococcus</i> spp.		
<i>S. gordonii</i>	15	
<i>S. cristatus</i>	5	
<i>S. mitis</i>	3	
<i>S. oralis</i>	3	
<i>S. sanguinus</i>	3	
<i>S. thermophilus</i>	1	
<i>S. downey</i>	1	
<i>S. infantis</i>	1	
<i>Staphylococcus</i> spp.		
<i>S. caprae</i>	3	
<i>S. epidermidis</i>	2	
<i>S. warneri</i>	2	
<i>S. hominis</i>	1	
<i>S. haemolyticus</i>	1	
<i>S. equorum</i>		10
<i>S. vitulinus</i>		3
<i>Fingoldia magna</i>	9	
<i>Corynebacterium</i> spp.		
<i>C. tuberculostrictum</i>	26	
<i>C. singulare</i>	4	
<i>C. glucuronolyticum</i>	2	
<i>C. matruchotii</i>	1	
<i>C. sundsvallense</i>	1	
<i>C. tuscaniae</i>		3
<i>C. afermentans</i>		1
<i>C. callunae</i>		1
<i>Brevibacterium stationis</i>		1

genera (table 3). The rank-abundance distribution curves (fig. 5) and the evenness measures (table 3) indicated a more even distribution of bacterial genera in barn dust, in which no taxa reached dominance such as *Corynebacterium* and *Streptococcus* in house dust (fig. 3). The dissimilar bacterial composition of the dusts is reflected by the low similarity measures (table 3).

Discussion

Substantial evidence exists that infections and unhygienic living conditions may confer protection against atopic diseases whereas modern lifestyle and urbanization are associated with an increased risk for these conditions [27]. However, the precise factors responsible for this increase remain unknown.

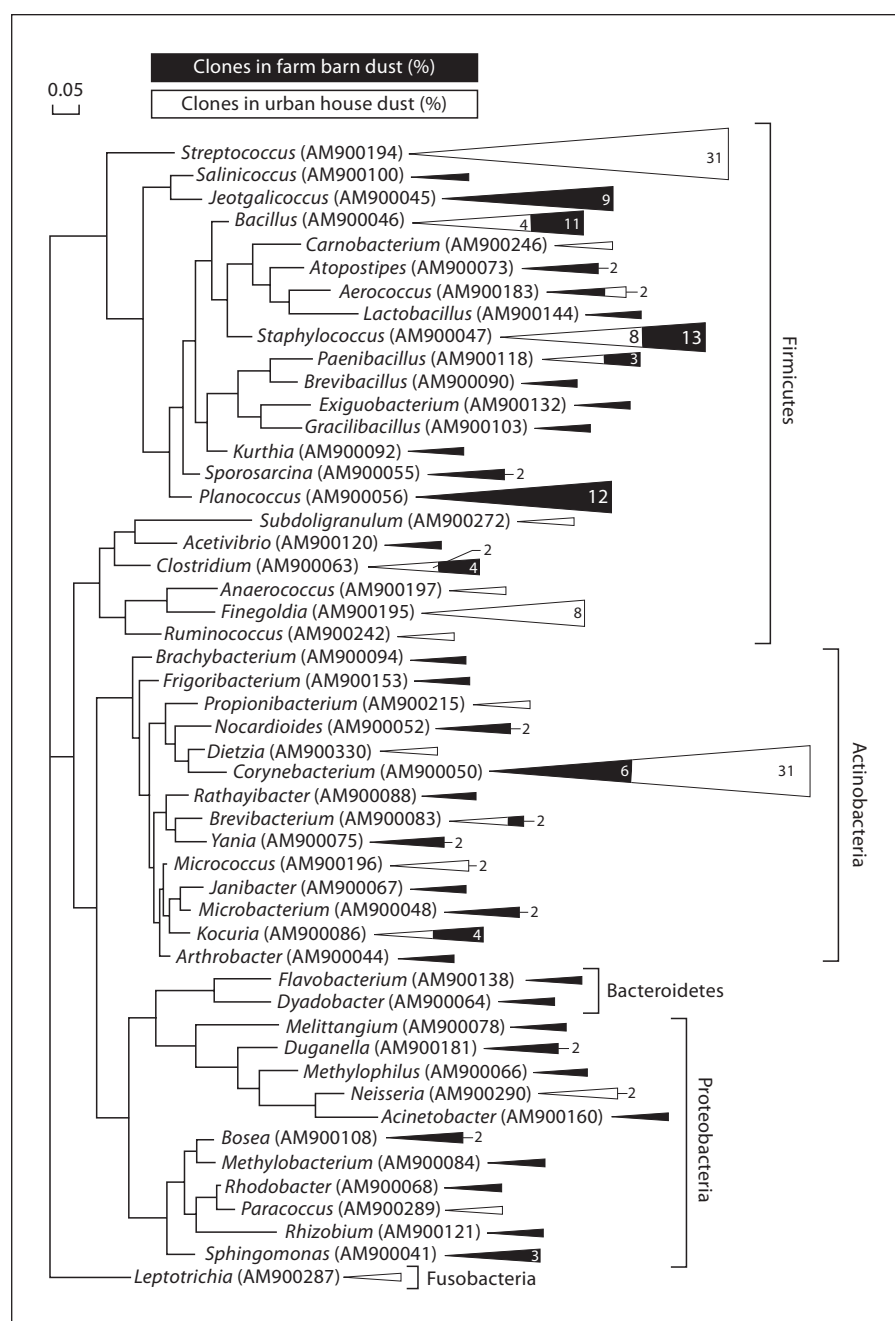


Fig. 3. Maximum-parsimony phylogenetic tree of the 16S rRNA gene sequences representing the genera cloned from urban house dust (108 clones) and from barn dust (102 clones). The triangles are divided into areas in proportion to the percentages of the genera (values >1% displayed) in the clone libraries in the barn (black) and urban house dusts (white).

This study revealed that repeated exposure to house dust elicited pulmonary eosinophilia in mice, which is one of the hallmarks of atopic diseases. Elicitation of pulmonary eosinophilia upon exposure to house dust extracts together with ovalbumin allergen was also reported by Ng et al. [28]. Using MyD88 knock-out mice, they showed that the Th2 response following exposure to house dust extracts/ovalbumin was mediated by Toll-like receptors, thus caused by microbial components. In con-

trast to pulmonary eosinophilia induced by house dust, we found that exposure to barn dust elicited a completely different response in mice, i.e. neutrophilic and lymphocytic airway inflammation together with expression of corresponding chemokines. Thus microbial components in different dusts may either prevent or promote allergic inflammation when present during the sensitization process.

Fig. 4. Maximum-parsimony phylogenetic tree of the 16S rRNA gene sequences representing the genera of isolates cultured from urban house dust and barn dust. The triangles are divided into areas in proportion to the numbers of cultured bacteria [$\log_{10} (\text{CFU} \times \text{g}^{-1})$ values >1 displayed] belonging to the genera in barn dust (black) and urban house dust (white).

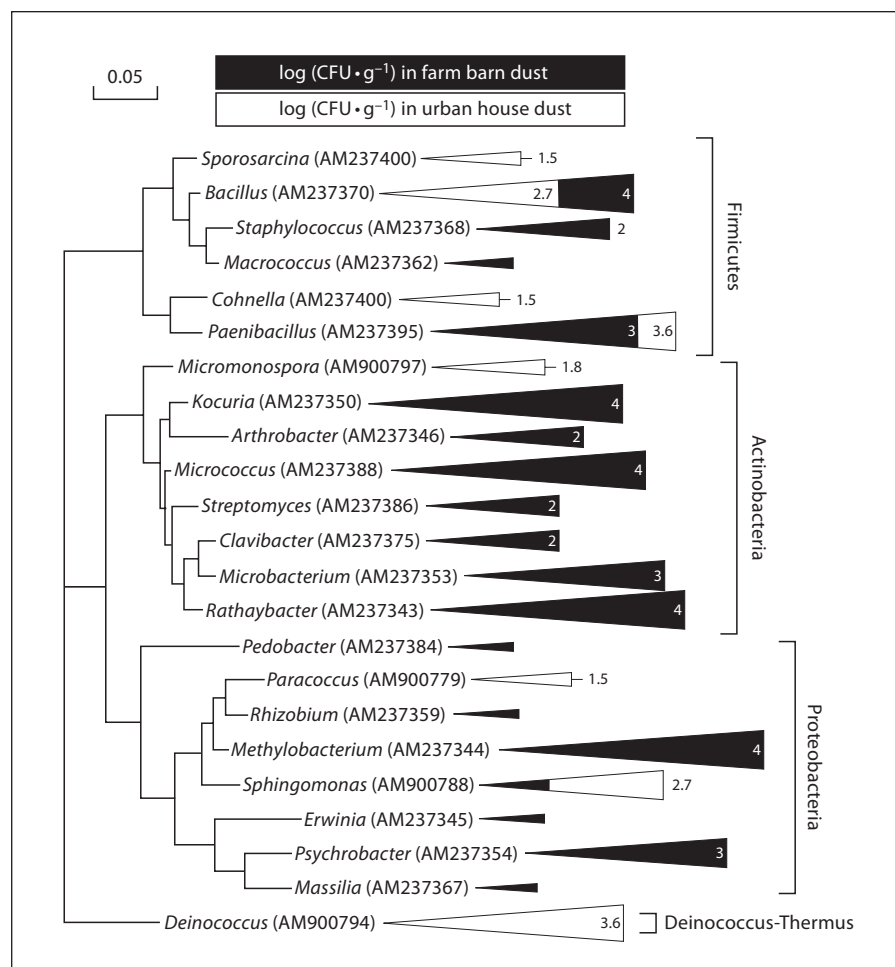


Fig. 5. Rank-abundance curves of the bacterial genera in the 16S rRNA gene clone libraries from DNA of urban house dust (Δ) and barn dust ($\bullet$). The figure shows the percentages of bacterial genera in the order of prevalence (rank 1 for the most abundant genus).

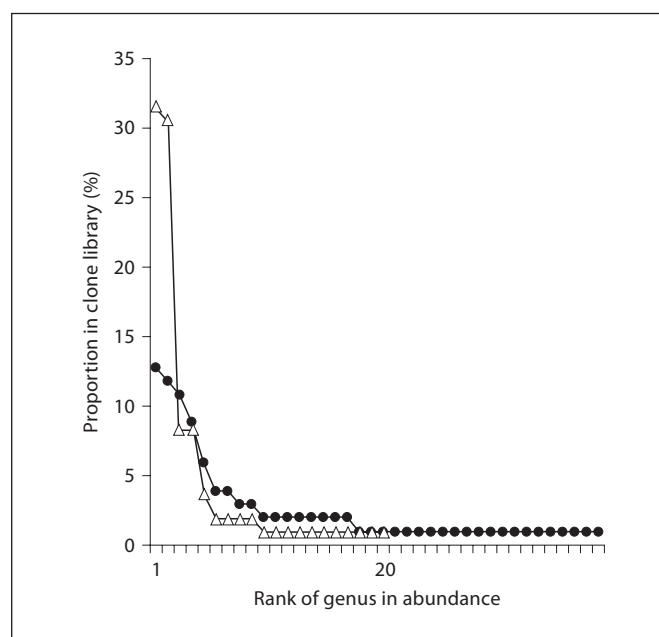


Table 3. Diversity and similarity of bacteria detected in urban house dust and in barn dust used for the analysis of Th1 and Th2 immunological responses (fig. 1, 2)

Biodiversity	Barn dust		Urban house dust	
	cloned	cultured	cloned	cultured
Clones or CFU, n	108	5.4×10^4	102	8.8×10^3
Genera, n	38	18	20	8
Shannon-Wiener index	3.17	1.85	2.03	1.16
Simpson index (1-D)	0.94	0.82	0.79	0.62
Evenness	0.87	0.64	0.68	0.55
Similarity	Barn dust vs. urban house dust			
	cloned	cultured		
Percentage similarity	0.212	0.080		
Sørensen coefficient	0.276	0.207		

Diversity analysis was executed at genus level based on the 16S rRNA gene libraries obtained from the dusts by cloning the 16S rRNA gene from the contained DNA or from the aerobic heterotrophic bacterial isolates. Simpson's index (D) represents the probability that two randomly selected isolates belong to the same genus.

Consensus exists that DCs are central in the process of allergic sensitization. Certain DC-derived costimulatory molecules promote the development of a Th2-type immune response. Indeed, stimulation of human moDCs with house dust elicited increased expression of OX40L and Jagged-1 costimulatory molecules, which both have been reported to play an important role in the development of Th2 immunity [25, 26]. In line with this, house-dust-educated DCs elicited the generation of Th2-polarized cells from naïve T cells. In sharp contrast to house dust, barn dust increased the expression of proinflammatory cytokine IL-23 and the notch ligand Delta-4, known to promote Th1 immunity [26]. DCs exposed to barn dust induced the generation of Th1-polarized T cells. Taken together, both our in vivo experiments in mice and the in vitro data from human DCs and T cells clearly demonstrate that house dust alone without any artificial adjuvant was able to induce Th2-type immunity, mandatory for the development of atopic diseases.

Gehring et al. [29] suggested that lipopolysaccharide, a cell wall component of Gram-negative bacteria, might confer protection against atopic eczema. The dusts in this study had similar contents of total bacteria, limulus-active endotoxin and β -D-glucan, providing no explanation for the polarized immune responses to the dusts. The

higher content of hydroxy fatty acids in barn dust compared to urban house dust indicates the presence of limulus-inactive endotoxin [30] found in old farm dusts [13].

House dust contained a high prevalence of few taxa. DNA of *Streptococcus* and *Corynebacterium* dominated in urban house dust (together >60% of the clone library). The 4 predominant genera (together 78% of the library) among the house dust clones mainly represented species occurring in the human skin and oral flora. Five streptococcal species, all *Corynebacterium*, *Staphylococcus* and *Finnegoldia* spp. in the urban house dust clone library were reported as colonizers of human skin [31, 32]. All the streptococci among the house dust clones, except for *Streptococcus thermophilus* and *Streptococcus downei*, were found among oral bacterial DNA clones reported by Aas et al. [33]. Interestingly, *Streptococcus mitis* in urban house dust elicited a Th2 response when administered to DCs or mononuclear cells [34]. *Corynebacteria* contain mannosylated lipoarabinomannans [35], known to inhibit the production of IL-12, a Th1-inducing factor of DCs [36, 37], which may further promote the development of Th2 response.

Barn dust contained numerous genera (about 30) at a low prevalence (1–5% of total). Thus, the bacterial communities in barn dust were (1) more diverse and (2) the taxa were more evenly distributed within the community as compared to house dust. Eleven genera of Gram-negative bacteria and 11 genera of low guanine + cytosine Gram-positive bacteria were exclusively found in barn dust, among others *Acinetobacter* and lactic acid bacteria. These were reported to induce the Th1 response in DCs [38].

To summarize, urban house dust, containing mainly bacterial debris of human commensal species, may stimulate pattern recognition receptors in a suboptimal way, promoting the development of Th2 immunity rather than being innocent bystanders only. Barn dust, which is rich in diverse viable bacteria, carries a wide spectrum of pattern-recognition-receptor-stimulating conserved microbial components and thus strongly stimulates DCs to drive the response towards Th1 immunity. Our findings give an impetus to test this concept in a large sample material.

Acknowledgements

J. P. is a recipient of a PhD scholarship (ABS Graduate School). The research was supported by the Academy of Finland (grant No. 53305), Helsinki University Hospital Grant (No. 5201) and a grant from the Sigrid Juselius Foundation.

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