Charles University in Prague

First Faculty of Medicine

UNIVERZITA KARLOVA
1. lékařská fakulta

Summary of the Doctoral Thesis

The Role of Microbiota in the Pathogenesis of Psoriasis

Role mikrobioty v patogenezi psoriázy

MSc. Zuzana Stehlíková

Prague, 2021

Doctoral Programs in Biomedicine

Charles University in Prague and Czech Academy of Sciences

Study Program:	Immunology
Chair:	doc. RNDr. Magdaléna Krulová, Ph.D.
Research Centre:	Institute of Microbiology of the Czech Academy of Sciences
Thesis Supervisor:	RNDr. Zuzana Jirásková Zákostelská, Ph.D.

The dissertation thesis will be available in printed form for public viewing at the Department for Scientific Activities and International Relations of the Dean´s Office of the 1st Faculty of Medicine at least five working days before the defense.

Dizertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

TABLE OF CONTENTS

ABSTRACT

Psoriasis is a chronic, immune-mediated inflammatory skin disease. Its pathogenesis is associated with dysregulated cooperation among keratinocytes, innate and adaptive immune cells, coupled with environmental triggers, including microbiota.

The aim of our study was to describe the microbiota composition in psoriasis and explore the role of bacteria and fungi in the pathogenesis of this disease.

We used a mouse model of psoriasis induced by topical application of imiquimod (IISI) in both germ-free (GF) mice and conventional (CV) mice with microbiota manipulated by administration of a mixture of broad-spectrum antibiotics (ATB). ATB treatment markedly changed the intestinal but not the skin bacterial diversity and led to higher resistance to IISI in CV mice. Metronidazole was the most effective antibiotic, alleviating IISI symptoms in CV, but not in GF mice. This confirms that the effect of metronidazole on IISI was microbiota-dependent.

Additionally, we characterized the microbiota composition of psoriatic lesions and unaffected skin in psoriatic patients compared to healthy controls, as well as the impact of different sampling approaches on uncovering cutaneous microbiota composition. We observed significant differences in α- and β-diversities when comparing identical samples sequenced on V1V2 and V3V4 regions of 16S rRNA. Sampling methods, i.e. swab, scraping, and biopsy, uncovered similar α-diversity, but each method revealed some specific bacterial and fungal species. For the first time, we showed a psoriasis-specific co-occurrence pattern between bacterial and fungal species. We also found elevated serum levels of intestinal fatty acids binding protein in psoriatic patients, suggesting intestinal barrier disruption.

Our results emphasize the importance of microbiota composition, as well as the integrity of intestinal barrier in the pathogenesis of psoriasis. It is still unclear whether the observed co-occurrence pattern has etiological significance or is secondary to the disease.

Keywords: psoriasis, skin microbiota, mouse model of psoriasis, sequencing

ABSTRAKT

Psoriáza je chronické zánětlivé kožní onemocnění. Patogeneze psoriázy je asociována s aberantní kooperací keratinocytů s imunitním systémem, s výrazným přispěním environmentálních faktorů včetně mikrobioty.

Hlavním záměrem naší studie bylo popsat složení kožní mikrobioty u pacientů s psoriázou a prozkoumat roli bakterií a hub v patogenezi tohoto onemocnění.

Využili jsme myší model psoriázy indukované imikvimodem (IISI), a to jak u bezmikrobních, tak u konvenčních myší. Změny ve složení mikrobioty u konvenčních myší jsme docílili orálním podáváním směsi širokospektrých antibiotik (ATB). Podávání ATB výrazně změnilo mikrobiální profil ve střevě, nikoliv však na kůži těchto myší a vedlo k jejich snížené vnímavosti na IISI. Ze směsi širokospektrých ATB byl nejúčinnější metronidazol, jehož podání zmírnilo projevy IISI u konvenčních, ale ne u bezmikrobních myší. Naše výsledky tak potvrzují, že vliv metronidazolu na IISI závisí na přítomnosti a složení mikrobioty.

Dále jsme se zabývali rozdíly ve složení kožní mikrobioty psoriatických lézí a zdravé kůže člověka s psoriázou v porovnání se zdravými kontrolami. Zkoumali jsme také vliv různě zvolených metodik na zjištěné složení kožní mikrobioty. U identických vzorků sekvenovaných pomocí primerů specifických pro V1V2 a V3V4 regiony 16S rRNA jsme pozorovali velké rozdíly mezi α- a β-diverzitou. Psoriatická a zdravá kůže, stejně tak jako způsoby odběru vzorku, tj. stěry, seškraby a biopsie, vykazovali podobnou α-diverzitu, ale každý z nich poskytoval specifické druhy bakterií a hub. Jako první jsme popsali korelační vztahy mezi kožními bakteriemi a houbami, specifické pro psoriázu. Zjistili jsme také zvýšenou hladinu sérového proteinu vázajícího mastné kyseliny ve střevě u psoriatických pacientů, což naznačuje možné porušení jejich střevní bariéry.

Naše výsledky zdůrazňují důležitost složení mikrobioty a integrity střevní bariéry v patogenezi psoriázy. Stále však ještě zbývá objasnit, zda jsou pozorované změny v zastoupení bakterií a hub etiologicky významné nebo jen sekundárně přidružené k onemocnění.

Klíčová slova: psoriáza, kožní mikrobiota, myší model psoriázy, sekvenace

1. INTRODUCTION

1.1. Human skin, mucosa and associated immune system

Human skin is an upper-most multilayer surface of the body. As a complex organ the skin represents a physical, chemical and microbial barrier against unfavorable conditions of the external environment. Skin is the largest organ of the human body, with an area of 2 m^2 forming up to 16% of the body´s overall weight (*1*). Anatomy of the matured skin comprises three main layers such as epidermis, dermis and hypodermis.

Skin is an active immunological environment which includes regional specialization represented by skin associated lymphoid tissue (SALT). Immune system within the skin is located in both epidermis and dermis. The main skin-resident immune cells in epidermis are Langerhans cells (LCs) and melanocytes, while the dermis is occupied by various dendritic cell (DCs) subpopulations, macrophages, mast cells and several T cell types (*2*). Highly important immune cells of the skin are keratinocytes, which actively participate in innate immune responses.

Similarly to skin, mucosal surfaces are in everyday contact with the external environment. While the skin surface with an area of 2 m^2 is mechanically protected by several layers of cells, the mucosal surface is covered mostly with a single layer of epithelium. Mucosal surfaces comprise gastrointestinal, respiratory and urogenital tract, eye conjunctivas and ducts of salivary and mammary glands forming together area of approximately 300 m^2 (*3*).

1.2. Skin microbiota

Skin is colonized by vast amount of microbes that are essential in maintaining a healthy environment. The term microbiota comprises the live communities of all microorganisms and include bacteria, archaea, viruses, fungi or protists.

The skin microbiota protects against opportunistic pathogens, sustains skin homeostasis and educates the immune system (*4-6*). There are diverse factor having impact on the skin microbiota composition. Apart from the distinct character of skin microenvironments those factors could be of internal nature, such as age, sex, genetic traits or ethnicity; or external nature, such as hygiene or other specifics of the environment.

By means of skin specific topography there are diverse microenvironments formed on the human skin, which vary in temperature, pH, in the presence of sweat or sebaceous glands, in number of skin folds or hair follicles. The skin surface, rather unfavorable environment in terms of nutrient content, hosts thousands of microbiota species inhabiting the small skin niches (*7, 8*). Moreover, microbiota is considered to inhabit also the sub-epidermal compartments such as dermis and dermal adipose tissue (*9*). Shifts in relative abundances of complex skin microbiota could lead to skin dysbiosis further possibly causing or exacerbating skin diseases (*4, 7, 10*).

The four most dominant bacterial phyla found on human skin are Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria (*7*). Sebaceous sites are dominated by lipophilic *Cutibacterium* species, while the bacteria which prefer humid conditions like *Staphylococcus* and *Corynebacterium* are preferentially present in the bends of the elbows and the feet (*7, 11-13*). Fungal community composition was found to be similar across body sites (*14, 15*). The predominant species on all body sites is *Malassezia* and the most complex location recognized on the body is the feet with representation of *Malassezia*, *Aspergillus*, *Cryptococcus*, *Rhodotorula* and others.

1.3. The gut-skin axis

The skin and the mucosa of gastrointestinal tract are the most exposed surfaces having contact with the external environment, thus possessing the vast majority of microbiota of our body. The local and systemic homeostasis is being regulated via the communication of microbiota with immune system. It is widely accepted that both skin and gut are immunology-wise closely related and their interconnection provides the overall body homeostasis. The mechanisms of how gut microbiota influences the areas beyond the GIT, particularly the skin are not completely uncovered yet.

It is considered that the linkage between gut and skin takes place through the combination of several ways. There is the production of biologically active molecules, such as hormones or molecules from breaking down the dietary compounds; influence through the immune system programming or the direct effect of probiotics. Microbial endocrinology concerns the interplay between host, microbes, and the secreted hormones they both produce. This field lies at the crossing of microbiota-gut-skin axis, and could be applied beyond the diseases as a result of the presence of shared neurochemicals between the host and the microbiota (*16*).

The intestinal microbiota also produces metabolites having the potential to modulate host´s immunity and alter the balance between tolerance and inflammation by affecting differentiation of naive T cells into Th17 or Treg lineage (*17*). The linkage between the gut and skin is further described in studies reporting that administration of oral probiotics together with the beneficial prebiotics could dampen the manifestation of some dermatoses (*18, 19*). Another example of favorable effect of probiotics is increased hydration of corneocytes, decreased transepidermal water loss and skin sensitivity after 12 weeks of using oral supplements with probiotics (*20, 21*).

1.4. Microbial dysbiosis and associated diseases

The imbalance in cutaneous or intestinal microbiota composition, termed as microbial dysbiosis, is mainly characterized by decrease in the most abundant commensal microbiota (*22, 23*). However, dysbiosis does not imply that the microbial diversity must be reduced (*24, 25*). Hence, decreased microbial diversity, although often described, should not be considered as an exclusive part of the disease manifestation (*24-29*).

The diseases associated with dysbiosis comprise skin, intestinal and extra-intestinal disorders. Skin diseases include psoriasis, acne, atopic dermatitis, vitiligo, systemic lupus erythematosus, seborrheic dermatitis and many others (*30-32*). Among intestinal diseases belongs inflammatory bowel disease, irritable bowel syndrome or coeliac disease and to extra-intestinal disorders associated with dysbiosis are usually ranked allergy, asthma, metabolic syndrome, obesity or cardiovascular disease (*30, 31*).

Majority of studies dealing with skin and intestinal disorders are usually based on monitoring differences in microbial composition between diseased and healthy individuals. Observed alterations are then considered as a possible trigger of a disease. On one hand, shift in microbial composition could certainly be a hallmark of a particular disease. On the other hand, it could only be its accompanying phenomenon, perhaps driven by other circumstances related to the disease. However, in the context of host biology it is complicated to thoroughly interpret the communication and diverse associations of microbiota with its host, since this interplay is not fully understood yet.

1.5. Psoriasis

Psoriasis is a chronic immune-mediated inflammatory disease affecting primarily the skin. Although the exact etiology of psoriasis is unknown, it is considered to be a multifactorial disease. Psoriasis is nowadays perceived as a systemic disease associated with higher incidence of other chronic diseases such as type II diabetes, psoriatic arthritis, inflammatory bowel disease or coeliac disease (*33, 34*). The prevalence is estimated to be 2-3% worldwide equally manifested in both sexes, although men tend to be prone to more severe manifestation than women (*35*).

Psoriasis can be triggered predominantly in genetically predisposed individuals by non-specific factors like sunburn, scratching, administration of systemic drugs, infection, stress and others (*36*).

The pathogenesis of psoriasis involves dysregulated interplay among keratinocytes, innate and adaptive immune cells and environmental triggers including microbiota (*37*). Plasmacytoid dendritic cells in the skin usually sense viral and microbial DNA through endosomal TLR receptors. In psoriasis, pDCs could get activated through the complexes of antimicrobial peptide LL-37 coupled with self-DNA (present in psoriatic lesioned skin after cell damage) in a TLR-9 dependent manner (*38*). Thus, the aberrant cascade of activating pDC and dermal DCs, followed by IL23 release and stimulation of T cells against LL37/self-DNA complexes leads to expansion of Th17 cells and production of IL17 (*39, 40*).

Composition of skin and gut microbiota is an important factor in modulation of inflammation and disease course in psoriasis (*41, 42*). Even though no single pathogen has been identified yet to strongly contribute to psoriasis onset, the dysbiosis in microbial ecosystems is considered to be one of the main triggers. Alekseyenko and colleagues described two different cutaneotypes of potentially pathophysiological significance associated with psoriasis, which differed in terms of the relative abundance of major phyla (Alekseyenko et al. 2013). Fungi share the spotlight with bacteria since it was shown that *Malassezia* species could be associated with exacerbations of psoriasis (*43, 44*). Intestinal dysbiosis is another phenomenon accompanying many skin diseases, including psoriasis (*26, 45, 46*). Intriguingly, in mouse model of psoriasis it has been shown that neonatal antibiotic treatment dysregulates the gut and skin microbiota in adults, which led to higher sensitivity to experimental psoriasis in those mice (*47*).

2. AIMS OF THE STUDY

The main purpose of this study was to assess the bacterial and fungal composition in relation to psoriasis and to evaluate the changes in microbiota composition associated with healthy and diseased state. The main goal can be further subdivided into following aims:

1. To explore the role of skin and gut microbiota in the in mouse model of psoriasis (imiquimod-induced skin inflammation, IISI) and analyze if the disease development can be altered by microbiota changes in adult mice.

2. To compare and unify current approaches in human skin microbiota research and map the overall bacterial and fungal composition of healthy and diseased skin. Furthermore, to find specific features of microbiota co-occurrence potentially associated with psoriasis incidence.

3. To test whether the intestinal barrier damage could be associated with psoriasis and if it could serve as a marker in the preventive care.

3. MATERIAL AND METHODS

3.1. Mouse studies

Mice: We use female BALB/c or C57BL/6 mice (7-10 weeks old) reared in conventional or germ-free conditions at the Institute of Microbiology of the Czech Academy of Sciences in Prague and Novy Hradek.

Murine model of psoriasis: The animals were treated daily for up to 7-8 consecutive days on their shaved back and left ear by either 62.5 mg of imiquimod cream (Aldara) or similar amount of control cream (vaseline). The severity of erythema and scaling in imiquimod-induced skin inflammation (IISI) was monitored daily by a scale based on Psoriasis Area and Severity Index (PASI).

Antibiotic treatment: Mice were treated with antibiotics (ATB) 2 weeks prior IISI induction and the treatment continued until the end of the experiment. In Study I, a mix of metronidazole, colistin, and streptomycin was administered daily by gavage and vancomycin was added to autoclaved drinking water. To protect the mice from potential *Candida* overgrowth, mice were gavaged daily with amphotericin-B starting 3 days prior antibiotic treatment until the end of the experiment. In Study II, mice in each of the experimental groups were given 300 μl of the mixture of all aforementioned ATBs or each ATB alone daily by oral gavage.

Histology: The dorsal skin and ear samples were fixed in 5% buffered formalin, dehydrated and embedded in paraffin. Next, 4 μm sections were cut and stained with H&E for histopathological examination by an experienced pathologist (Pavel Rossmann), unaware of the treatment of the mice.

Flow cytometry: We analyzed major T cell phenotypes using anti-CD3, anti-γδTCR, and anti-RORγt antibodies. For intracellular staining of produced cytokines we stimulated cells with PMA and Ionomycin, followed by treatment with mixture of Brefeldin A and Monensin. We then stained the cells with anti-CD3, anti-IL17, and anti-IFN-γ antibodies.

Cell cultivation and cytokine measurement: The cell viability, generally around 90%, was analyzed using Trypan Blue exclusion. Cells were stimulated for 48 hours with plate-bound anti-CD3 and soluble anti-CD28 antibodies. ELISA sets were used to measure the levels of IFN-γ and IL-17 in the supernatants.

Microbiota analysis: In Studies I and II, stool samples from ATB-treated and control mice were collected on day 0, 14 (just before IISI induction) and 21 (the last day of the

experiment). In Study II, skin swabs from the mouse shaved back skin were collected at the same time points as the stool samples. DNA from stool and swab samples was extracted by commercial kits. To measure the microbial load in Study I, extracted DNA was analyzed by qPCR assay using universal bacterial primer pair (*48*).

Gene expression in the skin – RNA isolation and qRT-PCR: In Study II, total RNA was isolated from approx. 50 mg of mouse skin tissue and 400 ng of total RNA was reverse transcribed and the resulting cDNA was used as a template for qPCR analysis. Changes in mRNA levels were shown as the fold change of expression in monocolonized mice compared to that in conventional mice.

Statistical analysis: We used unpaired Student's t-test to compare two experimental groups or one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test to compare multiple groups. All data were expressed as the mean ± standard deviation (SD) unless otherwise stated, and differences were considered statistically significant at $p \leq 0.05$. In Study II, the data from gene expression analysis were expressed as mean ± SEM of the values obtained in all experiments.

3.2. Human study

Study participants and sample collection: Study was conducted on 34 patients with chronic plaque psoriasis, who were recruited from the Department of Dermatovenerology at Bulovka Hospital. For microbiota analysis, participants provided skin swabs, scrapings and some of them also punch biopsy samples. In psoriatic patients both psoriatic and contralateral unaffected sites were sampled.

Microbiota analysis: DNA from swabs, scrapings and biopsies was extracted using commercial kits.

Bacteria-fungi correlation: Only fungi and bacteria present in at least one-third of the patients in any group of samples (psoriatic, unaffected, and healthy skin) were kept for further analysis. Pearson correlation coefficients and p-values were calculated for each bacterium–fungus pair and for each group of samples separately.

ELISA: Serum levels of intestinal fatty acid binding protein (I-FABP), caspase-cleaved cytokeratin 18 fragment (ccCK18) and total cytokeratin 18 (CK18) were determined by commercially available ELISA kits. All assays were performed according to the manufacturer's instructions.

Statistical analyses: ELISA statistics was performed using Mann-Whitney test. All data were expressed as the mean ± standard deviation (SD) unless otherwise stated, and differences were considered statistically significant at $p < 0.05$.

3.3. Material and methods used across studies

PCR amplification, sequencing: Mouse DNA samples were amplified using primers for V3V4 region of bacterial 16S rRNA. Human samples were amplified using primers for both V3V4 and V1V2 region of 16S rRNA. Moreover, DNA was also amplified using primers capturing the fungal ITS region of 18S rRNA in Studies II and III.

Sequencing data analyses: Sequencing data were processed using QIIME (Quantitative Insights Into Microbial Ecology) software package version 1.9.1 (Caporaso et al., 2010). The data are available in the Sequence Read Archive (SRA), http://www.ncbi.nlm.nih.gov/sra, under the accession numbers: Study I (SRP0678451), Study II (SRP156846), and Study III (SUB4321198). For microbiota analysis, several alpha diversity indices such as Chao1, Shannon, Gini-Simpson and PD-whole tree were calculated. Beta diversity was presented in principal coordinate analysis (PCoA) plots and assessed using several indices, including weighted and unweighted UniFrac distances for bacterial analysis, and Binary–Jaccard and Bray–Curtis metrics for fungal analysis. To determine the discriminative features for both taxonomic profiles of communities in Studies II and III, the LEfSe analysis tool was employed (Caporaso et al., 2010).

Statistical analyses: Statistical significance for alpha diversity measures was confirmed using Kruskal–Wallis test with Dunn's multiple comparison test or Mann–Whitney test. Statistical significance for beta diversity was confirmed using PERMANOVA.

4. RESULTS

4.1. Intestinal Microbiota Promotes Psoriasis-Like Skin Inflammation by Enhancing Th17 Response

Zakostelska Zuzana, Malkova Jana, Klimesova Klara, Rossmann Pavel, Hornova Michaela, Novosadova Iva, **Stehlikova Zuzana**, Kostovcik Martin, Hudcovic Tomas, Stepankova Renata, Juzlova Katerina, Hercogova Jana, Tlaskalova-Hogenova Helena, Kverka Miloslav.

PLoS ONE (2016): 11(7): e0159539

In this study we found that changes in intestinal microbiota achieved by antibiotic treatment of conventional mice (CV) reduced the sensitivity to imiquimod-induced skin inflammation (IISI), which was observable as a lower degree of local and systemic Th17 activation. To confirm that microbiota plays the major role in the development of IISI, we induced the inflammation also in germ-free (GF) background.

When compared to GF mice, CV mice manifested more severe erythema, scaling, thickening and other histological features of psoriasis, as well as higher leukocyte infiltration into the dermis. CV mice treated with mix of broad-spectrum antibiotics displayed altered intestinal microbiota composition, mainly decreased diversity and shifted composition towards higher abundance of Lactobacillales. Absence of microbiota or ATB treatment decreased the frequencies of γδ T cells and Th17 cells in spleen and axillary lymph nodes in IMQ-treated mice. Treatment with imiquimod (IMQ) itself had no effect on observed microbial changes.

Since GF and ATB-treated mice had significantly milder skin inflammation than CV mice, the protective effect was not limited to the absence of microbiota. The severity of skin inflammation could be thus modified by altering the gut microbiota in adult mice, supporting the evidence of the gut-skin axis.

4.2. Crucial Role of Microbiota in Experimental Psoriasis Revealed by a Gnotobiotic Mouse Model

Stehlikova Zuzana, Kostovcikova Klara, Kverka Miloslav, Rossmann Pavel, Dvorak Jiri, Novosadova Iva, Kostovcik Martin, Coufal Stepan, Srutkova Dagmar, Prochazkova Petra, Hudcovic Tomas, Kozakova Hana, Stepankova Renata, Rob Filip, Juzlova Katerina, Hercogova Jana, Tlaskalova-Hogenova Helena, Jiraskova Zakostelska Zuzana.

Frontiers in Microbiology (2019): 21; 10:236

In this study we observed that each component of a broad-spectrum antibiotic mixture, i.e. colistin (COL), vancomycin (VAN), streptomycin (STR) and metronidazole (MET) changed the susceptibility to IISI to a certain extent. Compared to controls and other ATB-treated groups, mice treated with MET developed the mildest skin inflammation. With respect to the widely discussed possible immunomodulatory effect of MET we repeated the experiment under GF conditions and found no differences in disease severity or Th17 proportions between MET and control germ free mice. However, the expression of *Nfkbiz* gene was increased in MET-treated mice ($p<0.05$), suggesting mild immunomodulatory microbiota-independent effect of MET. Taken together, the antimicrobial activity of MET is what contributes the most to its anti-inflammatory effect.

Regarding the intestinal microbiota composition, greater diversity differences from the control group were observed only in MET-treated mice. Generally, we observed the highest impact on intestinal diversity and microbial composition, particularly the marginal increase of Lactobacillales species, in the group of mice treated by mixture of all forenamed antibiotics (MIX).

Next, we found that monocolonization of mice with anti-inflammatory-acting *Lactobacillus plantarum* WCFS1 does not improve the IISI when compared to GF mice; on the other hand, all tested parameters were significantly lower than in CV mice. Monocolonization with pro-inflammatory-acting SFB bacteria was sufficient neither to change the skin clinical signs of IISI when compared to GF mice, nor to induce IISI comparable to that of CV mice. This suggests that colonization with only one bacterial species may not be enough to revert/induce signs of IISI inflammation.

4.3. Dysbiosis of Skin Microbiota in Psoriatic Patients: Co-occurrence of Fungal and Bacterial Communities

Stehlikova Zuzana, Kostovcik Martin, Kostovcikova Klara, Kverka Miloslav, Juzlova Katerina, Rob Filip, Hercogova Jana, Bohac Petr, Pinto Yshai, Uzan Atara, Koren Omry, Tlaskalova-Hogenova Helena, Jiraskova Zakostelska Zuzana.

Frontiers in Microbiology (2019): 21; 10:438.

In this study we found out how important is to follow the same procedures when it comes to microbiome data collection, analysis, interpretation and comparison across studies. We observed large differences in bacterial β-diversity, richness and evenness ($p<0.001$), when comparing identical samples sequenced both on V1V2 and V3V4 variable regions of 16S rRNA. Not only the V3V4 region provides wider diversity, but it also captures more *Staphylococcus* species in contrast to V1V2 region.

Different sampling approaches such as swabs, scraping or biopsies provided similar microbial α-diversity, and each approach revealed several discriminative features in bacterial and fungal distribution on the back and elbow skin. Each sampling site (psoriatic, unaffected psoriatic, and healthy) was also associated with presence of specific taxa. When comparing the oily and dry skin areas – back and elbow, psoriatic skin on the back dispose of increased fungal but not bacterial diversity than psoriatic skin on the elbow. We did not observe any niche-specific variations in the distribution of the most abundant KEGG-pathways in the back and elbow skin, only ethylbenzene-degradation pathway common for unaffected skin of both areas.

We found a specific pattern of taxonomic correlations between bacteria and fungi related to skin condition and sampling site. For example, we observed a strong negative correlation of *Micrococcus* species with *Capnodiales* in psoriatic skin on the elbow ($r = -0.69$), while on the healthy elbow skin this correlation was positive ($r = 0.91$).

Patients with psoriasis had significantly increased level of I-FABP but not ccCK18 in the serum when compared to healthy controls ($p = 0.0413$), suggesting that the intestinal barrier integrity play a role in the pathogenesis of psoriasis.

5. DISCUSSION

Constant interactions between microbiota and the immune system are essential for priming the immune system since birth. Mounting evidence of the communication axis between different organs underscores the crucial role of microbiota in our everyday life. The widely discussed gut-skin axis and associated dysbiosis is often described in patients suffering from diverse skin diseases including psoriasis (*32, 49*). In immune-mediated chronic diseases with unknown etiology such as psoriasis, the dysbiotic phenomenon is often involved in the interpretation of the cause and consequence of the disease.

Using IISI, we have analyzed the effect of microbiota on the development of psoriatic skin inflammation in CV, as well as in GF mice. To change the intestinal microbiota composition in CV mice, we treated mice with a mixture of broad-spectrum ATBs (MIX), starting 2 weeks before IISI induction and lasting for the whole duration of the experiment. Mice treated with MIX displayed the most prominent gut microbiota changes and together with GF mice developed lower skin and systemic inflammation (*50*). This is in agreement with Zanvit et al. (*47*), who used adult CV mice treated with a mixture of only vancomycin and polymixin B. In contrast to our study, Zanvit et al. (*47*) applied the antibiotic mixture not only orally but also topically in an IISI model. They found improved skin symptoms of IISI in adult CV mice, such as decreased acanthosis and skin thickness, after using both routes of administration (*47*). Both studies agreed on the observed microbiota abundance, e.g. increase of Lactobacillales in MIX-treated mice (*47, 50*). This goes along with many studies reporting a beneficial effect of lactobacilli on cutaneous health (*20, 21, 51, 52*), as well as their beneficial role in improving intestinal barrier, leading to decreased sensitization to allergens (*53*).

To further explore our findings, we used the individual components of the antibiotic mixture to check the specific effect of each antibiotic, namely colistin, vancomycin (VAN), streptomycin, and metronidazole (MET). We found the most profound changes, such as decrease of skin thickness and decrease of Th17 cells in inguinal lymph nodes in MET-treated mice. Since MET may have anti-inflammatory properties (*54-56*), we used GF mice to find out whether MET influences the IISI in a microbiota-dependent or independent manner (*57*). Importantly, we found that MET treatment did not change the severity and other parameters of IISI under GF conditions, suggesting that the anti-inflammatory effect of MET

observed in CV mice is microbiota-dependent (*57*). Our observation of antimicrobial effect of MET is further supported by studies showing the efficacy of MET in alleviating experimental uveitis via changing the microbiota composition (*58*) or in improving the SIBO syndrome, which is primarily caused by small intestinal dysbiosis (*59, 60*).

We observed significant diversity changes in microbiota composition in VAN, MET, and MIX-treated groups of mice before the IISI induction. The observed changes in skin microbiota were significant and associated with VAN treatment, while microbiota shifts in the intestine were even more extensive and associated with VAN, MET, and also MIX treatment (*57*). The decrease in skin microbiota diversity in VAN-treated mice before IISI is consistent with previous findings of Ahlawat and Sharma (*22*), who also reported changes in skin microbiota composition after treatment with an antibiotic mix including VAN. VAN is described as not easily absorbed via intestinal mucosa, therefore its effect is expected to be site-specific, localized rather to the intestine. Despite this fact, its effect is probably wide-range as observed in the research focused on wound healing (*61*).

It was recently observed that staphylococci and streptococci found in mouse fecal samples worsened experimental psoriasis manifestation (*62*). These results lend support to our findings, since we observed a correlation between IISI improvement and decreased skin abundance of staphylococci and streptococci species, while Okada with coauthors exacerbated the disease by administering those bacteria orally (*62*).

We have further found that MET treatment profoundly changed the gut microbiota abundances by decreasing the overall diversity, which led to an enormous increase of lactobacilli species in the intestine. A member of lactobacilli, species *L. plantarum*, recently showed protective effect in human as well as mouse models of cutaneous and intestinal inflammation (*63-68*). Furthermore, certain microbial species were shown to populate Th17 or Treg cells (*69-71*). Consistently with the anti-inflammatory properties of *L. plantarum* reported in the literature, we found that LP monocolonization led to a comparable degree of IISI as in GF mice. On the other hand, monocolonization with SFB promoted neither higher Th17 expansion nor an increase of proinflammatory cytokines in the inflamed skin when compared to CV mice. When compared to GF mice, monocolonization with SFB led to significantly increased Th17 expansion only in the spleen (*57*). It may seem that this is partially inconsistent with other mice studies reporting the role of SFB bacteria in inducing the proinflammatory response (*72, 73*). However, the reason we did not observe

worsening of IISI might be that SFB bacteria need the presence of other commensals to fully reach their pro-inflammatory potential (*74*).

Research of the human skin microbiome presents some unique challenges, such as low microbial biomass on the skin compared to the gut content, high contamination risk, diversity of cutaneous habitats, or site-specific microbiota (*75-77*). Many host factors, such as gender, ethnicity, handedness, living with animals, hygiene and cosmetics habits, can impact the composition of skin microbiota (*78, 79*). Skin microbiome studies are also heavily influenced by experimental design. Each method has its strengths and weaknesses, making the study of microbiome extremely challenging, with results that are difficult to compare. The major influencing factor, however, seems to be the choice of 16S rRNA region for sequencing, as this can profoundly impact the perceived diversity and microbial community composition (*80-83*). For example, the V1V3 region could better distinguish among *Staphylococcus* species (*84, 85*), and using primers for the V4 region results in underrepresentation of *Cutibacterium* species (*86*). The V3V4 region has been described to sufficiently cover the skin microbial diversity (*7, 81, 87*) and Teng et al. (*88*) also confirm that V3V4 provides more reproducible data than, for example, V1V3.

Because results vary across publications, we aimed to conduct a comprehensive study comparing all previously published methodological approaches using one data set. To deal with the issue of 16S rRNA region choice, one possible way is to study the identical samples using different 16S rRNA regions (*80*). Therefore, we have compared the V1V2 and V3V4 sets of primers on identical samples from psoriatic patients and healthy controls (*32*). As described above, primers for the V3V4 regions were probably not sufficient to classify the majority of *Staphylococcus* species, unlike V1V3 primers used by Alekseyenko et al. (*89*) whose finding was further supported by Meisel et al. (*86*). Nevertheless, although primers for V1V2 regions were better in classifying *Staphylococcus* to the species level, sequencing the V3V4 region recovered greater overall diversity (*32*). This is in line with Graspeuntner and colleagues, who also confirmed greater number of taxa identified using the V3V4 region of 16S rRNA (*90*).

To overcome other potential bias we combined and compared 3 previously described techniques of sample collection, e.g. swabs, scrapings and biopsies. Unlike some other studies, that investigated microbiota of various body sites and summarized their results across these localities (*91, 92*), we focused only on two common sites frequently affected

by psoriasis, which differ in their specific microenvironments – the oily back and the dry elbow skin (*32*). Similarly to Tett et al. (*93*), we sought to reduce the intra- and inter-individual variation by using control samples from unaffected contralateral skin of the same patient and also samples from healthy controls. Our study has the added value of analyzing the skin fungal composition and correlating bacteria and fungi from skin swabs (*32*).

Swabs and scrapings showed similar alpha diversity across affected and unaffected psoriatic skin and healthy control skin both on the back and elbow. Our data on healthy skin are supported by Bay et al. (*94*), who researched the moist and dry areas of the skin, and consistent with other data on healthy skin (*12*), suggesting that microbial alpha diversity might be similar among anatomic locations of healthy skin.

Despite non-significant differences in alpha diversity across sampled sites and localities on the body, we found a tendency to higher species richness (total number of species) and evenness (relative proportions of each species) on the elbow than on the back skin. This corresponds to the dry and oily skin areas investigated by Tett et al. (*93*) and possibly underscores the microbiota changes caused by the disease and reflects the conditions of distinct microenvironments as well. However, Tett et al. (*93*) did not include healthy controls in the analyses, so the microbiota variation between healthy and diseased skin is missing.

Biopsy samples, on the other hand, showed tendency to decreased richness and evenness in psoriatic skin, while the increasing trend in unaffected and healthy skin. Using LEfSe to analyze discriminative microbial species for each sampling methodology, we found many more bacterial and fungal biomarkers in biopsies, distinguishing biopsy samples from swabs or scrapings (*32*). This does not mean that swabbing the upper layers of epidermis would reflect lower amount of species on the surface of the skin in contrast to the dermal sites. In other words, the variability in bacterial community composition could change from epidermal to dermal locations – from the epidermal microbiota, being more affected by environmental factors, to the well-conserved, compositionally and functionally distinct dermal microbiota (*94*). Therefore, this could be the reason why we have detected so many distinguishing species in biopsy samples in contrast to swabs and scrapings (*32*).

The predominant species in biopsies from healthy controls was *Staphylococcus*, whereas biopsies from psoriatic patients had variable microbiota composition (unpublished data). This

partially contrasts with Fahlén et al. (*91*), who found the most common species to be *Streptococcus* in both psoriatic and healthy controls. Nevertheless, their findings correspond to our results in uncovering a higher abundance of *Staphylococcus* in healthy controls versus psoriatic patients (*91*). However, the data are not directly comparable, since Fahlén et al. (*91*) contrasted control samples obtained mostly from the back with psoriatic samples obtained mostly from the limb. This again goes back to the problems with interpretation of microbiota composition due to different localities, hence microenvironments, on the human body. To date, several other human studies described higher *Staphylococcus* and *Streptococcus* abundance in psoriatic skin in contrast to healthy skin, which is not in concordance with our human study (*89, 92*). On the other hand, despite the differences in sampling strategy, our data are mostly consistent with other studies that investigated human psoriasis (*91, 95*). The reasons for discrepancy might be the already discussed sequencing approaches, high interindividual variation, specific niches of different body sites, or low abundance of discriminatory taxa (*93, 96, 97*).

When oily and dry skin when compared in their beta-diversities, the oily skin showed larger beta diversity than the dry skin (*32, 93*). This possibly reflects the differences of oily and dry microenvironments (*12*). However, we observed no significant beta diversity differences between psoriatic and unaffected skin on any of the examined sites. This contrasts with the study of Tett et al. (*93*), where the authors found larger beta diversity in oily skin, particularly in psoriatic compared to unaffected skin. However, since Tett et al. (*93*) used shotgun metagenomics in their study, it is difficult to compare the results, since different methodological strategies vary in their outcomes (*98*).

The fungi on human skin are an integral part of the whole microbiota community, however, studies concerning the mycobiota composition in psoriasis are still rare. Studies in mice nevertheless proposed that cutaneous fungi could exacerbate experimental skin inflammation by inducing the accumulation of IL17-A producing Th, Tc and γδ-T cells within the skin (*99*). We also tried to influence the mycobiota composition with antifungals, but we did not achieve a change in the severity of IISI between treated and control mice (unpublished data).

In the human study, we aimed to characterize how different sampling techniques affect the uncovered skin fungal composition, since previous human studies did not address this issue (*15, 100-102*). We found no differences in alpha diversity between swabs, scrapings or biopsy samplings. Our results support previous findings about *Malassezia* being the most dominant

fungal species on the skin (*15*). For instance, *M. sympodialis* is known to enhance the production of proinflammatory cytokines in keratinocytes (*103*) and induce the activation of mast cells, which then release leukotriens, increased in atopic dermatitis and psoriasis patients (*104, 105*). Interestingly, in our study the psoriatic lesions on the oily back skin were predominated by *Malassezia restricta* and the dry elbow skin rather with *Malassezia sympodialis,* as revealed by LEfSe analysis (*32*). This contrasts with the study of Paulino et al. (*106*) who found the opposite, i.e. *M. restricta* to be the predominant species on the dry elbow skin, followed by *M. sympodialis*. Unfortunately, Paulino et al. (*106*) investigated only 3 psoriatic patients, therefore their results are not very conclusive. We observed a lower ratio of *Malassezia globosa* to *Malassezia restricta* in samples from psoriatic lesions on the back in contrast to healthy skin, which is consistent with previous findings (*102*).

Chang et al. (*107*) investigated bacterial interactions within the skin microbiome and identified clusters of bacterial species corresponding in their abundance. Even though we investigated bacteria-fungi interactions, we uncovered similar patterns in our results. For instance, *Corynebacterium* and *Peptoniphilus* clustered together in the study of Chang et al. (*107*) and both genera were positively correlated with Malasseziales in unaffected psoriatic skin on the back in our study (*32*). Furthermore, *Corynebacterium* clustered with *Finegoldia* (*107*) and both genera were positively correlated with *Aspergillus* on elbow psoriatic lesions (*32*). To better understand the observed bacteria-fungi interactions in relation to the pathogenesis of psoriasis, more studies expanding this knowledge by other –omics approaches are needed.

Not only skin microbiota changes should be considered in psoriasis, but attention should be given to the intestinal microbiota as well. New growing evidence suggests that psoriatic patients also suffer from intestinal dysbiosis (*26-28, 49, 108, 109*). It has been described that psoriatic patients display a marked increase in Actinobacteria species and some cohort-specific differences as well, such as significant overrepresentations of *Blautia*, *Coprococcus*, *Ruminococcus* or *Dorea* (*28*).

Since the microbial composition is individualized to a certain extent, there is no precise definition of a “healthy microbiome”. Despite this knowledge gap, it is generally accepted that the higher the microbial diversity, the better physiology and homeostasis (*110*). However, this hypothetical assumption, although based on many observations, does not have to be true in all cases (*24, 25, 107*).

6. CONCLUSIONS

1.

CV mice treated by a mixture of broad-spectrum ATBs were more resistant to IISI, similarly as GF mice. ATB treatment profoundly changed the gut microbiota profile of CV mice, which resulted in lower degree of local and systemic Th17 activation (*50*). MET was the most efficient antibiotic in mitigating the IISI symptoms due to its antimicrobial activity and not its immunomodulatory effect, as we showed on GF mice. Furthermore, monocolonization of mice with single bacteria species was not sufficient to change the course of IISI (*57*).

2.

Different techniques of sample collection provided similar richness, evenness and genera abundance of the present taxa, but each technique provided some specific bacterial or fungal taxa associated with the particular method (i.e. swab, scraping, and biopsy). Each sampling site, as well as body location was also characterized by specific microbial communities. Bacteria-fungi correlation pattern among psoriatic, psoriatic-unaffected and healthy skin suggests a link between niche occupancy and psoriatic changes on the skin (*32*).

3.

Elevated serum levels of I-FABP were found in patients with psoriasis, pointing at the intestinal barrier malfunction. Despite we did not find increase in serum levels of ccCK18, another marker of intestinal barrier impairment, the intestinal integrity certainly plays an important role in the pathogenesis of psoriasis (*32*).

Composition of cutaneous and intestinal microbiota is an influential aspect in the course of psoriasis.

7. REFERENCES

1. D'Orazio J. *et al.*, UV radiation and the skin. *Int J Mol Sci* **14**, 12222-12248 (2013).
2. Matejuk A., Skin Immunity. *Arch Immunol Ther Exp (Warsz)* **66**, 45-54 (2018).
3. Tlaskalova-Hogenova H. *et al.*, Mucosal immunity: its role in defense and allergy. *Int Arch Allergy Immunol* **128**, 77-89 (2002).
4. Gallo R.L. *et al.*, Microbial symbiosis with the innate immune defense system of the skin. *J Invest Dermatol* **131**, 1974-1980 (2011).
5. Belkaid Y. *et al.*, Compartmentalized and systemic control of tissue immunity by commensals. *Nat Immunol* **14**, 646-653 (2013).
6. Grice E.A., The intersection of microbiome and host at the skin interface: genomic- and metagenomic-based insights. *Genome Res* **25**, 1514-1520 (2015).
7. Grice E.A. *et al.*, The skin microbiome. *Nat Rev Microbiol* **9**, 244-253 (2011).
8. Probst A.J. *et al.*, Archaea on human skin. *PLoS One* **8**, e65388 (2013).
9. Nakatsuji T. *et al.*, The microbiome extends to subepidermal compartments of normal skin. *Nat Commun* **4**, 1431 (2013).
10. Cogen A.L. *et al.*, Skin microbiota: a source of disease or defence? *Br J Dermatol* **158**, 442-455 (2008).
11. Costello E.K. *et al.*, Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694-1697 (2009).
12. Grice E.A. *et al.*, A diversity profile of the human skin microbiota. *Genome Res* **18**, 1043-1050 (2008).
13. Oh J. *et al.*, Temporal Stability of the Human Skin Microbiome. *Cell* **165**, 854-866 (2016).
14. Oh J. *et al.*, Biogeography and individuality shape function in the human skin metagenome. *Nature* **514**, 59-64 (2014).
15. Findley K. *et al.*, Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**, 367-370 (2013).
16. Neuman H. *et al.*, Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiol Rev* **39**, 509-521 (2015).
17. Omenetti S. *et al.*, The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut Microbiome. *Front Immunol* **6**, 639 (2015).
18. Kalliomaki M. *et al.*, Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* **357**, 1076-1079 (2001).
19. Volkova L.A. *et al.*, [Impact of the impaired intestinal microflora on the course of acne vulgaris]. *Klin Med (Mosk)* **79**, 39-41 (2001).
20. Gueniche A. *et al.*, Randomised double-blind placebo-controlled study of the effect of Lactobacillus paracasei NCC 2461 on skin reactivity. *Beneficial microbes* **5**, 137-145 (2014).
21. Ogawa M. *et al.*, Effects of oral intake of heat-killed Lactobacillus brevis SBC8803 (SBL88™) on dry skin conditions: A randomized, double-blind, placebo-controlled study. *Experimental and therapeutic medicine* **12**, 3863-3872 (2016).
22. Ahlawat S. *et al.*, Gut–organ axis: a microbial outreach and networking. *Letters in Applied Microbiology*, (2020).
23. Duboc H. *et al.*, Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* **62**, 531-539 (2013).
24. Fredricks D.N. *et al.*, Molecular identification of bacteria associated with bacterial vaginosis. *New England Journal of Medicine* **353**, 1899-1911 (2005).

25. Srinivasan S. *et al.*, Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PloS one* **7**, e37818 (2012).
26. Codoñer F.M. *et al.*, Gut microbial composition in patients with psoriasis. *Scientific reports* **8**, 1-7 (2018).
27. Chen B. *et al.*, Presence of Segmented Filamentous Bacteria in Human Children and Its Potential Role in the Modulation of Human Gut Immunity. *Front Microbiol* **9**, 1403 (2018).
28. Shapiro J. *et al.*, Psoriatic patients have a distinct structural and functional fecal microbiota compared with controls. *The Journal of dermatology* **46**, 595-603 (2019).
29. Tan L. *et al.*, The Akkermansia muciniphila is a gut microbiota signature in psoriasis. *Experimental Dermatology* **27**, 144-149 (2018).
30. Catinean A. *et al.*, Microbiota and Immune-Mediated Skin Diseases-An Overview. *Microorganisms* **7**, (2019).
31. Kobayashi T. *et al.*, Dysbiosis and Staphylococcus aureus Colonization Drives Inflammation in Atopic Dermatitis. *Immunity* **42**, 756-766 (2015).
32. Stehlikova Z. *et al.*, Dysbiosis of Skin Microbiota in Psoriatic Patients: Co-occurrence of Fungal and Bacterial Communities. *Front Microbiol* **10**, 438 (2019).
33. Sundarrajan S. *et al.*, Comorbidities of Psoriasis - Exploring the Links by Network Approach. *PLoS One* **11**, e0149175 (2016).
34. Singh S. *et al.*, An update on psoriasis and metabolic syndrome: A meta-analysis of observational studies. *PLoS One* **12**, e0181039 (2017).
35. Hagg D. *et al.*, The higher proportion of men with psoriasis treated with biologics may be explained by more severe disease in men. *PLoS One* **8**, e63619 (2013).
36. Boehncke W.H. *et al.*, Psoriasis. *Lancet* **386**, 983-994 (2015).
37. Lowes M.A. *et al.*, Immunology of psoriasis. *Annual review of immunology* **32**, 227-255 (2014).
38. Lande R. *et al.*, Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564-569 (2007).
39. Nestle F.O. *et al.*, Psoriasis. *N Engl J Med* **361**, 496-509 (2009).
40. Boutet M.A. *et al.*, Role of the IL-23/IL-17 Axis in Psoriasis and Psoriatic Arthritis: The Clinical Importance of Its Divergence in Skin and Joints. *Int J Mol Sci* **19**, (2018).
41. Drago F. *et al.*, Psoriasis and small intestine bacterial overgrowth. *Int J Dermatol* **57**, 112-113 (2018).
42. Scher J.U. *et al.*, Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. *Arthritis Rheumatol* **67**, 128-139 (2015).
43. Gomez-Moyano E. *et al.*, Do Malassezia species play a role in exacerbation of scalp psoriasis? *Journal de mycologie medicale* **24**, 87-92 (2014).
44. Narang T. *et al.*, Malassezia and psoriasis: Koebner's phenomenon or direct causation? *Journal of the European Academy of Dermatology and Venereology: JEADV* **21**, 1111 (2007).
45. Abrahamsson T.R. *et al.*, Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* **129**, 434-440, 440 e431-432 (2012).
46. Ellis S.R. *et al.*, The Skin and Gut Microbiome and Its Role in Common Dermatologic Conditions. *Microorganisms* **7**, (2019).
47. Zanvit P. *et al.*, Antibiotics in neonatal life increase murine susceptibility to experimental psoriasis. *Nature communications* **6**, 1-10 (2015).

48. De Gregoris T.B. *et al.*, Improvement of phylum-and class-specific primers for real-time PCR quantification of bacterial taxa. *Journal of microbiological methods* **86**, 351-356 (2011).
49. Hidalgo-Cantabrana C. *et al.*, Gut microbiota dysbiosis in a cohort of patients with psoriasis. *British Journal of Dermatology* **181**, 1287-1295 (2019).
50. Zakostelska Z. *et al.*, Intestinal Microbiota Promotes Psoriasis-Like Skin Inflammation by Enhancing Th17 Response. *PLoS One* **11**, e0159539 (2016).
51. Baba H. *et al.*, Oral intake of Lactobacillus helveticus-fermented milk whey decreased transepidermal water loss and prevented the onset of sodium dodecylsulfate-induced dermatitis in mice. *Bioscience, biotechnology, and biochemistry* **74**, 18-23 (2010).
52. Zhao M. *et al.*, Treatment efficacy of probiotics on atopic dermatitis, zooming in on infants: a systematic review and meta-analysis. *International journal of dermatology* **57**, 635-641 (2018).
53. Kozakova H. *et al.*, Colonization of germ-free mice with a mixture of three lactobacillus strains enhances the integrity of gut mucosa and ameliorates allergic sensitization. *Cellular & molecular immunology* **13**, 251-262 (2016).
54. Al-Banna N. *et al.*, Impact of antibiotics on the microcirculation in local and systemic inflammation. *Clinical Hemorheology and Microcirculation* **53**, 155-169 (2013).
55. Mencarelli A. *et al.*, Development of non-antibiotic macrolide that corrects inflammation-driven immune dysfunction in models of inflammatory bowel diseases and arthritis. *European journal of pharmacology* **665**, 29-39 (2011).
56. Miner J. *et al.*, Steroid-refractory ulcerative colitis treated with corticosteroids, metronidazole and vancomycin: a case report. *BMC gastroenterology* **5**, 3 (2005).
57. Stehlikova Z. *et al.*, Crucial Role of Microbiota in Experimental Psoriasis Revealed by a Gnotobiotic Mouse Model. *Front Microbiol* **10**, 236 (2019).
58. Heissigerova J. *et al.*, The microbiota determines susceptibility to experimental autoimmune uveoretinitis. *Journal of immunology research* **2016**, (2016).
59. García-Collinot G. *et al.*, Effectiveness of Saccharomyces boulardii and Metronidazole for Small Intestinal Bacterial Overgrowth in Systemic Sclerosis. *Digestive Diseases and Sciences* **65**, 1134-1143 (2020).
60. Soifer L.O. *et al.*, Comparative clinical efficacy of a probiotic vs. an antibiotic in the treatment of patients with intestinal bacterial overgrowth and chronic abdominal functional distension: a pilot study. *Acta gastroenterologica Latinoamericana* **40**, 323-327 (2010).
61. Zhang M. *et al.*, Oral antibiotic treatment induces skin microbiota dysbiosis and influences wound healing. *Microbial ecology* **69**, 415-421 (2015).
62. Okada K. *et al.*, The Role of Gut Microbiome in Psoriasis: Oral Administration of Staphylococcus aureus and Streptococcus danieliae Exacerbates Skin Inflammation of Imiquimod-Induced Psoriasis-Like Dermatitis. *International journal of molecular sciences* **21**, 3303 (2020).
63. Jang S.-E. *et al.*, Lactobacillus plantarum CLP-0611 ameliorates colitis in mice by polarizing M1 to M2-like macrophages. *International immunopharmacology* **21**, 186-192 (2014).
64. Kim H. *et al.*, Oral administration of Lactobacillus plantarum lysates attenuates the development of atopic dermatitis lesions in mouse models. *Journal of Microbiology* **53**, 47-52 (2015).
65. Kim W. *et al.*, Lactobacillus plantarum-derived extracellular vesicles induce anti-inflammatory M2 macrophage polarization in vitro. *Journal of extracellular vesicles* **9**, 1793514 (2020).

66. Kong H.H. *et al.*, Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome research* **22**, 850-859 (2012).
67. Mariman R. *et al.*, Lactobacillus plantarum NCIMB8826 ameliorates inflammation of colon and skin in human APOC1 transgenic mice. *Beneficial Microbes* **7**, 215-225 (2016).
68. Prakoeswa C. *et al.*, Beneficial effect of Lactobacillus plantarum IS-10506 supplementation in adults with atopic dermatitis: a randomized controlled trial. *Journal of Dermatological Treatment*, 1-8 (2020).
69. Atarashi K. *et al.*, Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* **331**, 337-341 (2011).
70. Round J.L. *et al.*, Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences* **107**, 12204-12209 (2010).
71. Wang Y. *et al.*, Induction of intestinal Th17 cells by flagellins from segmented filamentous bacteria. *Frontiers in immunology* **10**, 2750 (2019).
72. Koga T. *et al.*, The role of IL-17 in systemic lupus erythematosus and its potential as a therapeutic target. *Expert review of clinical immunology* **15**, 629-637 (2019).
73. Kwon M.-S. *et al.*, Lactobacillus sakei WIKIM30 ameliorates atopic dermatitis-like skin lesions by inducing regulatory T cells and altering gut microbiota structure in mice. *Frontiers in immunology* **9**, 1905 (2018).
74. Stepankova R. *et al.*, Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. *Inflammatory bowel diseases* **13**, 1202-1211 (2007).
75. Kong H.H. *et al.*, Performing skin microbiome research: a method to the madness. *Journal of Investigative Dermatology* **137**, 561-568 (2017).
76. Naik S. *et al.*, Compartmentalized control of skin immunity by resident commensals. *Science* **337**, 1115-1119 (2012).
77. Salter S.J. *et al.*, Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC biology* **12**, 87 (2014).
78. Fierer N. *et al.*, The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Sciences* **105**, 17994-17999 (2008).
79. Kim H.-J. *et al.*, Segregation of age-related skin microbiome characteristics by functionality. *Scientific reports* **9**, 1-11 (2019).
80. Bukin Y.S. *et al.*, The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific data* **6**, 190007 (2019).
81. Castelino M. *et al.*, Optimisation of methods for bacterial skin microbiome investigation: primer selection and comparison of the 454 versus MiSeq platform. *BMC microbiology* **17**, 23 (2017).
82. Willis C. *et al.*, Influence of 16S rRNA variable region on perceived diversity of marine microbial communities of the Northern North Atlantic. *FEMS microbiology letters* **366**, fnz152 (2019).
83. Yang B. *et al.*, Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC bioinformatics* **17**, 1-8 (2016).
84. Conlan S. *et al.*, Species-level analysis of DNA sequence data from the NIH Human Microbiome Project. *PloS one* **7**, e47075 (2012).
85. Jo J.-H. *et al.*, Research techniques made simple: bacterial 16S ribosomal RNA gene sequencing in cutaneous research. *Journal of Investigative Dermatology* **136**, e23-e27 (2016).

86. Meisel J.S. *et al.*, Skin microbiome surveys are strongly influenced by experimental design. *Journal of Investigative Dermatology* **136**, 947-956 (2016).
87. Group J.C.H.M.P.D.G.W., Evaluation of 16S rDNA-based community profiling for human microbiome research. *PloS one* **7**, e39315 (2012).
88. Teng F. *et al.*, Impact of DNA extraction method and targeted 16S-rRNA hypervariable region on oral microbiota profiling. *Scientific reports* **8**, 1-12 (2018).
89. Alekseyenko A.V. *et al.*, Community differentiation of the cutaneous microbiota in psoriasis. *Microbiome* **1**, 31 (2013).
90. Graspeuntner S. *et al.*, Selection of validated hypervariable regions is crucial in 16S-based microbiota studies of the female genital tract. *Scientific reports* **8**, 1-7 (2018).
91. Fahlén A. *et al.*, Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. *Archives of dermatological research* **304**, 15-22 (2012).
92. Gao Z. *et al.*, Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. *PloS one* **3**, e2719 (2008).
93. Tett A. *et al.*, Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. *NPJ biofilms and microbiomes* **3**, 1-12 (2017).
94. Bay L. *et al.*, Universal dermal microbiome in human skin. *Mbio* **11**, (2020).
95. Drago L. *et al.*, Skin microbiota of first cousins affected by psoriasis and atopic dermatitis. *Clinical and Molecular Allergy* **14**, 1-11 (2016).
96. Kverka M. *et al.*, Intestinal microbiota: facts and fiction. *Digestive Diseases* **35**, 139-147 (2017).
97. Yan D. *et al.*, The role of the skin and gut microbiome in psoriatic disease. *Current dermatology reports* **6**, 94-103 (2017).
98. Jarrin C. *et al.*, in *23. IFSCC Conference Zurich 2015*. (Zurich, 2015).
99. Hurabielle C. *et al.*, Immunity to commensal skin fungi promotes psoriasiform skin inflammation. *Proceedings of the National Academy of Sciences* **117**, 16465-16474 (2020).
100. Amaya M. *et al.*, Molecular analysis of Malassezia microflora in the lesional skin of psoriasis patients. *The Journal of dermatology* **34**, 619-624 (2007).
101. Jagielski T. *et al.*, Distribution of Malassezia species on the skin of patients with atopic dermatitis, psoriasis, and healthy volunteers assessed by conventional and molecular identification methods. *BMC dermatology* **14**, 3 (2014).
102. Takemoto A. *et al.*, Molecular characterization of the skin fungal microbiome in patients with psoriasis. *The Journal of dermatology* **42**, 166-170 (2015).
103. Watanabe S. *et al.*, The effects of Malassezia yeasts on cytokine production by human keratinocytes. *Journal of investigative dermatology* **116**, 769-773 (2001).
104. Buentke E. *et al.*, Natural killer and dendritic cell contact in lesional atopic dermatitis skin–Malassezia-influenced cell interaction. *Journal of investigative dermatology* **119**, 850-857 (2002).
105. Selander C. *et al.*, TLR2/MyD88-dependent and-independent activation of mast cell IgE responses by the skin commensal yeast Malassezia sympodialis. *The Journal of Immunology* **182**, 4208-4216 (2009).
106. Paulino L.C. *et al.*, Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *Journal of clinical microbiology* **44**, 2933-2941 (2006).
107. Chang H.-W. *et al.*, Alteration of the cutaneous microbiome in psoriasis and potential role in Th17 polarization. *Microbiome* **6**, 154 (2018).
108. Dei-Cas I. *et al.*, Metagenomic analysis of gut microbiota in non-treated plaque psoriasis patients stratified by disease severity: development of a new Psoriasis-Microbiome Index. *Scientific reports* **10**, 1-11 (2020).

109. Yegorov S. *et al.*, Psoriasis Is Associated With Elevated Gut IL-1α and Intestinal Microbiome Alterations. *Frontiers in immunology* **11**, 2431 (2020).
110. Eckburg P.B. *et al.*, Diversity of the human intestinal microbial flora. *Science* **308**, 1635-1638 (2005).

8. CURRICULUM VITAE

PERSONAL DATA

Zuzana Stehlíková, MSc.

Date and Place of Birth:	17. 10. 1987, Prague, Czech Republic
Nationality:	Czech
Address:	Nová Ves 17, Konstantinovy Lázně, 34952, Czech Republic
E-mail:	zuzana.stehlikova@biomed.cas.cz

BRIEF EDUCATIONAL AND PROFESSIONAL HISTORY

2015 – now

Ph.D. student at Charles University in Prague, First Faculty of Medicine
Field of Study: Immunology

Dissertation thesis: **The role of microbiota in the pathogenesis of psoriasis**
Supervisor: RNDr. Zuzana Jirásková Zákostelská, Ph.D.
Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Cellular and Molecular Immunology

2014 – 2015

Ph.D. student at Charles University in Prague, Second Faculty of Medicine
Field of Study: Microbiology

Dissertation thesis: **Detection and characterization of bacterial pathogens from chronic infections**
Supervisor: doc. MVDr. Oto Melter, Ph.D.
Institute of Medical Microbiology, Motol Medical Hospital

2011 – 2014

Master´s student at Charles University in Prague, Faculty of Natural Sciences
Field of Study: Microbiology

Diploma thesis: **Functional analyses of Spr1057 protein *Streptococcus pneumoniae***
Supervisor: RNDr. Pavel Branny, CSc.
Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Cell Signaling

2008 – 2011

Bachelor´s student at University of South Bohemia in České Budějovice
Field of Study: Biology
Bachelor's thesis: **Presence of tetracycline resistance genes in ecosystems with distinct levels of human impact.**
Supervisor: Mgr. Martina Slaninová Kyselková, Ph.D.
Institute of Soil Biology, Biology Centre CAS, České Budějovice

RESEARCH FUNDING

2017 – 2019

Principal Investigator
Grant Agency of Charles University: GA UK 908217
Project title: Fungal microbiota and TLR9 signaling in the pathogenesis of mouse model of psoriasis.

ACADEMIC RESEARCH TRAININGS

Internship

Short 1-month stay in laboratory of Omry Koren, Ph.D.
May 2017, Bar-Ilan Faculty of Medicine, Safed, Israel

Courses

The Basics of cellular and Molecular Immunology
February-June 2019, Institute of Microbiology of the CAS, Prague, Czech Republic

1st Old herborn University Summer School – Gut Microbiota for Health
June 2018, Herborn, Germany

Advances in Molecular Biology and Genetics

September 2017, Institute of Molecular Genetics ASCR, Prague, Czech Republic

Elements of Science

Science Communication, Paper & Grant Writing, Poster & Lecture Preparation

March 2016, Institute of Molecular Genetics ASCR, Prague, Czech Republic

Multiplexing ELISPOT Workshop

February 2016, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Cytometry Mini-course

February 2016, Czech Society for Analytical Cytometry, Prague, Czech Republic

LINGUISTIC KNOWLEDGE

English: Cambridge Assessment English (CAE), Cambridge English Level 2 Certificate in ESOL International (Advanced), 2018; grade B, Council Europe Level C1 (Total Score 195)

9. PUBLICATION ACTIVITY

9.1. List of publications used in this thesis

Zakostelska Z, Malkova J, Klimesova K, Rossmann P, Hornova M, Novosadova I, **Stehlikova Z**, Kostovcik M, Hudcovic T, Stepankova R, Juzlova K, Hercogova J, Tlaskalova-Hogenova H, Kverka M (2016) *Intestinal Microbiota Promotes Psoriasis-Like Skin Inflammation by Enhancing Th17 Response*. PLoS ONE 11(7): e0159539. doi: 10.1371/journal.pone.0159539.

IF 2.806

Stehlikova Z, Kostovcikova K, Kverka M, Rossmann P, Dvorak J, Novosadova I, Kostovcik M, Coufal S, Srutkova D, Prochazkova P, Hudcovic T, Kozakova H, Stepankova R, Rob F, Juzlova K, Hercogova J, Tlaskalova-Hogenova H, and Jiraskova Zakostelska Z (2019) *Crucial Role of Microbiota in Experimental Psoriasis Revealed by a Gnotobiotic Mouse Model.* Front Microbiol 21;10:236. doi: 10.3389/fmicb.2019.00236.

IF 4.235

Stehlikova Z, Kostovcik M, Kostovcikova K, Kverka M, Juzlova K, Rob F, Hercogova J, Bohac P, Pinto Y, Uzan A, Koren O, Tlaskalova-Hogenova H, and Jiraskova Zakostelska Z (2019) *Dysbiosis of Skin Microbiota in Psoriatic Patients: Co-occurrence of Fungal and Bacterial Communities*. Front Microbiol 21;10:438. doi: 10.3389/fmicb.2019.00438.

IF 4.235

9.2. List of other publications

Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, **Stehlikova Z**, Brezina J, Wohl P, Spicak J, and Drastich P (2017) *Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis*. World J Gastroenterol 23(25): 4548-4558. doi: 10.3748/wjg.v23.i25.4548.

IF 1.409

Stehlikova Z, Tlaskal V, Galanova N, Roubalova R, Kreisinger J, Dvorak J, Prochazkova P, Kostovcikova K, Bartova J, Libanska M, Cermakova R, Schierova D, Fassmann A, Borilova Linhartova P, Coufal S, Kverka M, Izakovicova-Holla L, Petanova J, Tlaskalova-Hogenova

H, and Jiraskova Zakostelska Z (2019) *Oral Microbiota Composition and Antimicrobial Antibody response in Patients with Recurrent Aphthous Stomatitis*. Microorganisms 1;7(12). doi:10.3390/microorganisms7120636.
IF 4.167

Coufal S, Galanova N, Bajer L, Gajdarova Z, Schierova D, Jiraskova Zakostelska Z, Kostovcikova K, Jackova Z, **Stehlikova Z**, Drastich P, Tlaskalova-Hogenova H, Kverka M (2019) *Inflammatory Bowel Disease Types Differ in Markers of Inflammation, Gut Barrier and in Specific Anti-Bacterial Response.* Cells 13;8(7):719. doi: 10.3390/cells8070719.
IF 5.276

Raskova Kafkova L, Brokesova D, Krupka M, Stehlikova Z, Dvorak J, Coufal S, Fajstova A, Srutkova D, Stepanova K, Hermanova P, Stepankova R, Uberall I, Skarda J, Novak Z, Vannucci L, Tlaskalova-Hogenova H, Jiraskova Zakostelska Z, Sinkora M, Mestecky J, Raska M (2020) *Secretory IgA N-glycans contribute to the protection against E. coli O55 infection of germ-free piglets*. Mucosal Immunol 1-12. doi: 10.1038/s41385-020-00345-8.
IF 6.730

Krausova A, Buresova P, Sarnova L, Oyman-Eyrilmez G, Skarda J, Wohl P, Bajer L, Sticova E, Bartonova L, Pacha J, Koubkova G, Prochazka J, Spörrer M, Dürrbeck Ch, **Stehlikova Z**, Vit M, Ziolkowska N, Sedlacek R, Jirak D, Kverka M, Wiche G, Fabry B, Korinek V, Gregor M (2021) *Plectin Ensures Intestinal Epithelial Integrity and Protects Colon Against Colitis*. Mucosal Immunol 2020. Article in press. doi: https://doi.org/10.1101/2020.10.06.323493
IF 6.730