Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation

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Background: The human commensal microbiota interacts in a complex manner with the immune system, and the outcome of these interactions might depend on the immune status of the subject.
Objective: Previous studies have suggested a strong allergy-protective effect for Gammaproteobacteria. Here we analyze the skin microbiota, allergic sensitization (atopy), and immune function in a cohort of adolescents, as well as the influence of *Acinetobacter* species on immune responses *in vitro* and *in vivo*.
Methods: The skin microbiota of the study subjects was identified by using 16S rRNA sequencing. PBMCs were analyzed for baseline and allergen-stimulated mRNA expression. In *in vitro* assays human monocyte-derived dendritic cells and primary keratinocytes were incubated with *Acinetobacter lwoffii*. Finally, in *in vivo* experiments mice were injected intradermally with *A lwoffii* during the sensitization phase of the asthma protocol, followed by readout of inflammatory parameters.
Results: In healthy subjects, but not in atopic ones, the relative abundance of *Acinetobacter* species was associated with the expression of anti-inflammatory molecules by PBMCs. Moreover, healthy subjects exhibited a robust balance between anti-inflammatory and T_H1/T_H2 gene expression, which was related to the composition of the skin microbiota. In cell assays and in a mouse model, *Acinetobacter* species induced strong T_H1 and anti-inflammatory responses by immune cells and skin cells and protected against allergic sensitization and lung inflammation through the skin.
Conclusion: These results support the hypothesis that skin commensals play an important role in tuning the balance of T_H1, T_H2, and anti-inflammatory responses to environmental allergens. (J Allergy Clin Immunol 2014;134:1301-9.)

Key words: *Atopy, Gammaproteobacteria,* Acinetobacter *species, PBMC, anti-inflammatory gene expression, dendritic cells, keratinocytes, mouse asthma model*

The incidence of atopic disorders has increased steadily in developed countries for several decades,[1] now affecting approximately 40% of children in the United Kingdom.[2] This epidemic has been related to changes in lifestyle.[3] The "old driends"[4-6] and biodiversity[3] hypotheses postulate that the increase in chronic inflammatory disorders is caused by reduced exposure to environmental microbes, which in turn influences the composition of the human commensal microbiota and its interactions with the immune system. Microbes and vertebrates have coevolved over millennia,[4,7] and the sudden altered microbial contact in urban societies in developed countries[8] might lead to dysregulation of host immunity. Revealingly, children growing up on traditional farms have an especially low risk of allergic sensitization (atopy), with the protective phenotype sustained into adult life[9]; a farm environment exposes children to microbial pressure that has few equivalents in the developed world. In a study in eastern Finland, land use in the surroundings of children's homes was significantly associated with the prevalence of atopy, with children living in homes surrounded by much forest and agricultural land showing less atopy.[10] The causal factor might be microbial exposure because the generic diversity of Proteobacteria on the skin was significantly associated with environmental land use.[10]

Microorganisms inhabiting mammalian body surfaces have a highly coevolved relationship with the host's immune system. Although the immune system is essential in maintaining homeostasis with resident microbes, the latter shape immunity by inducing protective and regulatory responses.[11] The molecular and cellular pathways that sense and transduce signals leading to protection are still largely unknown, but they are likely to primarily target regulatory immune processes. Thus bacteria in the intestine can

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Abbreviations used
ACTB: β-Actin gene
AHR: Aryl hydrocarbon receptor gene
DC: Dendritic cell
FOXP3: Forkhead box P3 gene
GAPDH: Glyceraldeyde-3-phosphate dehydrogenase gene
moDC: Monocyte-derived dendritic cell
OVA: Ovalbumin
PE: Phycoerythrin
qPCR: Quantitative PCR
TLR2: Toll-like receptor 2
Treg: Regulatory T

promote the activity of regulatory T (Treg) cells by inducing IL-10 production. For instance, *Bacteroides fragilis* causes $CD4^+$ T cells to secrete IL-10 through the action of polysaccharide A on Toll-like receptor 2 (TLR2),[12] and a mixture of *Clostridium* strains promotes intestinal Treg cell activity, possibly through the induction of TGF-β by the production of short-chain fatty acids.[13] In germ-free mice IL-10 expression is markedly reduced and Treg cells in the colon are less abundant in comparison with those seen in normal mice.[14] Microbes can also influence the immune system and protect against allergies through induction of T_H1-type immune responses, which inhibit the development of T_H2 cells. Endotoxins (constituents of the outer membrane of gram-negative bacteria) stimulate macrophages and antigen-presenting cells to produce IL-12, which triggers the development of T_H1 immune responses. Microbe-induced immune programming might further involve epigenetic modifications at immune-related genes. A recent study using a mouse model demonstrated modified histone acetylation of the IFN-γ promoter of $CD4^+$ T cells in the offspring of *A lwoffii*–exposed mothers.[15] The effect of microbes at other sites than the gut has been less extensively investigated, but there is evidence that skin commensals autonomously control local inflammatory responses[16] and intranasal delivery of microbial material provides significant protection from experimental allergy.[17,18]

Here we analyze associations between the relative abundances of bacterial genera on the skin and expression of selected genes coding for proinflammatory and anti-inflammatory molecules in a cohort of adolescents. We report significantly dissimilar patterns of association between the microbes and gene expression in PBMCs of healthy versus atopic subjects, suggesting that the interactions between the immune system and the microbiota are significantly altered by atopy (allergic sensitization). The analysis highlights a robust positive association between the genus *Acinetobacter* (Gammaproteobacteria) and anti-inflammatory molecules in healthy but not atopic subjects. Having identified the special role for *Acinetobacter* species among some hundreds of bacterial genera, we investigated the effect of heat-inactivated *A lwoffii* on immune responses and report strong induction of anti-inflammatory gene expression in cell assays and protection against allergic sensitization and lung inflammation after exposure to microbial material through the skin in a mouse model.

METHODS

Study subjects and atopic sensitization

The study subjects (n = 118) had been previously selected for a long-term allergy study.[19] All subjects provided written informed consent, and institutional ethics committees approved the protocol. Atopy was defined as a specific IgE level to inhalant allergens of greater than 2.5 kU_A/L. On the basis of the previous study,[10] the cutoff value was located between 2 clear humps in the log-transformed distribution of IgE values for this cohort.

Skin microbiota

The skin microbiota was analyzed by using 16s rRNA sequencing, as described previously.[10]

PBMCs

PBMCs were separated from whole blood and stimulated with allergens for 6 or 24 hours in complete RPMI.

Microbes for *in vitro* and *in vivo* experiments

A lwoffii strains from blood culture isolates were identified by means if 16S sequencing or API 20NE strips (BioMérieux, Lyon, France), grown on chocolate agar plates, collected, and heat inactivated.

In vitro cell assays

Monocytes were isolated from healthy donor buffy coats and differentiated into monocyte-derived dendritic cells (moDCs). The moDCs and human epidermal keratinocytes were stimulated for 6 hours with heat-killed *A lwoffii* (at 1:5 cell/bacteria ratio).

Real-time quantitative PCR and Luminex

RNA was extracted from PBMCs, moDCs, keratinocytes, and mouse tissue; reverse transcribed into cDNA; and analyzed by using real-time quantitative PCR (qPCR). As endogenous controls, we used 18S rRNA, β-actin gene *(ACTB)*, and glyceraldeyde-3-phosphate dehydrogenase gene *(GAPDH)* for human targets and 18S and TATA-binding protein for mouse targets. The results are expressed as relative quantity, which was calculated by using the comparative cycle threshold method, according to the manufacturer's instructions. Supernatants from the PBMC cultures were analyzed by using Luminex with Bio-Rad multiplex assays (Bio-Rad Laboratories, Hercules, Calif).

Animal model

All animal experiments were approved by the Social and Health Care Department of the State Provincial Office of Southern Finland. Mice were shaved on the back, tape stripped 3 times, and injected intradermally with ovalbumin (OVA) and heat-killed *A lwoffii*, followed by intranasal OVA challenge and collection of samples. Lung, skin, and blood samples were prepared for histologic analysis, RNA extraction, and measurement of IgE serum levels.

Statistical analysis

The association between and effect of gene expression, the relative abundance of *Acinetobacter* species on the skin, and atopy were examined by using Pearson correlation and analysis of covariance, respectively. Global associations of gene expression with the microbiota were inferred by using network analysis.

For a detailed description of the methods, see the Methods section in this article's Online repository at www.jacionline.org.

RESULTS

Associations between the skin microbiota and PBMC gene expression

The skin microbiota was identified to the genus level by sequencing the 16S rRNA gene from DNA samples obtained from the volar surface of the forearm. Altogether, 1017 bacterial genera were identified in the 118 study subjects. The PBMCs