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The Role of Microbiota in the Pathogenesis of Psoriasis

Role mikrobioty v patogenezi psoriázy

Doctoral Thesis

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Prague, 2021

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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

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LIST OF ABBREVIATIONS

AD	Atopic dermatitis
AMPs	Antimicrobial peptides
AP-1	Activator protein 1
ATB	Antibiotic
BALT	Bronchus-associated lymphoid tissue
DCs	Dendritic cells
CCR	C-C chemokine receptor
ccCK18	Caspase-cleaved cytokeratin 18
CD4+	CD4 positive T helper cells
CD8+	CD8 positive cytotoxic T cells
CLA	Cutaneous lymphocyte antigen
CLRs	C-type lectin receptors
COL	Colistin
CV	Conventional
CXCR	C-X-C chemokine receptor
ELISA	Enzyme-linked immunosorbent assay
GALT	Gut-associated lymphoid tissue
GF	Germ-free
GIT	Gastrointestinal tract
HMP	Human Microbiome Project
IBD	Inflammatory bowel disease
IgA	Immunoglobulin A
IISI	Imiquimod-induced skin inflammation
I-FABP	Intestinal fatty acid binding protein
IL17	Interleukin 17
IL22	Interleukin 22
IL23	Interleukin 23
ILFs	Isolated lymphoid follicles
IMQ	Imiquimod
ITS	Internal transcribed spacer
KEGG	Kyoto Encyclopedia of Genes and Genomes
LEfSe	Linear discriminant analysis Effect Size
LCs	Langerhans cells
LL37	Active form of cathelicidin antimicrobial peptide
LP	Lamina propria
MALT	Mucosa-associated lymphoid tissue
MET	Metronidazole
MIX	Mixture of antibiotics
MLNs	Mesenteric lymph nodes
MRSA	Methicillin-resistant *Staphylococcus aureus*
NALT	nasopharynx-associated lymphoid tissue

NF-κB	Nuclear factor kappa B
Nfkbiz	Nuclear factor kappa B inhibitor zeta
NIH	National Institute of Health
NKT	Natural killer T cell
NLRs	NOD-like receptors
NO	Nitric oxide
PAMPs	Pathogen-associated molecular patterns
pDCs	Plasmacytoid dendritic cells
PPs	Peyer´s patches
PRRs	Pathogen recognition receptors
16S rRNA	Ribosomal RNA of the prokaryotic 30S small ribosomal subunit
SALT	Skin-associated lymphoid tissue
SCFAs	Short chain fatty acids
SIBO	Small intestinal bacterial overgrowth
SFB	Segmented filamentous bacteria
STAT3	Signal transducer and activator of transcription 3
STR	Streptomycin
TLRs	Toll-like receptors
TNFα	Tumor necrosis factor alpha
Treg	T helper regulatory cell
Th1	T helper cell Type 1
Th2	T helper cell Type 2
Th17	T helper cell Type 17
VAN	Vancomycin
V1V2	Variable region 1 and 2 of prokaryotic 16S rRNA
V3V4	Variable region 3 and 4 of prokaryotic 16S rRNA
WGS	Whole genome sequencing

1. INTRODUCTION

1.1 Human skin

Skin is an upper-most multilayer surface of our body, densely covered by large amounts of microorganisms (Grice and Segre 2011). As a complex organ the skin represents a physical, chemical and microbial barrier against unfavorable conditions of the external environment (Figure 1). Among the main functions of human skin belong thermoregulation, regulation of water content, resorption of liposoluble substances and resident immune processes of an organism (Dabrowska et al. 2018, Nguyen and Soulika 2019). 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 (D'Orazio et al. 2013).

Anatomy of the matured skin comprises three main layers such as epidermis, dermis and hypodermis. The body surface is covered with a monolayer of cells during the early stages of fetus development and only around fourth month of prenatal age the epidermal basal cells start to proliferate to form the main epidermal layers (Sadler 2011), the process known as keratinization. The fully matured epidermis is then composed of diverse layers of differently matured keratinocytes and skin-associated structures such as sweat and sebaceous glands, nails and hair, which are being formed already in the dermis. The interface between epidermis and dermis constitutes of a basal layer on which the first layer of epidermal stem cells is situated (*stratum basale*). These cells continuously proliferate and gradually penetrate the higher levels of epidermis while they differentiate and lose intracellular organelles including nucleus by autophagy (Akinduro et al. 2016, Yoshihara et al. 2015). The process of gradual maturation of keratinocytes is estimated to take approximately 52-75 days in the skin (Deo and Deshmukh 2018), being profoundly impaired during diseases such as psoriasis (Bowcock and Krueger 2005).

Above the basal layer in the zone of spiny cells (*stratum spinosum*) the keratinocytes starts to produce keratin and later as granular cells (*stratum granulosum*) they produce keratohyalin granules with profilaggrin as the main component, which binds keratin filaments together. During the last transformation from granular cells to corneocytes the cells starts to secrete lamellar bodies rich in lipids and proteins, which leads to the formation of the protective lipid layer of the skin (Candi et al. 2005). The zone of dead translucent cells (*stratum lucidum*)

appears in the areas of thick skin on palms and soles. Translucent cells are rich in eleidin, a transmutation product of keratohyalin (Shlivko et al. 2015). The outermost cornified layer of the skin (*stratum corneum*) constitutes of terminally differentiated keratinocytes, which are subjected to regular desquamation and perform protective mechanical and physiological functions (Del Rosso and Levin 2011).

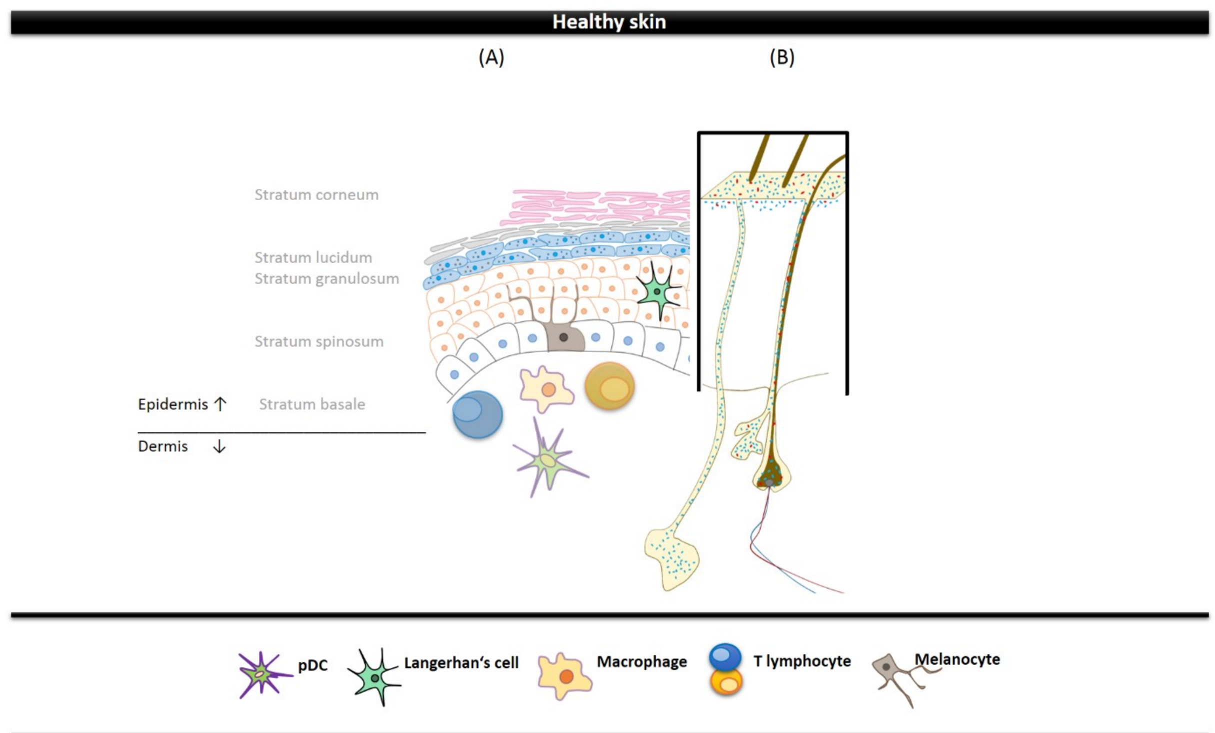

Figure 1: Schematic view on healthy skin structure.
(A) General structure of healthy skin; (B) Representative microbiota distribution on healthy skin (partially adopted from Grice and Segre (2011)). *pDC – plasmacytoid dendritic cell*.

1.2 Immune system of the skin

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, rarely also T cells such as CD8+ T cells (Nestle et al. 2009a).The dermis is occupied by various dendritic cell (DCs) subpopulations, macrophages,

mast cells and several T cell types including Th1, Th2, Th17, γδ-T cells and also natural killer T cells (NKT) (Matejuk 2018, Nestle et al. 2009a).

In contrast to other secondary lymphoid organs and tissues including mucosa associated lymphoid tissue (MALT), SALT does not contain high numbers of B cells, does not form lymphoid follicles and the present T cells are antigen-experienced memory T cells, therefore SALT serves as a peripheral lymphoid tissue with distinct function (Egawa and Kabashima 2011). Antigen presentation to memory T cells takes place within the skin (Clark 2010) yet the antigen presentation to naive T cells occur solely in skin-draining lymph nodes (Honda et al. 2019).

Adaptive immune system of the skin is formed mainly by resident and recirculating populations of T cells, which are nearly twice as much present in the skin than in the blood (Watanabe et al. 2015). T cells isolated from normal non-inflamed skin expressed high levels of skin homing receptor CLA and chemokine receptors CCR4, CCR6 and some of them also CCR8 and CXCR6 (Clark et al. 2006). Skin resident T cells include both CD4+ and CD8+ αβ-T cells and γδ-T cells, with the latter forming 1-10% of all skin T cells. Tissue resident memory T cells (Trm) do not recirculate, and transcriptionally, phenotypically and functionally differ from recirculating central and effector memory T cells (Schenkel and Masopust 2014).

Inconsiderable part of skin's immune system are keratinocytes. Keratinocytes actively participate in innate immune responses not only by expressing different pattern recognition receptors (PRRs) such as Toll-like receptor 2 (TLR2) or TLR4, but also by production of cytokines and chemokines (Pivarcsi et al. 2004, Pivarcsi et al. 2005). Furthermore, keratinocytes are able to respond to microbe-derived substances by production of nitric oxide (NO) or antimicrobial peptides (AMPs), such as cathelicidin (Braff et al. 2005).

1.3 Mucosa and its associated lymphoid tissue

Similarly to skin, mucosal surfaces are in everyday contact with the external environment. Most microorganisms do not enter the host via skin but rather via the mucosal path. 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 (Tlaskalova-Hogenova et al. 2002). 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.

The inner environment of an organism is protected by vastly developed mucosa-associated lymphoid tissue (MALT) located in specialized submucosal areas. MALT is a system of diffused lymphoid tissues that protects mucosal surfaces. In synchronicity with SALT it forms net effect to protect all surfaces of the body which are in contact with external environment. MALT can be further subdivided into specialized lymphoid tissues guarding a particular region of the body such as bronchus-associated lymphoid tissue (BALT), nasopharinx-associated lymphoid tissue (NALT) or gut associated lymphoid tissue (GALT) (Mestecky et al. 2005). MALT involves the majority of immunologically active cells (about 80%) such as macrophages, DCs, B and T cells most of which are present in gastrointestinal tract since the greater number of immunogenic stimuli are components of intestinal commensals or arrive there with food (Tlaskalova-Hogenova et al. 2002).

Some of the characteristic features of mucosal immunity which distinguish it from systemic immunity are strongly developed innate defense, oral tolerance, transport of polymeric immunoglobulins through the epithelium, preferential induction of tolerogenic immune responses or the, so called, common mucosal system (Mestecky et al. 2005). Common mucosal system corresponds to the colonization of the mucosal and exocrine glands by cells originating from MALT, so that secretions of exocrine glands that are not directly stimulated by certain antigens contain natural antibodies to those antigens (Mestecky 1987). The hallmark of mucosal immunity is secretory IgA, which neutralizes antigens on the mucosal surfaces (Simecka 1998).

1.3.1 Mucosa of the gastrointestinal tract

Gastrointestinal tract begins already in the oral cavity, which contains important structures helping the initial process of digestion. The oral cavity is protected by oral mucosa, providing a shelter of the deeper tissues from the external environment, e.g. from the microbiota living on its surface. Oral epithelium also secretes variety of antimicrobial peptides which contributes to the innate immunoprotective properties of the mucosa.

The intestinal mucosa is one of our largest and most exposed body surfaces (Flach and Diefenbach 2015). The single layered epithelium, further covered by secreted products such as immunoglobulins, mucous, or defensins (Arrieta et al. 2006) lays above the intestinal immune system which is divided into multiple compartments with unique properties. Mucosa of the small and large intestines has a similar structure composed of a single layer epithelium, the underlying lamina propria (LP) and a thin layer of smooth muscle under LP. Layers of

connective tissue with blood and lymphatic vessels are located beneath the mucosa. Blood and lymphatic vessels lead to and from a mesentery, which harbors the largest lymph nodes in the body that drain the small and large intestine: the mesenteric lymph nodes (MLNs). MLNs are secondary lymphoid organs receiving antigens from afferent lymphatics draining LP and submucosa and per definition they are not part of the GALT, but they are essential in intestinal immune responses (Newberry and Gustafsson 2016). The main lymphoid tissues of GALT are represented by isolated lymphoid follicles, appendix, and Peyer's patches (PPs) and serve as the major sites for initiation of the immune responses in the gastrointestinal tract (GIT) (Mestecky et al. 2005, Newberry and Gustafsson 2016, Simecka 1998).

Similarly to MLNs, PPs are the secondary lymphoid organs and develop according to age and relevant microbial colonization: they begin to form and contain follicular dendritic cells already in the week19 of gestation, followed by rapid development of germinal centers rich in B cells and surrounded by T cells after birth, when the intestine is exposed to antigens (Cornes 1965). PPs are distributed along the entire length of the gut; oval and irregularly distributed in small intestine, numerous and ring-shaped in distal ileum (46% of PPs concentrated here) with high numbers in the colon and rectum (Cornes 1965, Simecka 1998, Van Kruiningen et al. 2002). PPs do not have afferent lymphatics and acquire antigens from the gut lumen. They consist of aggregated lymphoid follicles surrounded and covered by follicle-associated epithelium (FAE) which contains specialized phagocytic microfold cells (M-cells). M-cells can be considered as the immune sensors of the intestine since they can transport luminal antigens and bacteria toward the underlying immune cells (Jung et al. 2010). Immune cells in PPs must then carefully differentiate harmful and non-harmful agents and coordinate immune responses, since unwarranted reactions against food antigens or gut microbiota could cause severe inflammation. This phenomenon goes hand in hand with active antigen-specific suppression of immune responses to orally administered antigens, the oral tolerance (Jung et al. 2010).

Isolated lymphoid follicles (ILFs) are clusters of B cells with few T cells interspersed between them, surrounded by thick layer of macrophages. Unlike the PPs which evolve already during the first weeks of gestation, ILFs develop only after microbial colonization of the intestine and thus function as induction sites for immune responses toward luminal content (Flach and Diefenbach 2015). Furthermore, strong evidence favors ILFs to be involved in the production of antigen-specific IgA in the gut (Tsuji et al. 2008, Wang et al. 2006).

Appendix is considered to play a prominent role as a sensory organ of the immune intelligence network in the gut (Bazar et al. 2004). It is believed to be ideally positioned to sample luminal antigens coming from digested environmental stimulants from the small intestine. Although the specific nature and putative function of human appendix are unknown, it could, furthermore, serve as a reservoir of beneficial commensal bacteria, provide support for their growth and promote re-inoculation of the colon (Bollinger et al. 2007).

1.4 Skin and mucosal microbiota

Skin and mucosa are colonized by vast amount of microbes that are essential in maintaining a healthy environment and that take an irreplaceable part in the development of the immune system. The term microbiota comprises the live communities of all microorganisms and include bacteria, archaea, viruses, fungi or protists. The term microbiome, although often used interchangeably, refers to the whole set of microbiota genes instead.

1.4.1 Skin microbiota

The skin microbiota protects against opportunistic pathogens, sustains skin homeostasis and educates the immune system (Belkaid and Naik 2013, Gallo and Nakatsuji 2011, Grice 2015). It has been shown that skin commensals can enhance the protective barrier by promoting higher expression of antimicrobial peptides (AMPs), and abrogating NF-κB suppression through their secreted factors (Wanke et al. 2011). Shifts in relative abundances of complex skin microbiota could lead to skin dysbiosis further possibly causing or exacerbating skin diseases (Cogen et al. 2008, Gallo and Nakatsuji 2011, Grice and Segre 2011).

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. Such microenvironments vary in temperature, pH, in the presence of sweat or sebaceous glands, in number of skin folds or hair follicles. Although the skin surface represents rather unfavorable environment in terms of nutrient content, thousands of bacterial, fungal, viral or archaeal species inhabit the small skin niches (Grice and Segre 2011, Probst et

al. 2013). Apart from skin topography, different skin localities enable diverse microbial composition to occur. Noteworthy, microbes do not only live on the surface of the skin, but inhabit also the sub-epidermal compartments such as dermis and dermal adipose tissue (Nakatsuji et al. 2013).

Different delivery mode is of importance for the initial microbial skin colonization coming from the outside environment. After vaginal delivery the infants acquire skin bacterial communities resembling their mother´s vaginal microbiota, e.g. *Lactobacillus*, *Prevotella*, or *Sneathia* spp., while C-section infants get mainly colonized by skin-surface bacteria such as *Staphylococcus*, *Corynebacterium*, and *Cutibacterium* spp. (Dominguez-Bello et al. 2010). Apart from colonization and perpetual microbial interaction the skin undergoes a significant change from an aqueous to a gaseous environment at the time of birth. Moreover, rapid skin colonization goes along with skin barrier function changes, e.g. trans-epidermal water loss, skin pH or sebaceous activity (Chiou and Blume-Peytavi 2004). Therefore the initial microbial settlement from the outside environment seems crucial for subsequent susceptibility to certain opportunistic pathogens such as *Staphylococcus aureus* (MRSA) (Centers for Disease and Prevention 2006).

Body sites with relatively low interpersonal microbial variability include locations inside and outside the nostrils or on the back skin, while high variability is usually observed between the toes and in the navel (Grice et al. 2008). Intrapersonal similarity of microbial composition reflects the skin microenvironments and remains stable during the time (Costello et al. 2009). Ethnicity and soap and shampoo practices were shown to be secondary factors compared to the ecological niches of the human body in assessing the cutaneous microbiota composition (Perez Perez et al. 2016). The overall composition of skin microbial communities thus seems to be driven by the individual rather than the environment (Dorrestein et al. 2016, Oh et al. 2016). Though host-specific behavior foster or hamper microbial populations and possibility of transmission as well.

Other factors which have impact on the composition of microbial communities are age, diurnal rhythm or drugs (Modi et al. 2014, Thaiss et al. 2014, Yatsunenko et al. 2012). Microbiota mainly has to withstand also a mechanical stress of being removed by fluid flow, scraping, shift in salt concentration, or epithelial turnover during the colonization (Otto 2014).

Despite a wide range of mechanisms and processes having impact on our skin microbial population, humans host unique and diverse collection of microbial strains (Lloyd-Price et al. 2017). Latest findings propose intriguing hypothesis of within-individual spatiotemporal diversity demonstrating how strain combination can effects virulence, evolution, and metabolism within the human skin microbiome, which underscores the role of strain diversity in skin health (Zhou et al. 2020).

Bacteria

The four most dominant bacterial phyla found on human skin are Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria (Grice and Segre 2011).

Modern research based on molecular biology techniques revealed that the sebaceous sites were dominated by lipophilic *Cutibacterium* species, while the bacteria which prefer humid conditions like *Staphylococcus* and *Corynebacterium* were preferentially present in the bends of the elbows and the feet (Costello et al. 2009, Grice et al. 2008, Grice and Segre 2011, Oh et al. 2016). *Cutibacterium*, *Corynebacterium* and *Staphylococcus* were found to be the most dominant species on human skin (Grice and Segre 2011, Ross et al. 2018).

Fungi

Fungal community composition was found to be similar across body sites (Findley et al. 2013, Oh et al. 2014). 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. Temporal diversity of fungal microbiota was also confirmed, suggesting strong community structure stability (Findley et al. 2013).

Viruses, archaea

Recent findings suggest that there exist a complex eukaryotic viral flora on the skin surface. Among the most frequent cutaneous viral flora belong polyomaviruses, papillomaviruses or circoviruses (Foulongne et al. 2012, Moens et al. 2011). Skin-colonizing viruses are, however, not yet well described, mostly due to the difficulty of their screening.

Archaea were previously thought to live solely in extreme environments. However, recent findings confirm archaea to cover up to 4.2% of the prokaryotic skin microbiome with the most gene signatures belonging to phylum Thaumarchaeota (Probst et al. 2013).

1.4.2 Microbiota of gastrointestinal tract

Particular nature and properties of skin and mucosa give rise to diverse microbial colonization of both compartments. For the successful development of adult microbiome the infant gut colonization is crucial (Turnbaugh et al. 2009). It is well known that exposure to microbial antigens early after birth shapes the composition of resident microbiota and thus the future immune responses (Hansen et al. 2012). For instance, it was shown that presence of gut microbiota influenced the maturation of B and T cells in PP and MLN in germ free mice (Hrncir et al. 2008).

The core of human microbiota structure and composition is being established during the first 3 years of life (Rodriguez et al. 2015). Within 2-5 years of age the microbiota than fully corresponds to the composition and diversity of an adult microbiota (Koenig et al. 2011, Yatsunenko et al. 2012). Among factors affecting infant's intestinal microbiota composition belongs the mode of birth and breast- versus formula-feeding. For example, vaginally delivered infants acquire gut microbial communities resembling those of mother's vagina and intestine, whereas the gut of C-section infants is colonized rather by skin-surface microbiota (Dominguez-Bello et al. 2010, Makino et al. 2013). As expected, the overall microbial communities of infants are undifferentiated across body habitats regardless of vaginal or C-section delivery, which is in contrast to highly differentiated communities of their mothers (Dominguez-Bello et al. 2010).

The adult human gut comprises up to 1000 species of indigenous microbiota (Qin et al. 2010). Microbiota inhabiting the small intestine is essential for host adaptation to dietary lipids and may contribute to conditions of over- and undernutrition (Martinez-Guryn et al. 2018). Significant nutritional role of resident microbiota in the large intestine is to help to breakdown dietary constituents, particularly carbohydrates not digested in the ileum to short-chain fatty acids (SCFAs) and to produce components such as vitamin K2 (Cooke et al. 2006, Cummings and Macfarlane 1997). SCFAs have a protective role against development of inflammatory diseases such as arthritis, allergy or colitis (Kim et al. 2014, Tian et al. 2018). Decreased levels of SCFAs were noticed in patients suffering atopic dermatitis (Song et al. 2016).

Bacteria

In general, the mucosal microbiota constitution is similar to the skin, but different in bacterial proportions, with Firmicutes and Bacteroidetes to be more abundant on mucosal surfaces of oral cavity and intestinal tract (Dewhirst et al. 2010, Eckburg et al. 2005). In the stomach the most abundant phylum is Proteobacteria (Bik et al. 2006).

Fungi

Although slightly individualised, the core set of fungal genera in healthy oral cavity was identified to be composed mainly of *Cladosporium*, *Candida*, *Malassezia*, *Aspergillus* or *Cryptococcus* species (Dupuy et al. 2014, Ghannoum et al. 2010, Stehlikova et al. 2019c). In the intestine the widely described species concern *Candida*, *Saccharomyces*, *Trichosporon* or *Cladosporium* (Hoffmann et al. 2013, Iliev et al. 2012). The dysbiosis in gut mycobiota could be associated with mucosal inflammation in inflammatory bowel disease (Li et al. 2014).

Viruses, archaea

Methanogenic archaea from the phylum Euryarchaeota (mostly the genus *Methanobrevibacter*) were found to be present on mucosa of large intestine or mouth; their levels in dental plaque could be associated with severity of periodontitis (Kulik et al. 2001), whereas their levels in large intestine with colon cancer or diverticulosis (Weaver et al. 1986).

1.4.3 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.

The ideas about the impact of gut microbiota on human health have been dated long back to 1930s and earlier, i.e. way before the discovery of the structure of DNA. Back then Stokes and Pillsbury tried to investigate a potential emotional linkage among the brain, gut and skin. They

hypothesized that emotional states change the secretion of hydrochloric acid and motility of GIT, which leads to microbial dysbiosis with the subsequent potential risk of small intestinal bacterial overgrowth (SIBO), increased intestinal permeability and local and systemic inflammation (Lombardo et al. 2010, Stokes and Pillsbury 1930). SIBO is a complex syndrome defined as an alteration of microbiota in the upper intestine, related to diverse symptoms and associated with gut discomfort of various kinds, followed by malabsorption in the worst case (Bures et al. 2010). Indeed, it was recently confirmed that low stomach acid and impaired small intestinal motility is a risk for development of SIBO syndrome (Lombardo et al. 2010, Roland et al. 2015). SIBO is often diagnosed in patients suffering diverse dermatoses including rosacea or psoriasis (Drago et al. 2018, Egeberg et al. 2017, Parodi et al. 2008). Interestingly, psoriatic patients suffering SIBO improved the cutaneous manifestation of psoriasis after SIBO treatment with antibiotics and prebiotics (Drago et al. 2018).

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. Microbiota produces hormones resembling those of mammals, such as acetylcholine, histamine, serotonin, corticotropin and others (Kawashima et al. 2007, Masson et al. 1996, Thomas et al. 2012). 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 (Neuman et al. 2015). The adaptive immune system both in the skin and in the gut is educated by residing microbes. For instance, the presence and interaction of segmented filamentous bacteria (SFB) with the Peyer´s patches in the gut promotes the maturation of the immune system and has been associated with Th17 immune response and development of Th17-mediated diseases (Chen et al. 2018, Stepankova et al. 2007b, Wu et al. 2010).

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 (Omenetti and Pizarro 2015). The regulatory metabolites involve molecules from breaking down the dietary compounds such as short-chain fatty acids (SCFAs) propionate, butyrate or acetate. SCFAs are formed mainly by microbial digestion of prebiotics, a non-digestible food supplements for humans, which selectively

stimulate the growth of beneficial intestinal microbiota (Gibson and Roberfroid 1995). The newest research suggests that SCFAs such as butyrate inhibit the action of histone deacetylases, while inositol-3-phosphate (IP3) generated by microbial digestion of phytate, activates histone deacetylases in epithelial cells of mammalian intestine. As a result, this activity could balance the mucosal response to diverse microbial signals (Wu et al. 2020). The levels of SCFAs positively contribute to the establishment of certain skin microbiota profiles and thus subsequently influence the cutaneous defense mechanisms (Schwarz et al. 2017).

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 (Kalliomaki et al. 2001, Volkova et al. 2001). For example, non-breastfed infants with decreased microbial conversion of lactic acid into butyrate, which was closely related to decreased fecal abundance of *Eubacterium* and *Anaerostipes* species, were shown to be more prone to suffer eczema (Wopereis et al. 2018). 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 (Gueniche et al. 2014, Ogawa et al. 2016).

1.4.4 Intra- and inter-species communication

Complex microbial composition on the skin or mucosal surfaces prevents colonization of pathogenic organisms either directly or indirectly in a process termed colonization resistance by enhancing the host immunity (Buffie and Pamer 2013, van der Waaij et al. 1971). Microbial species not only modulate the antimicrobial responses of the host, but they could also antagonize pathogens directly. Certain evidence suggests that Bacilli could inhibit growth of *Clostridium difficile* (Honda et al. 2011). For example, human isolate from fecal microbiota *Bacillus thuringiensis* produces bacteriocin and inhibit *C. difficile* and *Listeria monocytogenes*, but has no effect on other intestinal microbiota components (Rea et al. 2011, Rea et al. 2010). It was further shown that intestinal colonization of mice with *Bacteroides thetaiotamicron* and *Bifidobacterium longum* enhances antiviral immunity (Abt et al. 2012) or that *B. thetaiotamicron* itself competitively prevent *Citrobacter rodentium* by consuming monosaccharides essential for the pathogen´s growth (Kamada et al. 2012).

Colonizers of skin or mucosal surface use specific mechanisms not only for adhesion to host proteins but also for ensuring the microbial community integrity. For this purpose microbiota

forms networks enabling multi-cellular functions and regulation of certain genes through quorum sensing (Brandwein et al. 2016). During quorum sensing microbial cells produce, secrete and perceive small molecules which, detected at high concentrations, direct microbial cells to express genes associated with collective behavior. Microbial biofilms harbor single or multiple species (Roder et al. 2016) and in 65% are pathogenic in nature (Jamal et al. 2018). It is being recognized that many infections are rather caused by communities of microorganisms in biofilms than a single pathogen (Jenkinson and Lamont 2005).

Cooperation between distinct species and domains of organisms is less understood. However, it is known that bacteria form biofilms on many surfaces where the contact with other domains is rather inevitable. Imminent is the contact of viruses with any other species providing them favorable living conditions. Yet complex social interactions are essential also for the coexistence of bacteria and fungi, as well as for the relationship and communication between microbiota and its host. Small signal molecules regulating interactions with other species and domains may be recognized by target cells no matter if prokaryotic or eukaryotic (Lowery et al. 2008).

Innate immune system is the primary defense line against invading organisms. Innate immune system responds to pathogen-associated molecular patterns (PAMPs) using a complex set of pattern recognition receptors (PRRs) such as such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs) and others. On the other hand, the host immune system maintains symbiotic relationship with the microbiota, and vice versa, the commensals train and educate the immune system of the host. The conception that development and establishment of microbiota occur in the absence of inflammation is referred to as "homeostatic immunity" (Belkaid and Harrison 2017). This idea was demonstrated in mice pre-colonized with *S. epidermidis* on skin, which were better protected against fungal and parasitic skin infections, but intradermal application resulted in inflammatory response (Naik et al. 2015, Naik et al. 2012). It is therefore crucial for the host to tolerate and benefit from commensals on the skin but target them in the case of skin or mucosal barrier disruption.

The relationship between microbiota and its host could be of several forms, with same microbe either being commensal, mutualist or even parasite according to the genetic landscape, nutritional and health status and other conditions of its host (Belkaid and Harrison 2017). Breakdown of this dynamic dialogue could result in inflammatory diseases, allergies or

autoimmunity. Therefore, the idea of selective microbial therapies could be of interest for potential disease mitigation and prevention.

1.4.5 Microbial dysbiosis and associated diseases

The imbalance in cutaneous or intestinal microbiota composition, termed as microbial dysbiosis, is often characterized by decrease in the most abundant commensal microbiota (Ahlawat and Sharma 2020, Duboc et al. 2013), which usually brings about the lower alpha diversity measures when compared to healthy individuals (Turnbaugh et al. 2009). However, dysbiosis does not imply that the microbial diversity must be reduced (Fredricks et al. 2005, Srinivasan et al. 2012). Hence, decreased microbial diversity, although often described, should not be considered as an exclusive part of the disease manifestation (Codoñer et al. 2018, Fredricks et al. 2005, Chen et al. 2018, Shapiro et al. 2019, Srinivasan et al. 2012, Tan et al. 2018).

The instability of microbial composition is being connected with many common disorders, some of them characterized as autoimmune. 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 (Catinean et al. 2019, Kobayashi et al. 2015, Stehlikova et al. 2019a). 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 (Catinean et al. 2019, Kobayashi et al. 2015).

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. Patients with psoriasis more often develop metabolic disorders such as type II diabetes, obesity or dyslipidemia due to the systemic inflammation and have increased atherogenesis contributing to higher risk of myocardial infarction or stroke (Rob and Hercogová 2019). Among other comorbidities belongs psoriatic arthritis, inflammatory bowel disease (IBD) or celiac disease (Singh et al. 2017, Sundarrajan and Arumugam 2016). Together with some of the aforementioned comorbidities a common feature of psoriasis seems to be the elevated serum level of intestinal fatty acid binding protein (I-FABP), a marker of gut barrier damage (Sarikaya et al. 2015, Sikora et al. 2019b, Stehlikova et al. 2019a).

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 (Hagg et al. 2013). Five types of psoriasis have been reported so far: the most common plaque psoriasis (psoriasis vulgaris), guttate psoriasis, inverse psoriasis, pustular psoriasis (either palmoplantar or generalized) and erythrodermic psoriasis. Plague-type psoriasis comprises 90% of psoriasis cases with the typical manifestation of sharply demarcated plaques covered in silvery scales (Rendon and Schakel 2019). The scales results from hyperproliferation of premature keratinocytes and their incomplete cornification accompanied by the presence of cell nuclei in the cornified layer, parakeratosis. Since the mitotic rate of psoriatic keratinocytes is increased as compared with healthy skin, the epidermis is thickened with elongated rete pegs of the epithelium (Nestle et al. 2009b). Psoriasis can be triggered predominantly in genetically predisposed individuals by non-specific causes like sunburn, scratching, administration of systemic drugs, infection, stress and others (Boehncke and Schon 2015). Furthermore, mutations in the genes coding for *Caspase Recruitment Domain-Containing Protein 14* (CARD14) and *Interleukin-36-receptor agonist* (IL36Ra) proteins were found to be able to independently induce psoriasis (Jordan et al. 2012, Marrakchi et al. 2011). Genome-wide association studies for genes related to psoriasis pathogenesis uncovered several susceptibility *PSORS* loci, including regions containing innate immunity genes coding for *Signal transducer and activator of transcription 3* (STAT3) or *Nuclear factor kappa B inhibitor alpha* (NFKBIA) (Tsoi et al. 2015).

First choice therapies include local treatments such as emollients, topical corticosteroids, vitamin D3 derivatives, phototherapy or non-steroidal anti-inflammatory drugs. Treatment of mild to less severe forms of psoriasis involves systemic treatment such as methotrexate or cyclosporine A. Advanced therapy for severe forms of psoriasis comprises biological drugs targeting the key cytokines IL17, IL23 or TNFα with specific antibodies (Kamata and Tada 2018, Kircik and Del Rosso 2009). Targeting the IL23/IL17 axis has been shown effective in curing the psoriasis symptoms, however, the true reason why psoriasis occurs still remains uncovered.

1.5.1 Pathogenesis of psoriasis vulgaris

The pathogenesis of psoriasis involves dysregulated interplay among keratinocytes, innate and adaptive immune cells and environmental triggers including microbiota (Lowes et al. 2014). According to some studies the initiation of psoriasis is most probably accompanied by loss of immune tolerance to one of the three recently described potential psoriasis autoantigens: LL37/cathelicidin, ADAMTSL5, or neolipid antigens (Arakawa et al. 2015, Cheung et al. 2016, Lande et al. 2014).

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 (Lande et al. 2007). 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 (Figure 2) (Boutet et al. 2018, Nestle et al. 2009b).

There were several other opinions about the psoriasis pathogenesis, including the hypothesis that T cells driving the psoriatic outbreak originate in tonsils, from where they migrate to the skin (Valdimarsson et al. 2009). This hypothesis was supported by the fact that T cells isolated from psoriatic skin and tonsil of the same individual carried the same gene rearrangements, which pointed at the same origin of those cells (Diluvio et al. 2006).

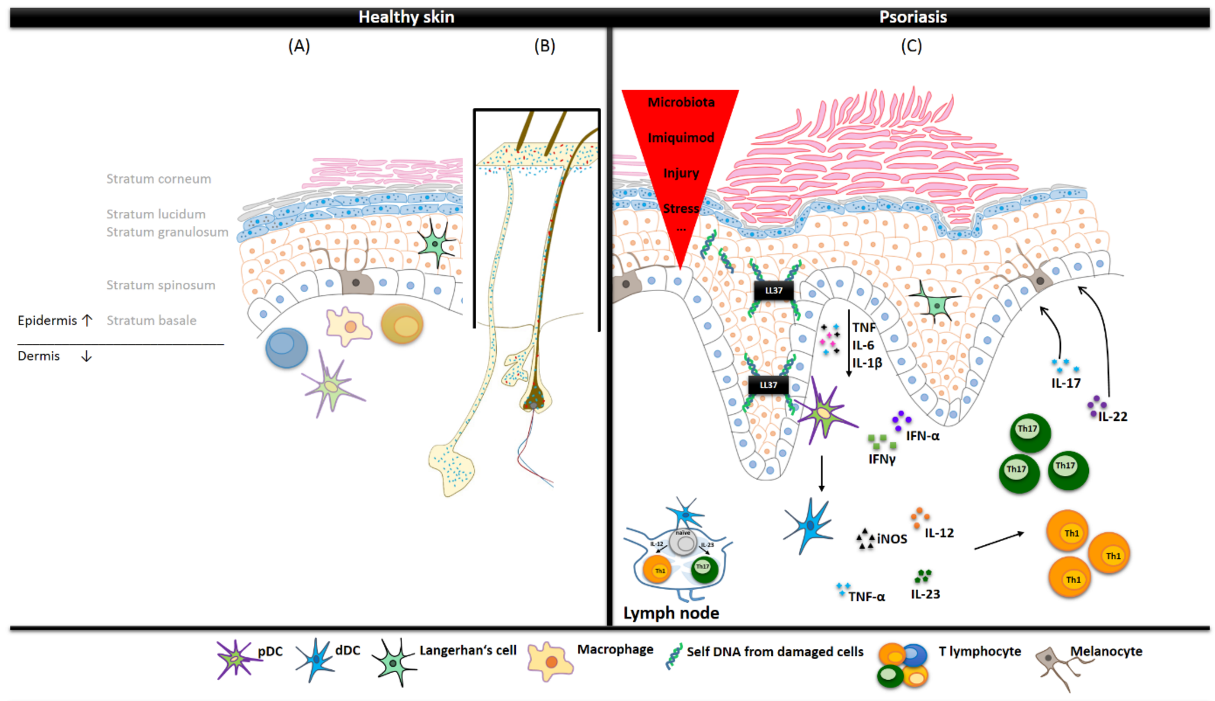

Figure 2: Schematic view on the pathogenesis of psoriasis.
(A) Healthy skin structure; (B) Representative microbiota distribution on healthy skin (partially adopted from Grice and Segre (2011)); (C) Pathogenic mechanisms in psoriasis. *pDC – plasmacytoid dendritic cells; dDC – dermal dendritic cells; LL37 – cathelicidin; TNF – tumor necrosis factor; IFN – interferon; IL – interleukin; Th – T-helper cell.*

1.5.2 The role of microbiota in psoriasis

Composition of skin and gut microbiota is an important factor in modulation of inflammation and disease course in psoriasis (Drago et al. 2018, Scher et al. 2015). Even though no single pathogen has been identified yet to strongly contribute to psoriasis onset, the dysbiosis in skin and gut microbial ecosystems is considered to be one of the main triggers.

Since 1950´s, there were attempts to link microbial infections to exacerbations of psoriasis (McFadden et al. 2009, Norrlind 1950, Pérez-Lorenzo et al. 1998). McFadden and coauthors postulated that streptococcal M proteins may mimic keratin determinants which could lead to subsequent activation of T cells, making M proteins relevant to the pathogenesis of post-streptococcal psoriasis onset (McFadden et al. 1991). Later studies described the impaired

composition of a skin residents *Staphylococcus aureus*, *Streptococcus pyogenes* or *Malassezia* species to contribute to the pathogenic processes of psoriasis (Munz et al. 2010, Rudramurthy et al. 2014, Tomi et al. 2005). Sigurdardottir and colleagues showed that during sore throat tonsils of psoriatic patients are more often infected by Group C *Streptococcus* and possessed higher frequencies of skin-homing CD4+ and CD8+ T cells, compared with infected tonsils from patients without psoriasis (Sigurdardottir et al. 2013). It was also found that patients suffering psoriasis could have bacterial DNA circulating in their blood, leading to a systemic inflammatory response (Munz et al. 2010, Ramirez-Bosca et al. 2015).

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. Cutaneotype 1 was dominated by Proteobacteria and associated with control specimens, while cutaneotype 2 was dominated by Actinobacteria and Firmicutes and was associated with lesioned specimens (Alekseyenko et al. 2013). Fungi share the spotlight with bacteria since it was shown that *Malassezia* species could be associated with exacerbations of psoriasis (Gomez-Moyano et al. 2014, Narang et al. 2007). In addition, new research proposed that skin fungi enhance the pathology of experimental psoriasis in mice by inducing accumulation of IL17-A producing Th and Tc cell within the skin (Hurabielle et al. 2020).

Microbial dysbiosis in the intestine is another phenomenon accompanying many skin diseases, including psoriasis (Abrahamsson et al. 2012, Codoñer et al. 2018, Ellis et al. 2019). Less diverse intestinal microbiota, i.e. decrease of *Coprococcus*, or *Akkermansia*, *Ruminococcus* and *Pseudobutyrivibrio*, was found in patients suffering psoriasis and psoriatic arthritis (Scher et al. 2015). Furthermore, 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 (Zanvit et al. 2015). The presence of complex microbiota is important in regulating the immune responses, since germ-free mice do not respond well to immunization (Lamousé-Smith et al. 2011), are more prone to late-onset sepsis due to dysregulated neutrophil homeostasis (Deshmukh et al. 2014), and have accelerated mortality due to viral infections (Gonzalez-Perez et al. 2016). Therefore, host-microbe interactions prime the immune system already early after birth and significantly impact the sensitivity to the diseases later in life.

1.6 Animal models of cutaneous inflammation

Inflammatory and autoimmune diseases are studied on wide range of transgenic or normal animal models, including mouse, fish, pig or rat. Since there are lots of variables between animals and humans, the disease pathogenesis often does not match absolutely. For example, the amount of free fatty acids, triglycerides or density of hair follicles are important factors in developing animal models for human skin diseases, as they bring about differences between skin barriers of animals and humans (Netzlaff et al. 2006). Moreover, there are also discrepancies in innate and adaptive immunity between human and animal skin, such as Toll receptors, antigen presenting function of endothelial cells or prevalence of γδ T cells in murine epidermis (Mestas and Hughes 2004). Aside other differences between mouse and human, the immune system also differs in subtypes of dendritic and other inflammatory cells (Haley 2003). Regarding skin dissimilarities, mouse epidermis is thinner, generally composed of only 2-3 keratinocyte layers with increased turnover and does not contain any rete ridges compared to human epidermis (Berking et al. 2002, Gudjonsson et al. 2007). Furthermore, mouse models are mostly raised on inbred background under controlled conditions, thus their genetic diversity is limited in contrast to outbred humans. However, mouse models have been successfully used to mimic some features of human skin diseases, such as atopic dermatitis or psoriasis (Avci et al. 2013).

Gnotobiotic animal models are especially important, as they have helped in clarifying the consequences of microbial colonization on immune system development. Animals can be reared in germ-free conditions and specifically colonized with defined microbes. In this way researches are able to define specific microbiota components or reveal the molecular mechanisms of beneficial or pathogenic effect of particular germs on its host physiology (Tlaskalová-Hogenová et al. 2011). Using GF mice it was shown, for example, that intestinal microbiota and bacterial components in the diet are important for the development of Treg cells (Hrncir et al. 2008). In another study, mono-colonization of GF mice at birth with recombinant *Lactobacillus plantarum* producing allergen helped to ameliorate the experimental allergy by shifting the usual allergen-specific Th2 response towards Th1/Th2 profile (Schabussova and Wiedermann 2008, Schwarzer et al. 2011). Moreover, colonization of GF mice with 3 strains of *Lactobacillus* was shown to strengthen the intestinal barrier accompanied by lower sensitization to allergens (Kozakova et al. 2016). Last but not least, by using GF mice it was recently shown that commensal microbes are necessary for migration of mast cells to the

intestine and their maturation there, which is crucial for experimental food allergy development (Schwarzer et al. 2019).

1.6.1 Mouse models of psoriasis

The ideal animal model of psoriasis should not only reflect the clinical hallmarks of psoriasis, but also histological and morphological features, pathogenesis and responsiveness to therapeutic agents. Spontaneous mouse mutants and the xenotransplantation of skin in the background of immunodeficient mice lacking both T and B cells, were probably the first attempts to achieve mouse models resembling human psoriasis (Boehncke and Schon 2015, HogenEsch et al. 1993). Later in 1996 it was shown that mice with healthy human skin transplants that received autologous blood lymphocytes from psoriatic patient, intradermally injected in the transplanted healthy skin, developed psoriasis (Wrone-Smith and Nickoloff 1996). Several other approaches were made in order to develop animal model of psoriasis, including manipulation of the Stat3 pathway or knocking out the AP-1 family members (Sano et al. 2005, Zenz et al. 2005).

One of the well-established and the most used mouse model of psoriasis nowadays is the imiquimod-induced murine model for which purpose the healthy mice are used. 5% imiquimod (IMQ) is an active substance of Aldara cream, which is applied topically to treat the human papillomavirus skin infections or certain forms of skin cancer. Strikingly, patients started to report the appearance of psoriatic lesions after Aldara treatment (Fanti et al. 2006, Rajan and Langtry 2006, Wu et al. 2004). IMQ contributes to a strong activation of immune system by binding to TLR7/8 of macrophages, monocytes and pDCs, enhancing the Langerhans cells migration and therefore the T cell response (Bocheńska et al. 2017).

It was first published in 2009 that the combination of IMQ with its carrier, the isostearic acid, applied daily on mouse shaved back induced a skin inflammation fully resembling the human psoriasis. The daily application led to formation of psoriatic plaques, accompanied by impaired differentiation and increased proliferation of keratinocytes, erythema and influx of immune cells to the skin. It has been shown that Aldara induces epidermal expression of key cytokines IL-17A, IL-17F and IL-23 and that mice deficient in IL-17 and IL-23 receptors does not develop psoriasis, conforming the crucial role of IL-23/IL-17 axis in the pathogenesis of psoriasis (Van der Fits et al. 2009). The short lasting presence of the experimental psoriasis is the

inconvenience of this model, since mice are able to revert the inflammatory process after the sixth day of imiquimod application.

1.7 Techniques of human microbiome research

The human microbiota forms an environment-specific ecosystem so that each site of the body represents a unique habitat of trillions of microbial cells (Huttenhower et al. 2012, Methé et al. 2012). During the rapid development of molecular biology techniques it became apparent that culture techniques are not sufficient enough for the thorough identification of microbiota, since the majority of microorganisms is not cultivable (Amann et al. 1995, Rhoads et al. 2012, Stewart 2012). In 2012 the National Institute of Health (NIH) funded Human Microbiome Project Consortium (HMP) established standardized methodologies for creating, processing and interpreting diverse types of fast-growing metagenomic data (Methé et al. 2012).

Currently, the era of high-throughput functional genomics enabled to extend the research in microbial communities tremendously. Advances in DNA-sequencing allow researchers to observe the microbial composition in culture-independent manner. By sequencing the 16S rRNA or ITS amplicons, researchers could profile the bacterial and fungal communities in taxonomic, phylogenetic, and also functional way. By shotgun metagenomics it is possible to create millions of random genomic fragments of the whole breadth of the community in a given sample. Once analyzed and compared to a reference database, the results from shotgun metagenomics could be then used for additional analyses such as metabolic (function) profiling (Mallick et al. 2019). Whole genome sequencing (WGS) provides deep insight into entire genome of a given microbial community or individual species. For physiological insight into microbial communities and investigating the dynamics of ever-changing cellular transcriptome the RNA sequencing can be used.

1.8 Translational microbiota research

The Human Microbiome Project, launched by the NIH in 2007 and finished in 2016 was established in order to generate knowledge and expertise to explore the human microbiome and its role in health and disease. The finding that each person´s microbiome is unique but at the same time certain communities could be used to predict possible predisposition to a disease, belongs to one of the major outcomes of this project (Ding and Schloss 2014).

Ever since microbes were known to be associated with human diseases, it has been intriguing to manipulate the microbiota composition. For example, the first use of today´s modern, helpful and much-discussed method of fecal microbiota transplantation is dated back to ancient times (Johns Hopkins Medical Center 2013). However, thanks to the exponential increase in the so called omics technologies enabling large-scale analysis we are now able to better understand the human microbiota composition and its possible function and probable role in diseases. Thus, there is a great potential in manipulating the microbiota composition by using either antibiotics, pre- and probiotics or even microbiota transplantation. Fecal microbiota transplantation is successfully used to treat recurrent enterocolitis caused by antibiotic-resistant *Clostridium difficile* strains, and other intestinal disorders, in order to restore the normal microbial composition (Matsuoka et al. 2014, Stebel et al. 2018, Šturdík et al. 2016).

Apart from the aforementioned fecal microbiota transplantation, researchers recently reported the first in-human topical microbiota transplantation in mouse model of atopic dermatitis. The skin commensal *Roseomonas mucosa*, collected from healthy individuals but not from patients with AD, improved both mouse and cell culture models of atopic dermatitis (Myles et al. 2018). In psoriasis, highly promising seems to be the interim data about treatment of psoriasis with high dose of EDP1815, a *Prevotella histicola* formulation, at Phase 1b clinical trial. Patients with mild to moderate psoriasis treated with this microbial drug showed statistically significant reduction of skin lesions severity, when compared to placebo receiving group ($p<0.05$) (Evelo Biosciences 2019).

In mouse model of dextran sulphate sodium (DSS)-induced colitis, oral administration of *Parabacteroides distasonis* components decreased the disease severity and stabilized the intestinal microbiota ecology (Kverka et al. 2011). Furthermore, oral administration of *Bifidobacterium longum* CCM 7952 was able to ameliorate DSS-induced colitis by strengthening the intestinal epithelial barrier function (Srutkova et al. 2015). Also manipulation of gut microbiota composition using bacteriophages, which was tried in gnotobiotic mice, brought hopeful results (Hsu et al. 2019).

The research has yielded insight into the nature of complex microbial communities; understanding the inter-kingdom interactions can enrich the development of new diagnostic techniques and research strategies not only in human health care, but also in ecology, agriculture or even in extraterrestrial field of research (Cullen et al. 2020).

2. AIMS OF THE STUDY

Rich and diverse composition of microbiota is generally, but not exclusively, attributed to better physiology and homeostasis, whereas less diverse microbiota was found in patients suffering various diseases. 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.

Psoriasis is being described as an inflammatory skin condition with multifactorial triggers including the involvement of microbiota composition. To analyze the role of gut microbiota in experimental psoriasis and adjacent T-cell response to IISI, we induced the skin inflammation in germ-free (GF) and conventional mice (CV). In CV mice, we used the IISI model with 14 days pre-dosing and 7 days co-dosing the mixture of broad-spectrum antibiotics to assess the role of microbiota under conventional conditions (Zakostelska et al. 2016). Furthermore, we evaluated whether the individual components of antibiotic mixture on their own could mitigate IISI and what nature the subsequent expected changes in microbiota composition and immune response will be (Stehlikova et al. 2019b). To test whether a single species is able to influence the course of IISI, we monocolonized germ-free mice with either SFB or *Lactobacilllus plantarum* WCFS1 (Stehlikova et al. 2019b).

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.

There are many attempts to research human skin microbiota composition. The main issue is that the methodology differs across publications so the results are difficult to compare. Therefore, we aimed to investigate how different approaches reflect the skin microbiota composition. In this comprehensive study we sampled the psoriatic lesions, unaffected psoriatic skin and healthy skin. In addition, we compared two typical sites of psoriasis incidence,

covering two niches with different microenvironments – the dry skin on the elbow and the oily skin on the back. On all the aforementioned sites we used three methods of samples collection: swab, scraping and biopsy. After obtaining the results we searched for specific features of microbiota co-occurrence which could be potentially associated with psoriasis lesions (Stehlikova et al. 2019a).

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.

Psoriasis is a systemic disease which could be associated with elevated serum levels of intestinal fatty acid binding protein (I-FABP), indicating intestinal barrier damage. We wanted to test whether there is an increased level of markers associated with gut barrier disruption such as ccCK18 and I-FABP in the serum of our cohort of psoriatic patients and could therefore serve as a marker for preventive psoriatic care (Stehlikova et al. 2019a).

3. LIST OF PUBLICATIONS

3.1 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.

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.

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.

3.2 List of other impacted 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.

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.

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.

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.

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

I hereby confirm that the author of this thesis, MSc. Zuzana Stehlíková, has substantially contributed to the publications included in this thesis. She performed the majority of the experimental work and significantly contributed to the manuscript preparation in the case of her first-author publications.

……………………………….

RNDr. Zuzana Jirásková Zákostelská, Ph.D.

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.

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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). This was manifested, among other things, 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 this skin inflammation also in germ-free (GF) mice.

When compared to GF mice, CV mice manifested more severe erythema, scaling, thickening and other histological features resembling human psoriasis, as well as higher leukocyte infiltration into the dermis. CV mice treated with mix of broad-spectrum antibiotics displayed altered intestinal microbiota composition, which was demonstrated mainly by decreased diversity and by shifted composition towards higher abundance of order Lactobacillales. Absence of microbiota or ATB treatment decreased the frequencies of $\gamma\delta$ T cells and Th17 cells in spleen and axillary lymph nodes in IMQ-treated mice. Treatment with 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 limited neither to the absence of microbiota nor to its composition. The severity of skin inflammation could be thus modified by altering the gut microbiota composition in adult mice, which strenghtens the importance of the gut-skin axis.

My contribution: sample collection, data analysis and interpretation

PLOS ONE

RESEARCH ARTICLE

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

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Abstract

Psoriasis is a chronic inflammatory skin disease in which Th17 cells play a crucial role. Since indigenous gut microbiota influences the development and reactivity of immune cells, we analyzed the link among microbiota, T cells and the formation of psoriatic lesions in the imiquimod-induced murine model of psoriasis. To explore the role of microbiota, we induced skin inflammation in germ-free (GF), broad-spectrum antibiotic (ATB)-treated or conventional (CV) BALB/c and C57BL/6 mice. We found that both mice reared in GF conditions for several generations and CV mice treated with ATB were more resistant to imiquimod-induced skin inflammation than CV mice. The ATB treatment dramatically changed the diversity of gut bacteria, which remained stable after subsequent imiquimod application; ATB treatment resulted in a substantial increase in the order *Lactobacillales* and a significant decrease in *Coriobacteriales* and *Clostridiales*. Moreover, as compared to CV mice, imiquimod induced a lower degree of local and systemic Th17 activation in both GF and ATB-treated mice. These findings suggest that gut microbiota control imiquimod-induced skin inflammation by altering the T cell response.

Introduction

Psoriasis is a chronic inflammatory disease that affects approximately 2–4% of the world's population [1]. It is characterized by scaly red plaques of epidermal hyperplasia, dilatation of dermal blood vessels, accumulation of inflammatory cells in dermis and activation of the IL-23/Th17 axis [2]. In genetically predisposed individuals, psoriasis can be initiated by various environmental triggers, including bacterial infection, antibiotic treatment or profound changes in

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diet [3–5]. These triggers suggest involvement of microbiota in the disease pathogenesis, although the exact molecular mechanisms of this host-microbe interaction are still largely unknown [6].

There is a close association between microbiota and psoriatic attacks [7]. Microbial infections are not only a well-known risk or aggravating factor for psoriasis, but they may even be a tool of natural selection for a pro-inflammatory genotype that favors psoriasis development [8, 9]. The microbiota associated with psoriatic lesions significantly differs from this on healthy skin [10, 11]. But the connection between psoriasis and microbiota may not be limited only to skin microbiota. In murine models of inflammatory diseases, gut microbiota profoundly influenced the immune system development and reactivity [12, 13]. During the early postnatal period, the host-microbe interactions may significantly influence the immune system development and thus change the sensitivity to inflammatory diseases later in life [14]. The outcome of host-microbe interaction could change during the individual's development. So while treatment with antibiotics (mixture of vancomycin and polymyxin B) decreases the severity of psoriasis-like skin inflammation in adult mice, it worsens it in mice born to parents exposed to these antibiotics [15]. Such antibiotic treatment can shift the microbiota composition significantly, although its effect on gut microbiota bacterial load is usually only marginal [16]. Therefore, in this study, we used mice reared in germ-free conditions for several generations to analyze the role of gut microbiota in psoriasis and T cell response as a link between them. Next, we focused on the gut microbiota and analyzed the development of psoriasis-like skin inflammation in conventional mice treated orally with antibiotics.

Materials & Methods

Mice

We used female BALB/c or C57BL/6 mice (7–10 weeks old) reared either in conventional or germ-free conditions at the Institute of Microbiology of the CAS. The GF mice were reared in sterile Trexler-type plastic isolators for several generations before they were used in experiments [17]. Mice were fed with Altromin 1414 diet (Altromin, Lage, Germany). All experiments were approved by the Animal Care and Use Committee at the Institute of Microbiology, CAS, approval ID: 050/2011 and 39/2015.

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 (IMQ) cream (Aldara, 3M Health Care Limited, Great Britain) or similar amount of control cream (vaseline, Aromatica CZ, Czech Republic). The severity of erythema and scaling was monitored daily by a scale based on the clinical Psoriasis Area and Severity Index (PASI), and ear swelling and skin thickening were measured at the end of the experiment, as described previously [18]. During our preliminary experiments, we found that irradiated IMQ cream (25 kGy), which was used in our experiments, induces skin inflammation of similar nature and degree as the non-irradiated cream (data not shown).

Antibiotic treatment

Mice were treated with antibiotics 2 weeks before psoriasis induction and the treatment continued until the end of the experiment. A mix of metronidazol (0.4 mg; B. Braun, Melsungen AG, Germany), colistin (0.3 mg) and streptomycin (2 mg) (both Sigma-Aldrich) was administered daily by gavage and vancomycin (0.25 mg/ml; Sigma-Aldrich) was added to autoclaved drinking water. Administration of this ATB mixture by gavage was performed in order to prevent

severe dehydration and weight loss, because the mice refused to drink these ATB in their drinking water as described by others [19, 20]. This treatment was well tolerated by all mice and led to significant changes in microbiota composition and to gut phenotype resembling GF animals. To protect the mice from subsequent *Candida* overgrowth, mice were gavaged daily with 20 μg of amphotericin-B (Sigma-Aldrich, St. Louis, MO), starting 3 days before antibiotic treatment until the end of the experiment.

Histology

The dorsal skin and ear samples were fixed in 5% buffered formalin, dehydrated and embedded in paraffin. Next, 4μm sections were cut and stained with H&E for histopathological examination by an experienced pathologist (P.R.), unaware of the treatment of the mice. The degree of psoriatic skin inflammation was scored on a scale of 0–2 (Table 1).

Flow cytometry

We processed spleen and axillary lymph nodes into single cell suspensions immediately after animals were sacrificed and blocked them as described previously [21]. Then, we performed three different flow cytometry experiments. To analyze major T cell phenotypes, the cells were stained with extracellular fluorochrome-labeled anti-mouse antibodies: PE conjugated anti-CD3 (clone 145-2C11, dilution 1:100) and FITC conjugated anti-γδTCR (clone EbioGL3, dilution 1:50) (all eBioscience, San Diego, CA, USA). To analyze intracellular RORγt, the cells were first stained with FITC conjugated anti-CD3 (clone 145-2C11, dilution 1:100) and then fixed, permeabilized by Intracellular fixation & permeabilization buffer set (eBioscience, San Diego, CA, USA) and stained intracellularly for PE-conjugated anti-RORγt (clone AFJKS-9, dilution

Table 1. Disease severity grading using light microscopy.

Grade	Description
0.0 (Normal skin)	• thin epidermis without acanthosis or hyperkeratosis • no inflammatory cellularity or rare single lymphocytes in corium
0.5 (Minimal changes)	• discrete scatter of individual lymphocytes in corium
1.0 (Moderate non-characteristic dermatitis)	• low-degree thickening, hyperkeratosis and acanthosis of epidermis, but without parakeratosis and accumulation of leucocytes ("microabscesses") in corium • scattered lympho-monocytic infiltrates in corium possibly with sporadic polymorphonuclear leukocytes
1.5 (Psoriasis-like lesion with incomplete signs)	• well-expressed hyperkeratosis possibly with focal-discrete parakeratosis • marked irregular acanthosis without prominent "gothic vaults" of rete pegs • diffuse cellular infiltration of corium with focal clustering and scattered polymorphonuclear leukocytes
2.0 (Severe dermatitis with structures resembling human psoriasis)	• prominent diffuse hyperkeratosis with salient areas of parakeratosis • scattered focal aggregates of intracorneal polymorphonuclear leukocytes; • dysplasia of epidermis with architectural disarray and nuclear polymorphism, mainly in the basal cell layer • severe acanthosis, apparent "roman and gothic vaults" of rete pegs • prominent, focally massive cellular infiltration of corium with clusters of granulocytes and focal "microabscesses" • congestion of corial capillaries and venules with leukostasis and mural adhesion, edema in tela subcutanea

The degree of imiquimod-induced psoriasis-like skin inflammation was evaluated from histological sections and scored on a scale of 0–2 according the instructions above.

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1:50). For intracellular staining of produced cytokines, cells were first stimulated with 1 μg/ml PMA and 2.8 μM Ionomycin (both from Sigma-Aldrich) for 8 hours and then treated with a mixture of Brefeldin A and Monensin (both from eBioscience) for 4 hours, according to the manufacturer's instructions. The cells were stained with extracellular FITC conjugated anti-CD3 (clone 145-2C11, dilution 1:100), fixed, permeabilized and stained for intracellular PE conjugated anti-IL-17 (clone eBio17B7, dilution 1:50) and APC conjugated anti-IFN-γ (clone XMG1.2, dilution 1:50) both from eBioscience. Data were acquired using LSRII (BD Bioscience) and analyzed by FlowJo software v 9.6.2. (Tree Star, Inc., Ashland, OR). Cells that were dead at the time of surface staining were excluded using Fixable viability dye eFluor 780 (dilution 1:200) (eBioscience).

Cell cultivation and cytokine measurement

Single cell suspensions from spleen and axillary lymph nodes were prepared as described above. The cell viability, generally around 90%, was analyzed using Trypan Blue (Sigma-Aldrich) exclusion and cells were seeded at 2 x 10^5 of live cells per well in 96-well plates in RPMI 1640 (Sigma-Aldrich) culture medium supplemented with 10% fetal bovine serum (Bio-Clot GmbH, Aidenbach, Germany) and 1% Antibiotic-Antimycotic solution (Sigma-Aldrich). Then, we stimulated the cells for 48 hours with plate-bound anti-CD3 (5 μg/ml; clone 145-2C11) and soluble anti-CD28 (2 μg/ml; clone 37.51; both eBioscience) antibodies. The supernatants were collected and frozen at -20°C until analysis. Commercially available ELISA sets were used to measure the levels of IFN-γ and IL-17 (Invitrogen Corp.) in the supernatants. All tests were performed according to the manufacturers' recommendations.

Microbiota analysis

Stool samples from ATB-treated and control mice were collected on day 0, 14 (just before psoriasis induction) and 21 (the last day of the experiment). DNA was isolated with MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA).

Next, the V3-V4 region of 16S rRNA gene was amplified using degenerate bacterial 16S rRNA-specific primers 341F (5′-CCTACGGGNGGCWGCAG-3’) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3’) which were barcoded to enable multiplexing of sequencing libraries. PCR amplification was performed with KAPA 2G Robust Hot Start DNA Polymerase (Kapa Biosystems, Wilmington, MA, USA), with final concentrations: Buffer B 1x, Enhancer 1x, dNTP 0.2 mM each, primers 0.5 μM each, DNA sample 4 ng/μl, KAPA polymerase 0.5U. Cycle parameters were 3 min 94°C, 25 cycles of 30 s at 94°C, 1 min at 54.2°C, and 1 min 15 s at 72°C, final extension at 72°C for 10 min. Three PCR products were pooled to minimize random PCR bias and the length of PCR product was checked on the agarose gel electrophoresis. Equal amounts of each sample were plate-purified using the SequalPrep™ Normalization Plate (96) Kit (Invitrogen). Equimolar amounts of PCR product from each sample were than pooled and MiSeq platform compatible adapters were ligated using TruSeq DNA PCR-Free LT Kit (Illumina), library was quantified using KAPA Library Quantification Kit (Illumina) and sequenced on MiSeq platform using 2x300bp kit at CEITEC Genomics Core Facility. Sequencing data were processed using QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.1 [22]. The sequence data are available in the Sequence Read Archive (SRA) http://www.ncbi.nlm.nih.gov/sra under the accession number SRP068451. To measure the bacterial load, extracted DNA was analyzed by recently optimized qPCR assay, using universal bacterial primer pair [23]

Statistical analysis

Unpaired Student's t-test was used to compare two experimental groups and one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test was used to compare the multiple experimental groups with the control group. The data are presented as the mean ± standard deviation (SD), unless stated otherwise, and differences are considered statistically significant at $P \leq 0.05$. The GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA) was used for all statistical analyses.

Results

Imiquimod induces milder skin inflammation in germ-free than in conventional mice

To analyze the role of microbiota in the murine model of psoriasis, we treated ear and shaven dorsal skin of germ-free (GF) and conventionally reared (CV) BALB/c mice with imiquimod (IMQ). Control mice were treated with control cream in a similar manner. We have not observed any differences in skin morphology between GF and CV mice. While IMQ-treated mice developed typical signs of psoriasis, such as redness, scaling and increased epidermal thickening, skin of the control mice manifested neither any signs of macroscopic nor microscopic inflammation (Fig 1). All changes found in shaved dorsal skin were usually mirrored by these found in ear skin. As compared to CV mice, GF mice displayed less severe erythema and scaling (Fig 1A), less severe ear and skin thickening (Fig 1B) and less severe hyperkeratosis, acanthosis and leukocyte infiltration into the dermis (Fig 1C and 1D). Moreover, while quite

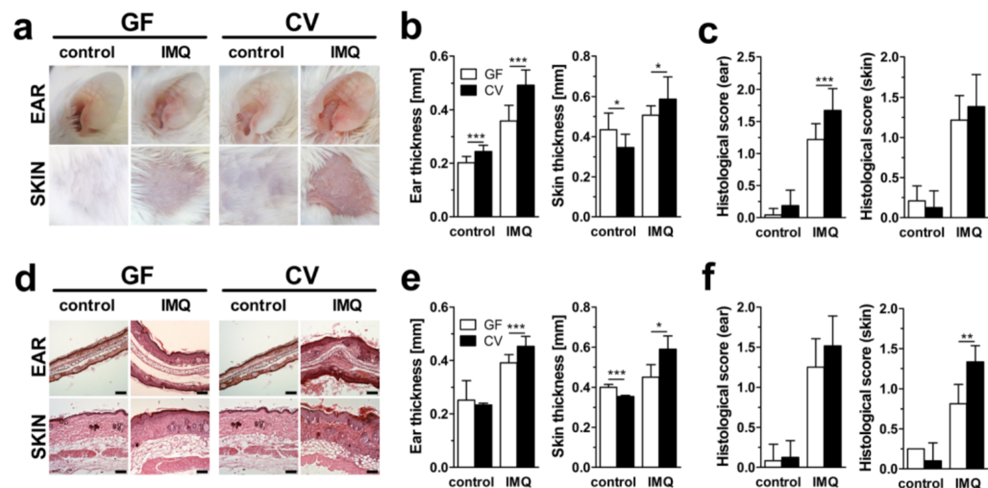

Fig 1. Microbiota enhances sensitivity to IMQ-induced skin inflammation in BALB/c and C57BL/6 mice. (a) Representative macroscopic pictures of healthy (control) and inflamed (IMQ) ear or dorsal skin of BALB/c mice, illustrating the disease severity. Quantification of skin and ear thickness of BALB/c (b) and C57BL/6 (e) mice. Quantification of histopathological score (0–2) after H&E staining of the ear and skin of BALB/c (c) a C57BL/6 (f) mice. (d) Representative H&E-stained ear and skin sections of BALB/c mice (scale bar, 100 μm). The values represent means ± SD as a pool of three independent experiments (n = 10–20 mice per group). $^{*}p < 0.05$, $^{***}p < 0.001$.

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common in skin of CV mice, we did not find any parakeratosis, acanthosis with prominent gothic vaults or focal microabscesses in the dorsal or ear skin of GF mice (Fig 1D).

As reported by others, genetic background of mice may influence the development of IMQ-induced skin inflammation, with BALB/c being more sensitive and C57BL/6 more resistant to disease induction [18]. To overcome this issue, we performed a similar experiment also in C57BL/6 mice. Although the disease severity differed slightly between C57BL/6 (Fig 1E and 1F) and BALB/c (Fig 1A, 1B, 1C and 1D) mice, both GF strains were still more resistant to IMQ-induced skin inflammation than CV. These results show that microbiota aggravates the disease development leading to psoriasis, regardless of genetic background.

Antibiotics change gut microbiota composition and prevent severe forms of skin inflammation

Microbiota significantly influences the immune system reactivity, and even the early germ-free period may have a major impact on the sensitivity to diseases later in life [14]. To analyze if the disease development can be altered by microbiota reduction in adult mice, we treated mice with a broad-spectrum antibiotic mixture (ATB), starting two weeks before the IMQ treatment and continuing until the end of the experiment. To analyze the effect of this antibiotic treatment on microbial load and composition, we performed 16S rRNA-based qPCR and next generation sequencing, respectively. We collected samples of feces before the ATB treatment (Day 0), before the imiquimod application (Day 14), and at the end of the experiment (Day 21). We found that oral ATB dramatically decreased the gut microbial diversity (Fig 2A) and shifted its composition (Fig 2B). This treatment, however, did not change the microbial load; because in

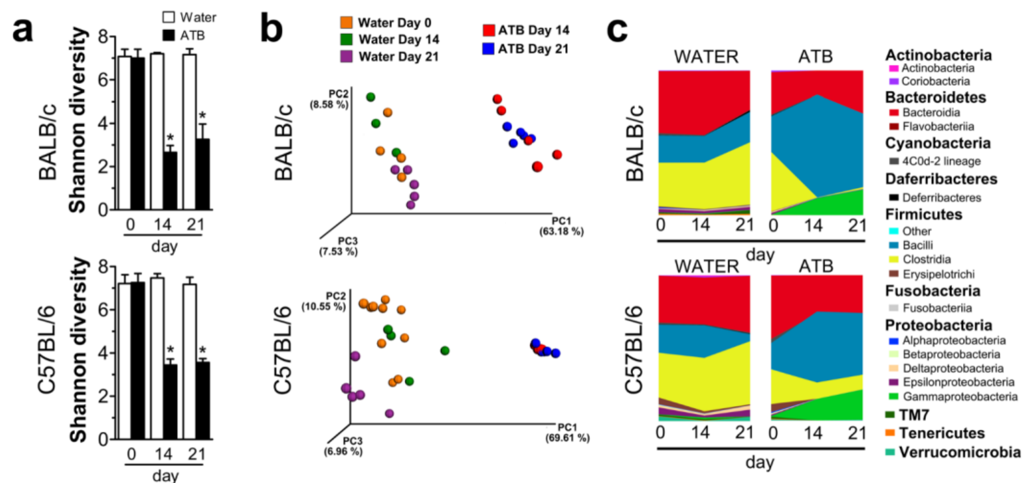

Fig 2. Antibiotic treatment changes the microbiota composition in both BALB/c and C57BL/6 mice. (a) Comparison of diversity in microbiota between the conventional (CV) and ATB-treated mice using Shannon diversity index. (b) Principal coordinates analysis (PCoA) plot using the unweighted UniFrac distance metric shows the compositional similarity before and after ATB treatment (Day 0, Day 14, Day 21). Each colored orb represents the microbiota composition in feces of one mouse. Each color represents each group of mice at the day 0, 14, 21. (c) The microbial composition in time is displayed as mountain plot before (Day 0) and after ATB treatment (Day 14), and after the induction of skin inflammation (Day 21) in comparison to CV mice. The figure shows (a) means ± SD or (c) means from pool of 5 mice. Statistical significance was determined by unpaired Student t test; *p < 0.05, **p < 0.01.

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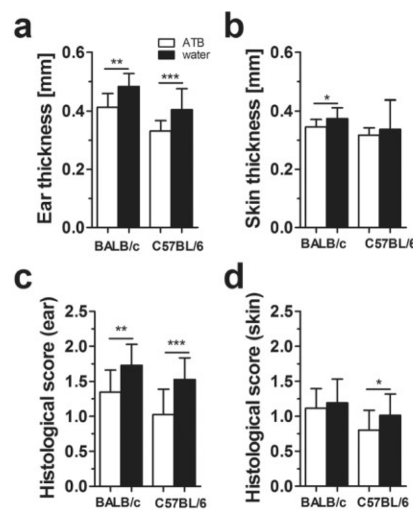

Fig 3. Antibiotic treatment decreases the skin inflammation in both BALB/c and C57BL/6 mice. Quantification of ear (a) and skin (b) thickness. Quantification of histopathological score (0–2) after H&E staining of the ear (c) a skin (d). The graphs show 18–19 mice per group (a pool of three independent experiments). Statistical significance was determined by unpaired Student t test; $*p < 0.05$, $**p < 0.01$.

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1g of stool there were $8.4 \pm 4.9 \times 10^{10}$, $6.2 \pm 3.7 \times 10^{10}$ and $7.1 \pm 1.2 \times 10^{10}$ (mean ± SD) copies of eubacterial 16S gene at day 0, 14 and 21, respectively. There were no significant changes in microbiota due to the IMQ treatment alone (Fig 2B and 2C). After ATB treatment, we found a substantial increase in the proportion of phylum *Firmicutes* which was caused by an increase in order *Lactobacillales* even though other members of the same phylum, such as *Clostridiales* and *Erysipelotrichiales*, were decreased (Fig 2C). On the other hand, members of orders *Coriobacteriales* and *Campylobacterales* (Fig 2C) were both significantly decreased after ATB treatment. We found that ATB had a similar effect on microbiota in both strains of mice, except for significant increase of *Enterobacteriales*, which was apparent in C57BL/6 but not in BALB/c mice. There were no significant changes in microbiota due to the IMQ treatment (Fig 2B and 2C).

We found that IMQ induced similar inflammatory changes in mouse skin, including edema (Fig 3A and 3B), erythema, scaling (data not shown) and histological changes (Fig 3C and 3D) in all treated groups but the degree of these changes was significantly lower in both strains of ATB-treated animals as compared to their respective controls.

Microbiota drives the skin inflammation by inducing stronger T cells response

Microbiota has a major impact on T-cell development and both psoriasis and its murine model result in significant local accumulation of $\gamma\delta TCR^+$ and Th17 cells [12, 24, 25]. Therefore, we evaluated how microbiota influences the frequency of $\gamma\delta TCR^+$ T cells and Th17 cells, both locally (in draining lymph nodes) and systemically (in spleen). We found that, in general, mice with reduced or absent microbiota have significantly lower frequencies of these T cells both

locally and systemically, although this effect is slightly less pronounced in ATB-treated mice as compared to GF mice (Fig 4). In both groups, there is even an indication of a lower number of IFN-γ^+ T cells in GF and ATB-treated mice, but these were only significant in spleen of GF and axial lymph nodes of ATB-treated mice (Fig 4B and 4C).

Another consequence of IMQ application is splenomegaly and activation of the IL17/IL-23 axis which leads in increased IL-17 and INF-γ production [18]. In our experiments, IMQ induced splenomegaly in CV mice which was less prominent in GF and ATB-treated mice (data not shown). To analyze dominant T cell response in lymphatic organs of IMQ-treated mice, we stimulated cells from their draining (axial) lymph nodes or spleens with anti-CD3 and anti-CD28 antibodies. We found that cells from CV mice produced more IL-17 than those from GF mice (Fig 5A). Moreover, IL-17 production in cells from axial lymph nodes was also higher in CV mice than in ATB-treated mice (Fig 5B). This is consistent with the high proportion of RORγt$^+$ and IL-17$^+$ cells we found in CV mice by flow cytometry (Fig 4C). We found a similar trend also for INF-γ production, but its production was always higher in spleen cells than in cells from axial lymph nodes (Fig 5A and 5B).

Discussion

Continuous interactions between microbiota and the immune system are important for the establishment and maintenance of host homeostasis. Thus change in microbiota composition could lead to a shift in immune system reactivity and ultimately to inflammatory diseases [26]. Here, we analyzed the role of microbiota in the early stages of psoriatic plaque formation using a murine model of psoriasis–IMQ-induced skin inflammation. We found that GF mice have significantly milder skin inflammation than CV mice. Unlike the ATB-treated animals, GF mice did not have any contact with live bacteria, because they were reared in germ-free condition for several generations. This is important, because even prenatal exposure to microbes permanently changes the immune system reactivity [27]. Although mice develop skin inflammation that closely resembles plaque-type psoriasis in humans, different murine strains may display specific disease characteristics [18, 28]. Therefore, we performed our experiments using two different mouse strains, each with distinct immune system reactivity. Nevertheless, the microbiota changes had a similar impact on the disease severity in both strains of mice. This suggests that microbiota influences mechanisms of skin inflammation that are not related to the genetic differences between these two strains.

There is emerging evidence supporting the existence of communication axes between organs, such as gut-skin axis [29, 30]. For example, atopic dermatitis or rosacea are both associated with marked changes in gut barrier and in intestinal microbiota [31, 32], suggesting that not only skin microbiota influences the disease pathogenesis. Altered gut microbiota (dysbiosis) is a hallmark of chronic gastrointestinal diseases, such as inflammatory bowel disease or celiac disease, which are often associated with skin inflammation [33, 34]. Interestingly, gut dysbiosis similar to this in inflammatory bowel disease was found in patients with psoriatic arthritis [35]. Moreover, gut dysbiosis is a common feature also in patients with rosacea, and treatment with oral nonabsorbed antibiotic markedly improves skin inflammation [36].

Since the GF animals lack microbiota both on skin and in gut throughout their life, we analyzed the microbiota-gut-skin axis by changing the gut microbial ecology in CV mice just before psoriasis induction using oral broad-spectrum ATB. We found that, similarly as in GF mice, ATB-treated animals have significantly milder skin inflammation than CV mice. These results are in agreement with a recently published study using even less complex antibiotic mixture of vancomycin and polymyxin B [15]. Therefore, this protective effect is not limited to the complete absence of microbiota during the early postnatal period and the skin

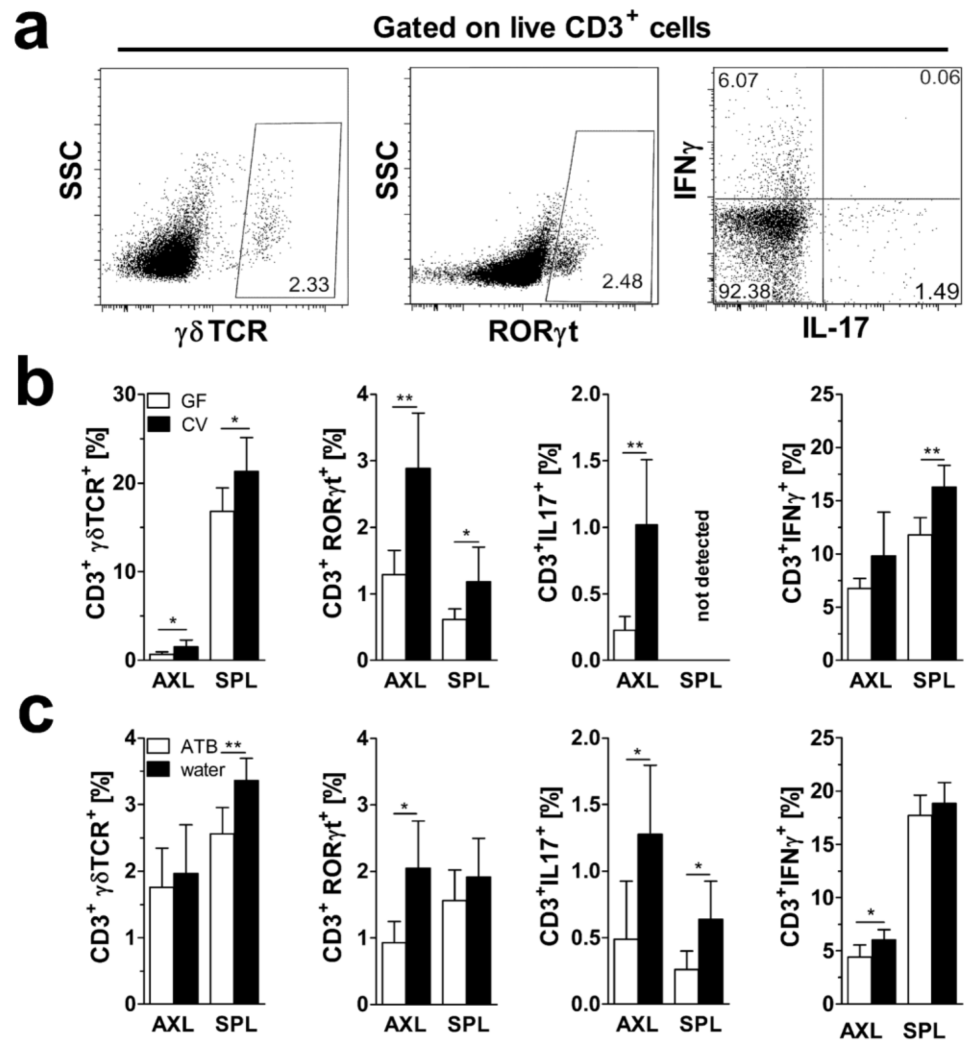

Fig 4. Absence of microbiota or ATB treatment decreases the percentage of γδ T cells and Th17 cells in spleen or axillary lymph nodes of IMQ-treated mice. Cells from spleen or axillary lymph nodes were isolated and either analyzed for surface γδTCR or intracellular RORγt or stimulated *in vitro* for 8 h by PMA and Ionomycin, the last 4h in the presence of Brefeldin A and Monensin, and analyzed for intracellular IL-17A and IFNγ production by flow cytometry. Cells were first gated for live cells and $CD3^+$ and subsequently on $γδTCR^+$, $RORγt^+$ or $IL\text{-}17^+$ and $INFγ^+$ cells as shown on the (a) example of gating strategy. These results from one representative experiment (n = 5–8 mice per group) out of three independent experiments with (b) GF versus CV mice or with (c) ATB-treated versus control mice are quantified in the graphs. Statistical significance was determined by unpaired Student t test; *$p < 0.05$, **$p < 0.01$.

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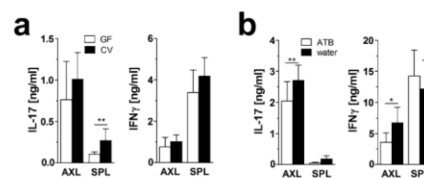

Fig 5. Absence of microbiota or ATB treatment reduces the production of pro-inflammatory cytokines by T cells from IMQ-treated mice. Cells isolated from spleen or axillary lymph nodes of (a) GF and CV mice or (b) ATB-treated and control mice were stimulated for 48hours *in vitro* by plate-bound anti-CD3 antibody and soluble anti-CD28 antibody. Cell culture supernatants were analyzed for IL-17A and INF-γ by ELISA. These data are representative of three independent experiments (n = 5–8 mice per group) with similar results. Statistical significance was determined by unpaired Student t test; $*p < 0.05$, $**p < 0.01$.

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inflammation severity could be modified by targeting gut microbiota in adult animals. Interestingly, even though the antibiotic regime used in our experiments was very harsh, it was not able to kill all gut bacteria and the resistant species quickly filled the vacated niche. This newly established ecosystem was, however, markedly different. We found that ATB treatment led to an increase in Firmicutes caused mainly by a massive increase of the *Lactobacilalles*, even though other members of this phylum, such as *Clostridialles* and *Erysipelotrichales*, were decreased. The potential protective role of lactobacilli in skin diseases is supported by findings of decreased intestinal lactobacilli in children with atopic dermatitis and by beneficial effect of their oral administration [37–39]. This immuno-modulatory effect of the gut lactobacilli can be mediated through their ability to suppress the IL-23/Th17 axis [40], which is intimately linked to the pathogenesis of psoriasis. The increase of these anti-inflammatory lactobacilli may then push this delicate balance back towards an anti-inflammatory phenotype.

To analyze the impact of gut microbiota on the IL-23/Th17 axis, we measured the Th17 and γδTCR-bearing lymphocytes in draining lymph nodes and in spleen of GF, ATB-treated and CV mice. We found that GF mice, and to a lesser extent also ATB-treated mice, had lower numbers of both $\gamma\delta TCR^+$ cells and Th17 cells as compared to CV mice. These data suggest that the absence of microbiota, or its change due to the ATB treatment, decreases the pro-inflammatory T cell response and thus decreases the severity of IMQ-induced skin inflammation. This is further supported by other studies describing the ability of commensal bacteria to modulate T cell development [12, 17]. The importance of this connection for psoriasis is still poorly understood, but a molecular link between microbe-dependent Th17 development and psoriasis was recently suggested [41, 42].

Taken together, our results suggest that host interactions with live microbes, possibly from the orders *Clostridiales* and *Erysipelotrichales*, are involved in the pathogenesis of IMQ-induced skin inflammation by influencing the Th17 cell reactivity. The positive effect of gut microbiota modulation by antibiotics on the severity of skin inflammation suggests the involvement of gut-skin axis and may represent the groundwork for novel approaches in psoriatic patient's management.

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Author Contributions

Conceived and designed the experiments: ZZ HTH M. Kverka. Performed the experiments: ZZ JM KK MH ZS. Analyzed the data: ZZ PR IN M. Kostovčík. Contributed reagents/materials/ analysis tools: KJ JH. Wrote the paper: ZZ HTH M. Kverka KK. Provided germ-free animals: RS TH.

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

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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. We noticed slight decrease in disease severity or frequencies of Th17 cells also in mice treated with COL, VAN or STR, however, these changes were not significant. 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, 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.

Treatment with STR was the most efficient in terms of decreasing the mRNA levels of key pro-inflammatory factors such as *Il17f*, *Il23a*, and *Cxcl1* in the inflamed skin, as well as *Nfkbiz*. We saw a trend towards decreasing the mRNA levels of *Il17f*, *Il23a*, and *Cxcl1* in MET-treated mice as well, but this effect was not significant. Furthermore, MIX group displayed the mildest skin inflammation, the lowest Th17 cell frequencies in inguinal lymph nodes and also decreased mRNA levels of *Il17f* and *Il23a* in the inflamed skin.

VAN was the most potent antibiotic which markedly changed the skin microbiota diversity in terms of abundance and evenness of the species present, as well as the microbiota profile in the intestine. The effect of other antibiotics on cutaneous microbiota was insignificant, with the application of IMQ having the greater impact especially in the control group of mice. Regarding the intestinal microbiota composition, greater diversity differences from the control group were only observed 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.

In mice treated with VAN, STR and MIX we did not detect any SFB bacteria compared to controls; on the other hand in MET-treated group there was a significant amount of SFB bacteria during the all experiment. Monocolonization with SFB bacteria did not change the skin clinical signs of IISI when compared to GF mice, however, the proportion of Th17 cell was higher in the spleen of SFB mice. In contrary to CV mice, monocolonization with SFB was not sufficient for development of IISI comparable to that of CV mice, suggesting that colonization with only one bacterial species may not be enough to revert/induce signs of IISI inflammation.

My contribution: sample collection and analyses, data analysis, interpretation of the results, manuscript writing

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

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Psoriatic patients have altered microbiota, both in the intestine and on the skin. It is not clear, however, whether this is a cause or consequence of the disease. In this study, using an experimental mouse model of psoriasis induced by imiquimod (IMQ), we show that oral treatment with a broad spectrum of antibiotics (MIX) or metronidazole (MET) alone mitigates the severity of skin inflammation through downregulation of Th17 immune response in conventional mice. Since some antibiotics, including MET, can influence immune system reactivity, we also evaluated the effect of MIX in the same model under germ-free (GF) conditions. GF mice treated with MET did not show milder signs of imiquimod-induced skin inflammation (IISI) which supports the conclusion that the therapeutic effect is mediated by changes in microbiota composition. Moreover, compared to controls, mice treated with MIX had a significantly higher abundance of the genus *Lactobacillus* in the intestine and on the skin. Mice treated with MET had a significantly higher abundance of the genera *Bifidobacterium* and *Enterococcus* both on the skin and in the intestine and of *Parabacteroides distasonis* in the intestine. Additionally, GF mice and mice monocolonized with either *Lactobacillus plantarum* or segmented filamentous bacteria (SFB) were more resistant to IISI than conventional mice. Interestingly, compared to GF mice, IMQ induced a higher degree of systemic Th17 activation in mice monocolonized with SFB but not with *L. plantarum*. The present findings provide evidence that intestinal and skin microbiota directly regulates IISI and emphasizes the importance of microbiota in the pathogenesis of psoriasis.

Keywords: psoriasis, antibiotics, microbiota, germ-free, animal model, imiquimod, intestine, skin

INTRODUCTION

Psoriasis is one of the most common immune-mediated inflammatory disorders of the skin that occurs in genetically predisposed individuals (Mak et al., 2009). It affects around 2–3% of the world population, and its incidence has been recently tightly linked to metabolic syndrome and other systemic inflammatory diseases (Armstrong et al., 2013; Boehncke, 2018). Psoriasis onset is triggered mainly by environmental factors, such as stress, bacterial infection, diet, and antibiotics (e.g., tetracycline) (Tsankov et al., 1988; Zeng et al., 2017). Recently, microbes and composition of microbiota have been prominently implicated in the etiopathogenesis of this disease (Yan et al., 2017). Several studies have demonstrated the differences in skin microbiota composition between healthy individuals and psoriatic patients (both in lesions and clinically unaffected skin), indicating that psoriasis can affect skin microbiome composition all over the human body (Tett et al., 2017; Yan et al., 2017). The importance of microbiota in psoriasis induction and pathogenesis is further highlighted by the observation that microbial infections are often associated with the development and/or aggravation of psoriasis (Naldi et al., 2001; McFadden et al., 2009). Disease exacerbation can be also associated with skin or mucosa streptococcal infection and colonization with *Staphylococcus aureus*, *Malassezia*, or *Candida albicans* (Noah, 1990; Waldman et al., 2001; Weisenseel et al., 2002).

Alteration of intestinal microbiota changes the systemic proinflammatory status of the host (Tlaskalova-Hogenova et al., 2011). Based on our studies, colonization of the gastrointestinal tract of germ-free (GF) animals with one bacterial strain or complex intestinal microbiota influences the host immune system at the local and systemic level, promoting proinflammatory or anti-inflammatory response, depending on the species used (Tlaskalova-Hogenova et al., 2011). The importance of the gut-skin axis in pathogenesis of psoriasis has been recently documented in humans as well as in animal models of psoriasis (Fry et al., 2013; Zanvit et al., 2015; Vlachos et al., 2016; Zakostelska et al., 2016; Drago et al., 2018). Recovery from intestinal dysbiosis, e.g., by healing the syndrome of small intestinal bacterial overgrowth, may mitigate the symptoms of psoriatic patients (Drago et al., 2018). Outbreaks of plague psoriasis may be connected to bacterial translocation into bloodstream which may result from increased intestinal permeability in psoriatic patients (Ramírez-Boscá et al., 2015). Moreover, changes in intestinal microbial diversity found in patients with IBD and obesity, particularly reduced abundance of *Akkermansia muciniphila*, have been recently observed also in patients with psoriasis (Tan et al., 2018).

To date, no study has investigated a potential causal relationship between changes in the gut and/or skin microbiota and psoriasis development and progression. However, numerous mice and human studies provide evidence for the influence of intestinal bacteria on skin condition (Salem et al., 2018). The important role of the skin-gut axis is highlighted by the findings that mice fed with the probiotic bacterium *Lactobacillus reuteri* developed thicker skin and denser and shinier fur and regained better reproductive fitness (Levkovich et al., 2013; Erdman and Poutahidis, 2014). Our previous research showed that broad spectrum antibiotic treatment (MIX) in conventional and GF mice leads to better resistance to imiquimod (IMQ)-induced skin inflammation (IISI) (Zakostelska et al., 2016). This effect goes hand in hand with downregulation of Th17 response. Moreover, the ATB treatment dramatically changed the diversity of intestinal bacteria, with an increase in Lactobacillales and a significant decrease in Coriobacteriales and Clostridiales (Zakostelska et al., 2016). Similarly, Zanvit et al. (2015) reported that antibiotic treatment in adult but not newborn mice resulted in amelioration of IISI. Moreover, the disease in neonatally ATB-treated mice was less severe when they were co-housed with untreated controls before the IISI induction, suggesting a protective role of unperturbed microbiota (Zanvit et al., 2015).

In the present study, we aim to investigate whether the individual constituents of antibiotic mixture used in our previous work have the potential to mitigate IISI on their own and to examine the resulting changes in microbiota composition and in the immune response both on the skin and in the intestine. Furthermore, we monocolonized mice with a well-known probiotic species *Lactobacillus plantarum* WCFS1 (LP) or with segmented filamentous bacteria (SFB) and compared them with conventional and GF mice to explore how a microbial diversity impact the severity of IISI.

MATERIALS AND METHODS

Mice

We used female BALB/c or C57BL/6 mice (7–10 weeks old) reared either in conventional or GF conditions at the Institute of Microbiology of the CAS. Mice were fed with Altromin 1,414 diet (Altromin, Lage, Germany; irradiated with 59 kGy for 30 min) and provided sterile water *ad libitum*. The GF mice were reared in sterile Trexler-type plastic isolators for several generations before being used in the experiments. Fecal samples were evaluated weekly by standard microbiological techniques to detect any contamination by bacteria, viruses, molds, and yeasts (Hrncir et al., 2008). The animals were kept in a room with a 12 h light-dark cycle at 22 °C (Kozakova et al., 2016). All experiments were approved by the Animal Care and Use Committee at the Institute of Microbiology, CAS, approval IDs: 34/2017 and 39/2015.

Monoassociation of Germ-Free Mice

We cultured *L. plantarum* WCFS1 in MRS broth (Oxoid, Basingstoke, UK) overnight. Then, we centrifuged the culture and washed it in sterile phosphate-buffered saline (PBS). We adjusted the concentration to 10^9 CFU/ml. After weaning, the BALB/c GF mice were colonized intragastrically by 2×10^8 CFU/0.2 ml of lactobacilli suspension. The colonization level of the animals was checked regularly by culturing their feces: appropriate serial dilutions were plated on MRS agar plates and colonies were counted after incubation at 37 °C for 48 h. Colonization remained stable throughout the whole experiment and reached levels of $2–3 \times 10^9$ CFU/g feces. The

littermates (second or third generation) of monocolonized mice were used for the experiments (Schwarzer et al., 2019). Monocolonization with SFB was described previously (Stepankova et al., 2007). Briefly, after weaning, C57BL/6 GF mice were colonized intragastrically with viable SFB (10^7–10^8 per dose) obtained from the stool of mice monoassociated with SFB. To check for the presence of SFB in the colon and cecum, we used an *in situ* hybridization probe SFB 1008-FITC (sequences 5′-GCGAGCTTCCCTCATTACAAGG-3′) (Snel et al., 1995).

Mouse Model of Psoriasis

To induce skin inflammation, the mice were treated daily for up to six to seven consecutive days with 62.5 mg of IMQ cream (Aldara, 3M Health Care Limited, Great Britain), applied on the shaved back skin and left ear. The severity of erythema and scaling was monitored daily, using a scale based on the clinical Psoriasis Area and Severity Index (PASI). Ear swelling and skin thickening were measured at the end of the experiment, as previously described (van der Fits et al., 2009). Histopathological examinations were performed in 4 μm sections stained with hematoxylin/eosin by an experienced pathologist (PR), who was blinded to the treatment status of the mice. The scoring system describing the degree of imiquimod-induced skin inflammation (IISI) on a scale of 0–2 has been previously developed in our laboratory (Zakostelska et al., 2016).

Antibiotic Treatment

Mice were treated daily with antimicrobials, starting 2 weeks prior to psoriasis induction until the end of the experiment (see experimental design in **Figure 1A**) (Zakostelska et al., 2016). Mice in each of the five experimental groups were given 300 μl of either metronidazole (5 mg/ml; B. Braun, Melsungen AG, Germany), vancomycin (5 mg/ml), colistin (1.66 mg/ml, Sigma-Aldrich), streptomycin (50 mg/ml, Sigma-Aldrich), or a mix of these antimicrobials by oral gavage. Administration by gavage was chosen in order to prevent severe dehydration and weights loss caused by unwillingness to drink water containing antimicrobials and to prevent the mice from bathing in the antibiotics. This treatment was well tolerated by all mice (Reikvam et al., 2011).

Microbiota Analysis

Changes in microbiota composition were analyzed in swabs from psoriatic-like skin lesions and in stool samples of experimental mice. Briefly, samples were collected from all mice at the beginning of the experiment (Day 0), after 14 days of ATB treatment before the IMQ application (Day 14), and after 7 days of IMQ application (Day 21). We isolated DNA from both swabs (PowerBiofilm DNA Isolation Kit, MoBio) and stool samples using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA). Next, we amplified the V3-V4 region of 16S rRNA gene using degenerate primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), which were barcoded to enable multiplexing of sequencing libraries. Subsequently, we processed them by PCR amplification, plate purification of amplicons, and ligating of adapters as previously described (Zakostelska et al., 2016). We quantified the amplicon library using the KAPA Library Quantification Kit (Illumina) and sequenced on MiSeq platform using 2 × 300 bp kit at the CEITEC Genomics Core Facility (Brno, CZ). 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 number SRP156846. For microbiota analysis, Shannon index and weighted and unweighted UniFrac distances expressed in the form of PCoA plots were used to describe alpha and beta diversity. To determine the discriminative features for both taxonomic profiles of communities, the LEfSe analysis tool was employed (Caporaso et al., 2010).

Flow Cytometry Analysis of the Induced Immune Response

Single cell suspensions of the spleen and inguinal lymph nodes were prepared and blocked as previously described (Zakostelska et al., 2011). The cells were then stained extracellularly with FITC-conjugated anti-CD3 (clone 145-2C11, dilution 1:100), and dead cells were excluded using the fixable viability dye eFluor 780 (dilution 1:200). Subsequently, the cells were fixed, permeabilized, and stained intracellularly with PE-conjugated anti-RORγt (clone AFJKS-9, dilution 1:50). Flow cytometry analysis was performed using LSRII (BD Bioscience) and evaluated by FlowJo software v 9.6.2. (Tree Star, Inc., Ashland, OR).

Gene Expression in the Skin/RNA Isolation and Quantitative Real-Time PCR

Total RNA was isolated from approx. 50 mg of mouse skin tissue using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. RNA samples were treated with TURBO DNA-free Kit (Thermo Fisher Scientific), and 400 ng of total RNA was reverse transcribed using oligo(dT)20 primers and SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific). The resulting cDNA served as a template for qPCR analysis with the CFX96 Real-Time PCR detection system (BioRad) using the iQ SYBR Green Supermix (BioRad). Each PCR reaction was performed in duplicates in a volume of 25 μl containing 4 μl of a 1:10 dilution of each cDNA preparation, 12.5 μl of SYBR Green Supermix, and 0.2 μM of each primer. The amplification protocol was as follows: 3 min at 95 °C followed by 40 cycles at 94 °C for 30 s, 60 °C for 35 s, and 72 °C for 50 s. The temperature was then gradually increased to 95 °C to obtain melting curves of the amplified fragments, which confirmed the specificity and uniformity of the PCR products. Serial dilutions of cDNA (1:5) were used in qPCR for each primer pair to determine the efficiency of amplification. Changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ (Livak) method. Quantitative measurements were normalized using elongation factor 2 (*Eef2*) mRNA levels as the reference gene. Changes in mRNA levels were shown as the fold change of expression in monocolonized mice compared to that in conventional mice. Data were expressed as mean ± SEM of the values obtained in all experiments.

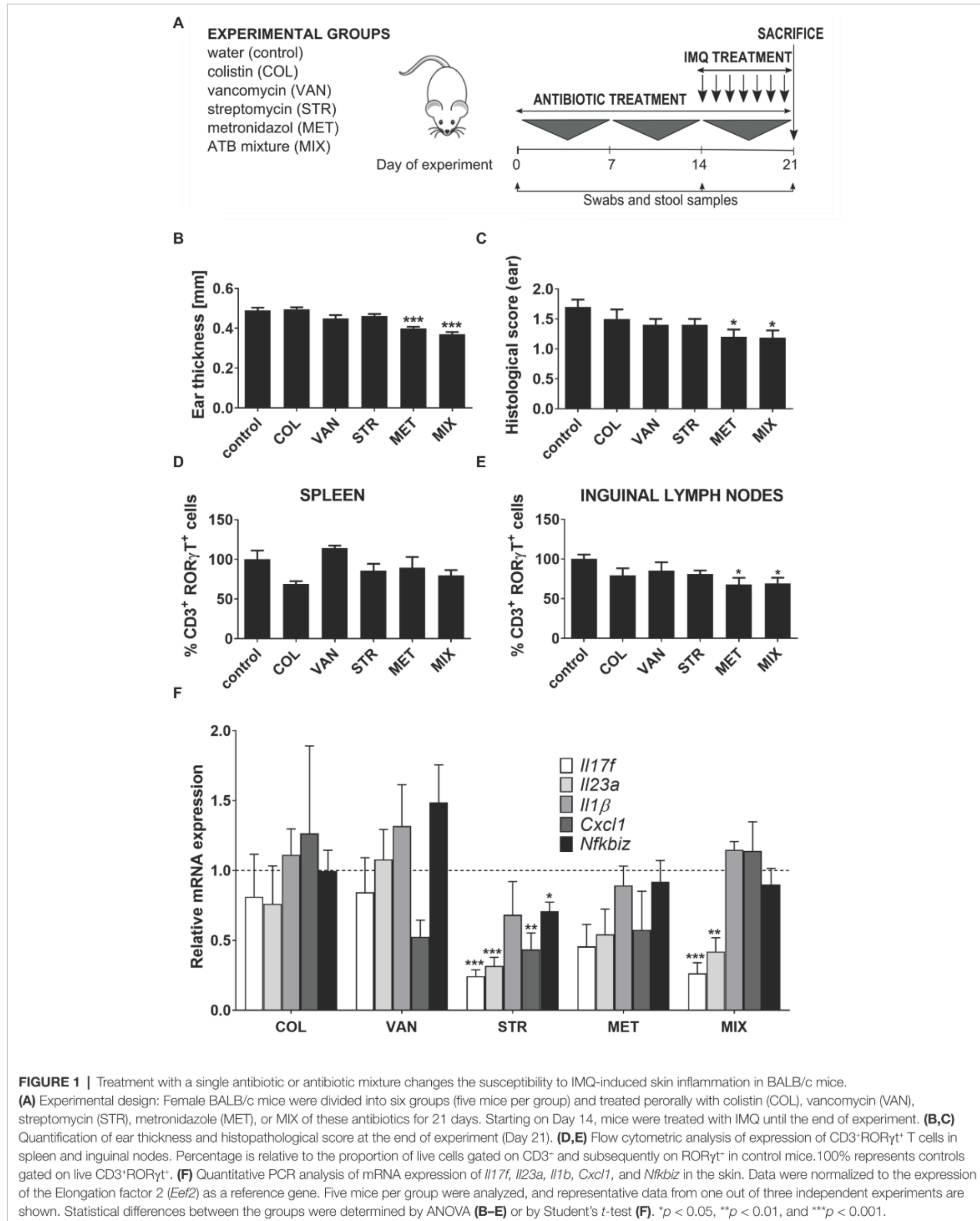

FIGURE 1 | Treatment with a single antibiotic or antibiotic mixture changes the susceptibility to IMQ-induced skin inflammation in BALB/c mice. **(A)** Experimental design: Female BALB/c mice were divided into six groups (five mice per group) and treated perorally with colistin (COL), vancomycin (VAN), streptomycin (STR), metronidazole (MET), or MIX of these antibiotics for 21 days. Starting on Day 14, mice were treated with IMQ until the end of experiment. **(B,C)** Quantification of ear thickness and histopathological score at the end of experiment (Day 21). **(D,E)** Flow cytometric analysis of expression of $CD3^{+}ROR\gamma t^{+}$ T cells in spleen and inguinal nodes. Percentage is relative to the proportion of live cells gated on $CD3^{-}$ and subsequently on $ROR\gamma t^{+}$ in control mice.100% represents controls gated on live $CD3^{+}ROR\gamma t^{+}$. **(F)** Quantitative PCR analysis of mRNA expression of *Il17f, Il23a, Il1b, Cxcl1,* and *Nfkbiz* in the skin. Data were normalized to the expression of the Elongation factor 2 (*Eef2*) as a reference gene. Five mice per group were analyzed, and representative data from one out of three independent experiments are shown. Statistical differences between the groups were determined by ANOVA **(B–E)** or by Student's *t*-test **(F)**. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

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 are expressed as the mean ± standard deviation (SD) unless otherwise stated, and differences were considered statistically significant at $p \leq 0.05$. For analyses, we used the GraphPad Prism statistical software (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

MET Decreases the Sensitivity to IISI Similarly as MIX

We have previously shown that the severity of IISI can be decreased by oral treatment with a broad-spectrum antibiotics mixture (Zakostelska et al., 2016), and here, we investigate the contribution of each of its components to the overall effect. We treated the animals with placebo (saline), or either metronidazole (MET), vancomycin (VAN), colistin (COL), streptomycin (STR), or their mixture (MIX) for 21 days. During the last 6 days of this treatment, we induced skin inflammation by daily application of IMQ on their ear and shaved back skin (**Figure 1A**). Similarly to the MIX-treated mice, mice treated with MET had significantly milder skin inflammation compared to controls (**Figures 1B,C**). There was some, albeit non-significant, decrease in disease severity in STR- and VAN-treated mice as well, which suggests a synergic effect responsible for the slightly stronger and more robust effect of MIX. On the other hand, COL-treated mice had psoriasis-like symptoms of comparable severity as control mice, including parakeratosis, acanthosis with prominent gothic vaults, or focal microabscesses. On the contrary, mice treated with MIX or MET exhibited only low-degree skin thickening, mild hyperkeratosis, and acanthosis of epidermis, but without parakeratosis and accumulation of leukocytes in corium, as shown by histological examination (**Supplementary Figure 2**) and quantification summarized in **Figure 1C**. Since Th17 cells play a crucial role in this model, we analyzed the impact of these antibiotics on the proportion of $CD3^{+}ROR\gamma t^{+}$ cells in spleen and inguinal lymph nodes by flow cytometry (for gating strategy, see **Supplementary Figure 1**). While there were no significant changes in the systemic circulation (spleen), MET decreased the proportion of $CD3^{+}ROR\gamma t^{+}$ cells to a similar degree as MIX (**Figures 1D,E**). Again, neither COL nor VAN decreased the proportion of $CD3^{+}ROR\gamma t^{+}$ cells as compared to controls. To further investigate these data, we analyzed the expression of key proinflammatory factors (*Il17f, Il17a, Il23a, Il1b, Cxcl1, Rorγt,* and *Nfkbiz*) directly in the skin by RT-qPCR. We found that MIX and STR reduced the expression of *Il23a* and *Il17f*, while neither VAN nor COL had this effect (**Figure 1F**). There was a trend toward reduced expression of *Il23a* and *Il17f* or *Il17a* in MET-treated group (**Figure 1F** and **Supplementary Figure 3A**). Nevertheless, none of the treatments reduced the expression of transcription factor *Rorγt* (**Supplementary Figure 3B**). There may be some differences in the immunomodulatory effects of these antibiotics, since STR treatment seemed to lead to a generalized decrease in the expression of proinflammatory factors, which was significantly lower for all genes but not for *Il1b*. All these results together suggest that most of the effect of MIX on the IISI is conveyed by MET, and the presence of VAN, COL, and (to some extent) STR is largely inconsequential.

Microbiota Is Necessary for the Protective Effect of MET in IISI

Oral antibiotics may possess immunomodulatory properties when administered in high concentrations (Grove et al., 1977; Al-Banna et al., 2013). Therefore, we analyzed whether MET influences the IISI in a microbiota dependent or independent manner, by repeating the experiments under GF conditions. There were no differences in disease severity or Th17 proportions between MET-treated and control GF mice (**Figures 2A–D**). This suggests that it is the antimicrobial activity of MET that is responsible for its anti-inflammatory effect. Interestingly, there was only one statistically significant difference between these two groups, *Nfkbiz* expression in the skin is higher in MET-treated GF mice than in controls, suggesting that there is some minor, immunomodulatory microbiota-independent effect of the drug (**Figure 2E**).

Treatment With Some Oral Antibiotics Leads to Profound Changes in the Intestine, but Not in the Skin Microbiome

We analyzed microbiota composition on the skin and in the intestine before the start of the ATB treatment (Day 0), before the IMQ application (Day 14), and at the end of the experiment (Day 21). Skin swabs and fecal samples were analyzed by sequencing the V3-V4 regions of the 16S rRNA gene. The mice treated with VAN and MIX reported significant reduction of microbial richness and evenness in the intestine but not on the skin (**Figures 3A,B**). There was similar trend toward decreased microbial richness and evenness in the intestine in MET-treated group (**Figure 3B**). Microbial diversity on the skin was significantly changed only after IMQ treatment in control or VAN group at the end of the experiment compared to microbiota composition on Day 0 (**Figure 3A**). The compositional similarity revealed that the skin (**Figure 3C**) and intestinal (**Figure 3D**) microbiota profiles after MET or MIX treatment, regardless of the presence or absence of the disease (Day 14 or Day 21), were strikingly different ($p = 0.001$) from those of the other tested groups and from the microbiota composition at the beginning of experiment. Consistently with our previous results (Zakostelska et al., 2016), antibiotic treatment did not decrease the total amount of bacteria (**Supplementary Figure 4A**). This suggests that both microbial composition and microbial load play important roles in psoriasis improvement. Antibiotic treatment and IMQ application generally changed the composition of microbiota

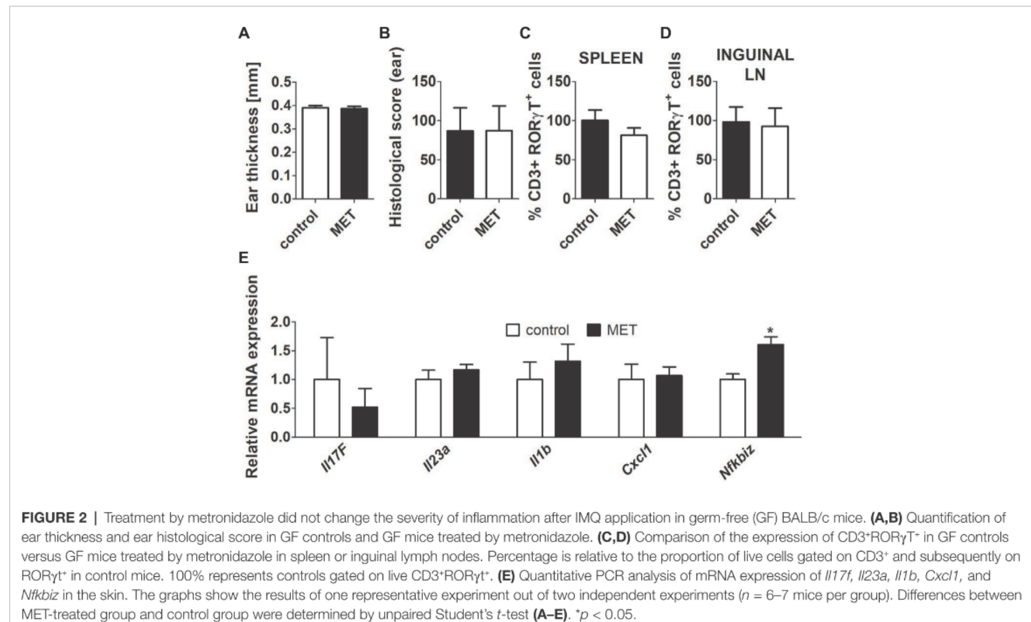

FIGURE 2 | Treatment by metronidazole did not change the severity of inflammation after IMQ application in germ-free (GF) BALB/c mice. **(A,B)** Quantification of ear thickness and ear histological score in GF controls and GF mice treated by metronidazole. **(C,D)** Comparison of the expression of CD3⁺RORγT⁺ in GF controls versus GF mice treated by metronidazole in spleen or inguinal lymph nodes. Percentage is relative to the proportion of live cells gated on CD3⁺ and subsequently on RORγt⁺ in control mice. 100% represents controls gated on live CD3⁺RORγt⁺. **(E)** Quantitative PCR analysis of mRNA expression of *Il17f, Il23a, Il1b, Cxcl1,* and *Nfkbiz* in the skin. The graphs show the results of one representative experiment out of two independent experiments (n = 6–7 mice per group). Differences between MET-treated group and control group were determined by unpaired Student's t-test **(A–E)**. $^{*}p < 0.05$.

in the intestine and on the skin (**Figures 3E,F**). MIX reduced the diversity of microbiota composition, and the vacant niche in the intestine was filled by Firmicutes, especially Lactobacillales, even though Clostridiales and Bacillales declined. Additionally, treatment with MIX led to reduction of Coriobacteriales in the intestine, in line with our previous results (Zakostelska et al., 2016). Similarly to the MIX-treated group, mice in the MET-treated group showed lower abundance of the family Ruminococcaceae, Clostridiales, and genus *Oscillospira* and *Dorea* in the intestine compared to the control group (**Figures 3E,F**). LefSe analysis revealed several discriminative features between MIX, MET, and control groups. For selected representatives, see **Table 1**; for detailed analysis, see **Supplementary Tables 1** and **2**. MET, but not MIX, treatment significantly increased the presence of *Parabacteroides distasonis* in the intestine. Moreover, MET significantly increased the abundance of the genera *Bifidobacterium* and *Enterococcus* compared to MIX or control groups, both in the intestine and on the skin (**Table 1**). Mice in the MIX group had a significantly higher abundance of genus *Lactobacillus* in the intestine and on the skin compared to controls (**Table 1**). Since significant reduction of *A. muciniphila* has been described in the intestine of patients with psoriasis (Tan et al., 2018), we analyzed the relative abundance of this species in our samples. We found that IMQ treatment itself did not significantly change the abundance of *A. muciniphila,* but treatment with VAN or MIX led to its eradication (**Supplementary Figure 5**). These results suggest that specific bacterial composition affects the severity of IMQ-induced inflammation.

Broad Spectrum of Bacterial Antigens and Fully Matured Immune System Is Required for IISI Development

We found that genus *Lactobacillus* is significantly increased in the intestine following the protective MIX treatment (**Supplementary Figure 4B**). Since *L. plantarum* has been previously used in the treatment of inflammatory skin diseases, here we further investigated whether monocolonization with *L. plantarum* WCFS1 (LP) itself changes the course of IISI compared to GF and conventional mice (Jang et al., 2014; Mariman et al., 2016). We found that monocolonization with LP led to a similar degree of skin inflammation as in GF mice in all the tested parameters. All these parameters were also significantly lower than in conventional mice (**Figures 4B,D,F,H**) or there was a trend toward reduced relative mRNA expression of *Il17f, Il23a, Il1b, Cxcl1,* and *Nfkbiz* (**Figure 4J**). Additionally, inflammation or VAN, STR, and MIX decreased the abundance of SFB in the intestine of conventional mice (**Supplementary Figure 4C**). Since MIX showed the greatest efficiency in mitigating the inflammation and SFB are known for their Th17 inducing capacity, we monocolonized mice with SFB to investigate their role in IISI. SFB and GF mice did not differ in clinical signs on the skin, except the CD3⁺RORγT⁺ cell proportion, which was higher in the spleen of SFB mice (**Figures 4A,C,E,G,I**).

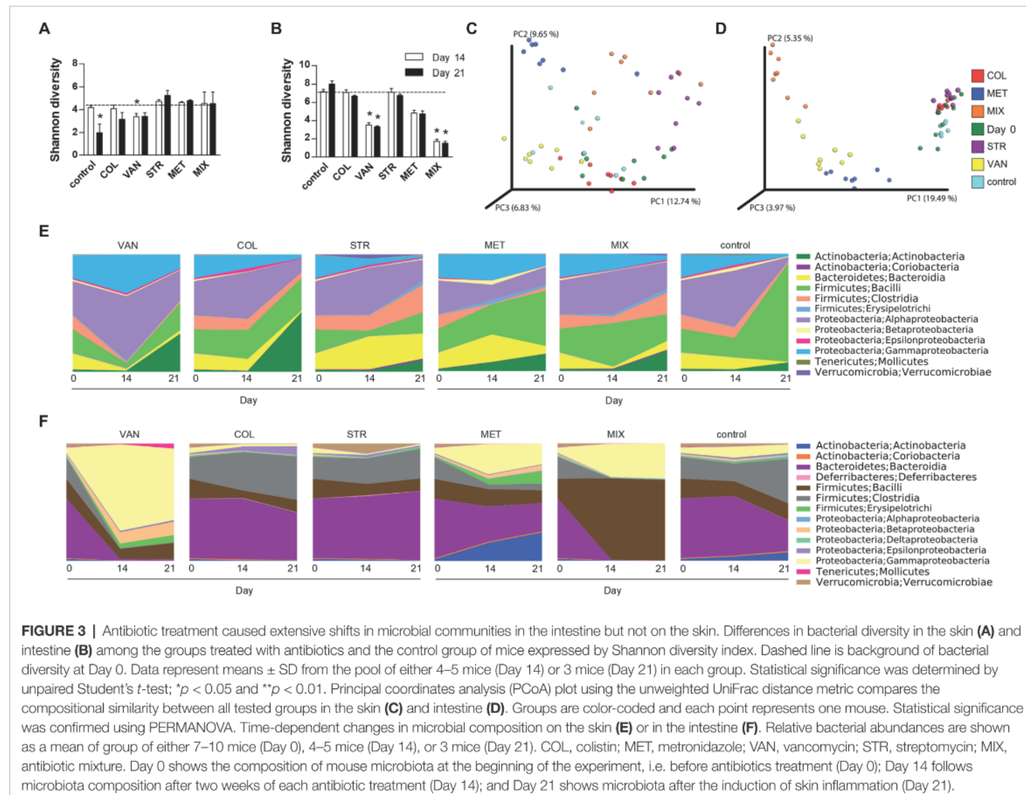

FIGURE 3 | Antibiotic treatment caused extensive shifts in microbial communities in the intestine but not on the skin. Differences in bacterial diversity in the skin **(A)** and intestine **(B)** among the groups treated with antibiotics and the control group of mice expressed by Shannon diversity index. Dashed line is background of bacterial diversity at Day 0. Data represent means ± SD from the pool of either 4–5 mice (Day 14) or 3 mice (Day 21) in each group. Statistical significance was determined by unpaired Student's *t*-test; **p* < 0.05 and ***p* < 0.01. Principal coordinates analysis (PCoA) plot using the unweighted UniFrac distance metric compares the compositional similarity between all tested groups in the skin **(C)** and intestine **(D)**. Groups are color-coded and each point represents one mouse. Statistical significance was confirmed using PERMANOVA. Time-dependent changes in microbial composition on the skin **(E)** or in the intestine **(F)**. Relative bacterial abundances are shown as a mean of group of either 7–10 mice (Day 0), 4–5 mice (Day 14), or 3 mice (Day 21). COL, colistin; MET, metronidazole; VAN, vancomycin; STR, streptomycin; MIX, antibiotic mixture. Day 0 shows the composition of mouse microbiota at the beginning of the experiment, i.e. before antibiotics treatment (Day 0); Day 14 follows microbiota composition after two weeks of each antibiotic treatment (Day 14); and Day 21 shows microbiota after the induction of skin inflammation (Day 21).

TABLE 1 | Selective distinctive features of changes in skin or intestinal microbiota composition distinguished by LEfSe (g_genus, s_species).

Distinctive composition of mouse microbiota using LEfSe				
	Day	Control	MIX	MET
Skin	14	–	*g_Lactobacillus*	*g_Enterococcus* *g_Bifidobacterium*
	21	–	*g_Lactobacillus*	*g_Enterococcus* *g_Bifidobacterium*
Feces	14	–	*g_Lactobacillus*	*g_Bifidobacterium* *g_Enterococcus* *s_Parabacteroides distasonis*
	21	–	*g_Lactobacillus*	*g_Bifidobacterium* *s_Parabacteroides distasonis* *g_Enterococcus*

As compared to conventional mice, SFB-monocolonized mice had milder inflammation in all tested parametres or there was a trend toward decreased expression of CD3$^+$RORγT$^+$ in inguinal lymph nodes (**Figure 4G**) or reduced relative mRNA expression of *Il17f, Il23a, Il1b, Cxcl1,* and *Nfkbiz* (**Figure 4I**). These results indicate that monocolonization with a single bacterial species or with a small group of specific bacteria, even though they possess some proinflammatory potential, may not be enough to induce full signs of IISI as observed in conventional mice.

DISCUSSION

Microbiota fundamentally influences the immune system development, and its perturbation, i.e., dysbiosis, is associated with many inflammatory diseases (Tlaskalova-Hogenova et al., 2011). While it is well established that the skin microbiota is involved in the pathogenesis of psoriasis (Fry et al., 2013), much less is known about the effect of intestinal microbiota on skin inflammation. By comparing germ-free and conventional mice, we have previously shown that the presence of intestinal microbiota promotes the IISI in mice by enhancing Th17 response and that by oral administration of broad-spectrum antibiotic mixture, a similar effect as in germ-free mice can be achieved (Zakostelska et al., 2016). Here, we analyzed which antibiotic drives this beneficial effect, which microbes are

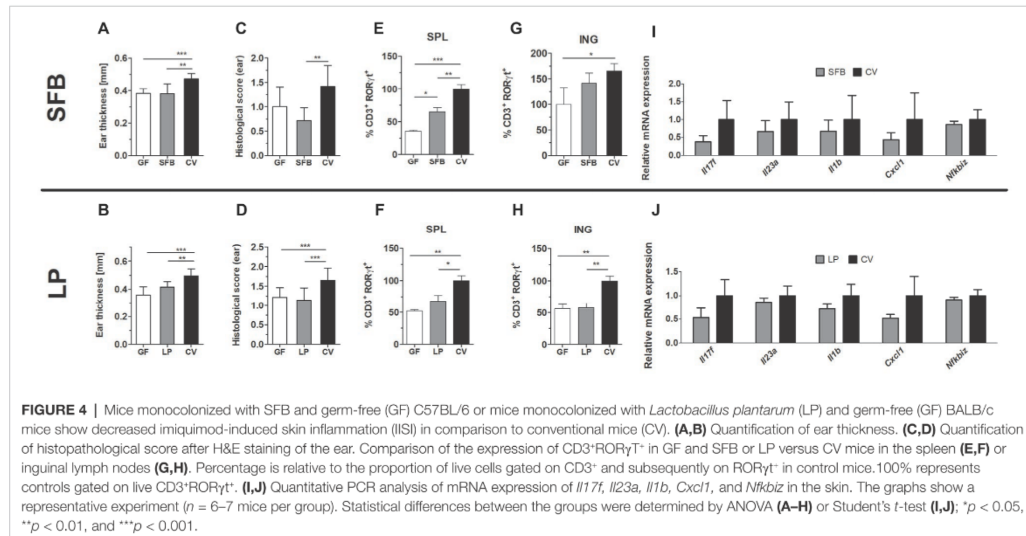

FIGURE 4 | Mice monocolonized with SFB and germ-free (GF) C57BL/6 or mice monocolonized with *Lactobacillus plantarum* (LP) and germ-free (GF) BALB/c mice show decreased imiquimod-induced skin inflammation (IISI) in comparison to conventional mice (CV). **(A,B)** Quantification of ear thickness. **(C,D)** Quantification of histopathological score after H&E staining of the ear. Comparison of the expression of $CD3^+ROR\gamma T^+$ in GF and SFB or LP versus CV mice in the spleen **(E,F)** or inguinal lymph nodes **(G,H)**. Percentage is relative to the proportion of live cells gated on $CD3^+$ and subsequently on $ROR\gamma t^+$ in control mice.100% represents controls gated on live $CD3^+ROR\gamma t^+$. **(I,J)** Quantitative PCR analysis of mRNA expression of *Il17f, Il23a, Il1b, Cxcl1,* and *Nfkbiz* in the skin. The graphs show a representative experiment (n = 6–7 mice per group). Statistical differences between the groups were determined by ANOVA **(A–H)** or Student's t-test **(I,J)**; $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

affected by this treatment, and whether the effect is microbiota-dependent or independent.

First, we found that MET has similar beneficial effect on IISI as the whole mixture of antibiotics. Since no other antibiotic contained in the MIX was able to reduce the severity of IISI on its own, we conclude that most of the protective effect of the MIX is due to MET. Both successful treatments – oral MET and MIX – decreased the proportion of Th17 cells ($CD3^+RoR\gamma T^+$) in the draining lymph nodes. Moreover, treatment with STR, MET, or MIX changed the expression of proinflammatory cytokines in the inflamed skin. Similarly to MIX, STR decreased cutaneous expression of *Il17f* and *Il23a*, but, unlike MIX or MET, it failed to improve IISI severity. MET slightly decreased *Il17f* and *Il23a* expression as well, but this effect was not statistically significant. This suggest that cutaneous Th17 response is not the only factor behind the beneficial effect of antibiotics.

Although microbes markedly change the reactivity of the immune system, MET may affect inflammation by its anti-inflammatory action (Al-Banna et al., 2013; Becker et al., 2016). MET is used as a supplemental treatment to patients with Crohn's disease, where it decreases the severity of early disease recurrence after ileum resection and improves healing of perianal fistulae (Rutgeerts et al., 1995; Dejaco et al., 2003). Nevertheless, MET is not efficient as a Crohn's disease monotherapy (i.e., without concurrent anti-inflammatory treatment), and even if combined with ciprofloxacin, its effect is only limited to a less common colonic Crohn's disease (Steinhart et al., 2002). The well-established involvement of gut microbiota in Crohn's disease pathogenesis and efficiency of oral MET in the treatment of small bowel bacterial overgrowth (common complication of Crohn's disease) suggest that MET's anticolitic effect is driven by its antimicrobial and not anti-inflammatory action (Castiglione et al., 2003). The effect of oral antibiotics on the inflammation outside gut may be less obvious, because mechanisms such as T cell polarization may be dependent on microbiota (Furusawa et al., 2013; Smith et al., 2013; Viaud et al., 2013; Scott et al., 2018), other mechanisms may not. In our previous studies, we found that oral treatment of mice with mixture of antibiotics, including MET, decreases severity of skin or retinal inflammation similarly as GF state (Heissigerova et al., 2016; Zakostelska et al., 2016). In humans, oral MET was successfully used as treatment for idiopathic lichen planus, even without clear parasitic infection (Wahba-Yahav, 1995; Buyuk and Kavala, 2000; Rasi et al., 2010). These results indicate that MET may have immunomodulatory effect in skin inflammatory disease beyond its antiparasitic effect, but none of these studies controlled for its ability to kill bacteria, mainly anaerobes. While skin and respiratory infections may play a role in triggering psoriatic disease, exposure to MET is not independently associated with disease risk (Horton et al., 2016). It is still unclear, if MET could influence the IISI directly, by immunomodulation or indirectly, by antibiotic effect. Therefore, we induced IISI in mice treated with MET under GF conditions and compared its severity with mice treated with placebo. Although the IISI is significantly less severe in GF than in conventional mice, oral MET did not have any effect on the severity of the inflammation in any of the studied parameters, suggesting that the anti-inflammatory effect of MET is microbiota dependent.

Next, we analyzed the changes in both intestinal and skin microbiome induced by antibiotics or IISI to identify the key microbes involved. Oral antibiotics induced significant changes in gut microbiota, but only minor changes in skin

microbiota, which decreased in diversity only with VAN treatment. Mice may actively transfer the oral antibiotics directly on their skin by bathing in the drinking water or by licking their backs (O'Neill et al., 2016). We administered the antibiotics by gavage, thereby avoiding this issue. The absence of similar decrease in other groups implies that this decrease in microbial diversity is caused by a different mechanism. There is a clear decrease in skin microbial diversity during the IISI development in the control group, which is probably the consequence of severe skin inflammation in these mice. Similar tendency was found in patients with inflammatory skin diseases such as psoriasis and atopic dermatitis (Kong et al., 2012; Alekseyenko et al., 2013). Similar to other studies in humans, we found that milder skin inflammation in mice is associated with higher abundance of Proteobacteria and lower abundance of Staphylococci and Streptococci in the MET- and MIX-treated groups (Gao et al., 2008; Alekseyenko et al., 2013). Reduction of *A. muciniphila* in the intestine has been recently found in patients with psoriasis (Tan et al., 2018). It indicates an interesting link between skin inflammation and intestinal microbiota, therefore we analyzed its changes during IISI induction. However, in contrast to the human studies, we did not find any significant changes in *A. muciniphila* during IISI induction, proposing differences between the mouse model of psoriasis and human disease.

In the intestine, both VAN and MIX significantly decreased microbial diversity and VAN-, MET-, and MIX-induced profound shifts in β-diversity. This manifested as marked overrepresentation of lactobacilli in the intestine of both MET- and MIX-treated animals on Day 14. This is in agreement with other studies analyzing intestinal and skin microbiota in mice treated with broad-spectrum antibiotic mixtures (Zanvit et al., 2015; Zakostelska et al., 2016). Interestingly, there are anti-inflammatory probiotics among lactobacilli, which can induce regulatory T cells even in the form of lysates (Zakostelska et al., 2011). Moreover, it is frequently shown that oral treatment with *L. plantarum* has a protective effect in mouse models of skin and gut inflammation (Jang et al., 2014; Kim et al., 2015; Mariman et al., 2016).

Based on these observations, we decided to monocolonize mice either with *L. plantarum* WCFS1 (LP) or with SFB. Both are well-characterized intestinal commensals, chosen for their ability to modulate the immune system. While the former is able to regulate inflammation by inducing high levels of regulatory cytokine IL-10, the latter shifts the T cell response towards Th17 (Ivanov et al., 2009; Gorska et al., 2014). Therefore, we expected that LP would decrease the severity of IISI by IL-10-dependent immune system regulation and that SFB would aggravate IISI by stimulating the Th17 cells. Although monocolonization with SFB led to a significant increase in RORγt$^+$ T cells in the mouse spleen, neither line of monocolonized mice differed from GF mice in IISI severity. The failure of SFB monocolonization to induce substantial aggravation of the IISI may be due to a similar mechanism as in experimental colitis, where SFB can worsen colitis only in the presence of other commensals (Stepankova et al., 2007). Taken together, these results indicate that microbial diversity is crucial for a full-fledged immune response in IISI.

CONCLUSION

In summary, we suggest that MET and MIX are sufficient to decrease the severity of IISI in a microbiota-dependent manner. While these beneficial changes are accompanied with downregulation of Th17 activity and an increase in abundance of intestinal lactobacilli in group treated with antibiotic MIX, monocolonization with neither lactobacillus nor Th17-promoting SFB is sufficient to change the IISI severity. These results emphasize the importance of gut-skin axis in the pathogenesis of inflammatory skin diseases. Our future studies will focus on causative effects of the changes in microbiota and more detailed study of antigen recognition and immune response to modulators released by bacteria mediating these changes. These data suggest a therapeutic potential *via* influencing the microbiota composition in psoriatic patients.

AUTHOR CONTRIBUTIONS

HT-H, JH, and ZZ conceived and designed the research. ZS, KK, PR, JD, SC, DS, PP, TH, HK, RS, FR, KJ, and ZZ performed the experiments. ZS, IN, MKo, MKv, JD, and ZZ analyzed and interpreted the data. ZS, KK, MKv, and ZZ wrote the manuscript. All authors revised and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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

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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 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. On the other hand, Planococcaceae were not detected by sequencing the V3V4 region of 16S rRNA.

Different sampling approaches such as swabs, scraping or biopsies provided similar microbial α-diversity, i.e. richness and evenness of the present taxa, as well as genera abundance. However, we found several discriminative features in bacterial and fungal distribution related to sampling approach both on the back and elbow skin. We also found that each sampling site (psoriatic, unaffected psoriatic, and healthy) is associated with presence of specific bacterial and fungal taxa, which is further dependent on the sampling approach.

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 while on the healthy elbow skin this correlation was positive On the other hand, we found a strong negative

correlation between *Malassezia* and species *Acinetobacter*, *Enhydrobacter* and *Pseudomonas* on the psoriatic back skin, however, these correlations were neither negative nor positive in healthy control skin.

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

My contribution: sample collection and analyses, data analysis, interpretation of the results, manuscript writing

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

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Psoriasis is a chronic inflammatory skin disease, whose pathogenesis involves dysregulated interplay among immune cells, keratinocytes and environmental triggers, including microbiota. Bacterial and fungal dysbiosis has been recently associated with several chronic immune-mediated diseases including psoriasis. In this comprehensive study, we investigated how different sampling sites and methods reflect the uncovered skin microbiota composition. After establishing the most suitable approach, we further examined correlations between bacteria and fungi on the psoriatic skin. We compared microbiota composition determined in the same sample by sequencing two distinct hypervariable regions of the 16S rRNA gene. We showed that using the V3V4 region led to higher species richness and evenness than using the V1V2 region. In particular, genera, such as *Staphylococcus* and *Micrococcus* were more abundant when using the V3V4 region, while *Planococcaceae*, on the other hand, were detected only by the V1V2 region. We performed a detailed analysis of skin microbiota composition of psoriatic lesions, unaffected psoriatic skin, and healthy control skin from the back and elbow. Only a few discriminative features were uncovered, mostly specific for the sampling site or method (swab, scraping, or biopsy). Swabs from psoriatic lesions on the back and the elbow were associated with increased abundance of *Brevibacterium* and *Kocuria palustris* and *Gordonia*, respectively. In the same samples from psoriatic lesions, we found a significantly higher abundance of the fungus *Malassezia restricta* on the back, while *Malassezia sympodialis* dominated the elbow mycobiota. In psoriatic elbow skin, we found significant correlation between occurrence of *Kocuria*, *Lactobacillus*, and *Streptococcus* with *Saccharomyces*, which was not observed in healthy skin. For the first time, we showed here a psoriasis-specific correlation between fungal and bacterial species, suggesting a link between competition for niche occupancy and

psoriasis. However, it still remains to be elucidated whether observed microbial shift and specific inter-kingdom relationship pattern are of primary etiological significance or secondary to the disease.

Keywords: psoriasis, microbiota, mycobiota, skin, sequencing

INTRODUCTION

The skin is our major interface with the outside environment. It harbors diverse site-specific microbial communities consisting of bacteria, fungi, and viruses (Grice and Segre, 2011). The skin microbiota protects against harmful microbes, maintains skin homeostasis and educates our immune system (Gallo and Nakatsuji, 2011; Belkaid and Naik, 2013; Grice, 2015). It has been shown that healthy microbiota can enhance the skin's protective barrier and strengthen the immune response of keratinocytes by inducing a higher expression of antimicrobial peptides and formation of biofilms (Wanke et al., 2011). On the other hand, microbial dysbiosis could cause or exacerbate skin diseases (Cogen et al., 2008; Gallo and Nakatsuji, 2011; Grice and Segre, 2011).

Psoriasis is a chronic inflammatory skin disease that involves a dysregulated interplay among immune cells, keratinocytes and environmental triggers, including microbiota (Nestle et al., 2009). Currently, psoriasis is perceived as a complex systemic immune mediated disease or syndrome, significantly associated with many chronic diseases including arthritis, heart disease, diabetes, metabolic syndrome, inflammatory bowel disease (IBD), or celiac disease (Sundarrajan and Arumugam, 2016; Singh et al., 2017). Pathogenesis of some of the aforementioned diseases, e.g., rheumatoid arthritis, obesity or IBD, has been also connected to microbial shifts (Berthelot and Le Goff, 2010; Befus et al., 2015; Chu et al., 2016). Indeed, changes in gut and skin microbiome have been recognized as important triggers for initiation or progression of psoriasis in humans as well as in animal models of psoriasis (Fry et al., 2013; Zanvit et al., 2015; Zakostelska et al., 2016; Drago et al., 2018). Moreover, changes in microbiota composition could be a one of the factors that leads to disruption of intestinal barrier function in psoriatic patients (Mattozzi et al., 2012; Yan et al., 2017).

Current studies describing the differences in microbiota composition in psoriatic patients suffer from inconsistent approaches, such as choosing different sampling techniques, different variable regions of the 16S rRNA gene and different skin sampling sites (Gao et al., 2008; Fahlen et al., 2012; Alekseyenko et al., 2013; Tett et al., 2017). Moreover, some of them are focused on microbiota composition, while others describe function and try to find connective features with disease state (Alekseyenko et al., 2013; Yan et al., 2017). The results differ in species richness (alpha diversity) and between sample diversity (beta) (Gao et al., 2008; Fahlen et al., 2012; Alekseyenko et al., 2013; Tett et al., 2017; Yan et al., 2017). Analyses of microbiota composition from swabs and biopsy samples revealed Firmicutes as the most dominant phylum in psoriatic lesions (Gao et al., 2008; Fahlen et al., 2012). In-depth analyses of microbiota composition in psoriatic patients compared to healthy controls revealed an increased abundance of the genus *Streptococcus* and an underrepresentation of the genus *Propionibacterium*, while presenting inconsistent findings on the abundance of *Staphylococcus* (Gao et al., 2008; Fahlen et al., 2012; Alekseyenko et al., 2013; Tett et al., 2017). To our knowledge, none of the published studies concerning the composition of mycobiome in patients with psoriasis used next generation sequencing and most of them focused mainly on *Malassezia* (Paulino et al., 2008; Jagielski et al., 2014; Takemoto et al., 2015). The most extensive study by Takemoto et al. (2015) described a higher fungal diversity and overall lower abundance of *Malassezia* in psoriatic lesions. Since no bacterial or fungal microbiota components have been robustly identified as being associated with psoriasis across the studies, new comparable standardized studies are necessary to extend the existing data on microbiome composition and function.

Here, we conduct a comprehensive study mapping the overall composition of bacterial and fungal communities in psoriatic lesions, unaffected psoriatic skin and healthy skin. We compare two typical sites of psoriasis incidence – the elbow and the back. Moreover, we compare three different sampling methods, namely swabs, scrapings and biopsies, since each of these techniques produces slightly different results of microbial composition.

Abbreviations: Bio, biopsies; He, healthy; Ps, psoriatic; Scr, scrapings; Swa, swabs; Un, unaffected.

MATERIALS AND METHODS

Patients and Sample Collection

Patients diagnosed with chronic plaque psoriasis were recruited at the Department of Dermatovenereology, Bulovka Hospital (Czechia). Altogether, 34 patients with chronic plaque psoriasis (6 females and 28 males) and 25 healthy controls (14 females and 13 males) were recruited. The average age ± standard deviation of patients was 45 ± 12 years and 44 ± 13.3 for healthy controls. Body Mass Index (BMI) was 20.6 ± 5.9, Psoriasis Area Severity Index (PASI) of psoriatic patients was 6 ± 7, and Physician Global Assessment (PGA) was 2 ± 1. Patients were clinically classified and their medical history was recorded. The majority of tested patients were under various treatment protocols depending on disease severity. However, seven psoriatic patients without prior treatment were included in our study. Majority of the study cohort, patients (85%) and controls (92%) alike, are residents of town municipalities. Characteristics and medical history of patients and healthy controls are summarized in **Supplementary Tables S1, S2**. The study was approved by The Ethical Committee of Bulovka Hospital with approval number 28.7.2014/7292/EK (Czech Republic) and all participants signed informed consent forms.

Sampling was performed by an accredited dermatologist using a protocol from the Human Microbiome Project Consortium [HMPC] (2012b) to minimize collection bias. Swabs, scrapings, and punch biopsy samples were taken either from the dorsal (back) or olecranon (elbow) skin areas. In psoriatic patients, both psoriatic and contralateral unaffected sites were sampled. Altogether 68 swabs, 68 scrapings, and 19 biopsies from psoriatic patients and 25 swabs, 23 scrapings, and 8 biopsies from healthy controls were analyzed (**Supplementary Table S3**). Briefly, swab samples were taken from a 2 × 2 cm area using flocked swabs (FLOQSwabs™ COPAN Diagnostics Inc., United States), soaked in sterile SCF-1 buffer [50 mM Tris buffer (pH 7.6), 1 mM EDTA (pH 8.0), 0.5% Tween 20] (Human Microbiome Project Consortium [HMPC], 2012b). Scraping samples were obtained from a 2 × 2 cm area using a scalpel similarly as previously described (Grice et al., 2008). Scraping samples were collected using a flocked swab soaked in sterile SCF-1 buffer. Samples were stored in 400 μl of SCF-1 buffer. Biopsies from psoriatic patients were taken at around 2 mm of size using a biopsy punch and stored dry (Fahlen et al., 2012). Biopsies from healthy controls were taken from the terminal end of elliptical specimens from patients undergoing wide excision of a birthmark. During biopsy sampling, the same local anesthetic (4% supracain) was used in both groups. No antiseptic treatment was used before sampling. All swabs, scrapings and biopsy samples were immediately frozen at −80°C.

DNA Extraction

Extraction of total DNA from swabs and scrapings was performed using DNeasy PowerBiofilm Kit (Qiagen, Germany) with minor changes in the protocol. Thawed swabs and scraping samples were thoroughly vortexed. Nipped off swabs were aseptically removed and samples were centrifuged for 10 min at 13,000 × *g*. The precipitate was diluted in 350 μl of the first kit buffer (MBL), and 100 μl of the second kit buffer (FB) was added to the sample, followed by homogenization at maximum speed (6.5 m/s) for 1 min (Fast Prep, MP Biomedicals, United States). Incubation of the bead tubes at 65°C for 5 min was excluded from the protocol as it resulted in a low DNA yield. The remaining steps were performed as per manufacturer's instructions. Total DNA from punch biopsies was extracted by MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, United States). Biopsies were homogenized at maximum speed (6.5 m/s) for 1 min in a solution of 300 μl Tissue and Cell Lysis Solution and 1 μl of Proteinase K (Fast Prep, MP Biomedicals, United States). Further isolation was performed following manufacturer's instructions.

PCR Amplification

To compare sequencing of different variable regions of the 16S rRNA gene (V1V2 and V3V4), PCR amplification of the V1V2 region was carried out with the 27F (5′-AATGATACGGCGACCACCGAGATCTACACGTACGTACGG TAGAGTTTGATCCTGGCTCAG-3′) and the 338R (5′-CAAGCAGAAGACGGCATACGAGATCGCTCACAGAATCC ACACTCATCATGCTGCCTCCCGTAGGAGT-3′) primers, already including Illumina adaptors and unique 12-nucleotide barcode on 338R (Hamady et al., 2008). The PCR reaction mixture consisted of 1X PrimeStar Max DNA polymerase (Takara Bio Co., Shiga, Japan), 0.3 μM primers and approximately 20 ng of DNA in 50 μl reaction (Koren et al., 2011). Cycle parameters were 33 cycles of denaturation (98°C, 10 s), annealing (55°C, 5 s) and extension (72°C, 5 s), and a final elongation at 72°C for 2 min. Microbial community profiling using the V3V4 region for bacteria and the ITS1 region for fungi was performed using 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) degenerate bacterial primers, and using ITS1-5.8Sfw (5′-AAGTTCAAAGAYTCGATGATTCAC-3′) and ITS1-5.8Srv (5′-AAGTTCAAAGAYTCGATGATTCAC-3′) degenerate fungal primers, which were all barcoded to enable multiplexing of sequencing libraries. Duplicates containing 25 μl of the reaction mixture were prepared for each sample. PCR amplification was carried out with 1X PrimeStar Max DNA polymerase (Takara Bio Co., Shiga, Japan), 0.4 μM primers and approximately 10 ng of template as the final concentrations. Thermal cycling parameters were 35 cycles of denaturation (94°C, 3 min), annealing (55°C, 5 s) and extension (72°C, 10 s), with a final extension at 72°C for 2 min. Replicate PCR products were pooled to minimize random PCR bias and the length of PCR products was determined using agarose gel electrophoresis. Extraction controls and no-template control were processed similarly.

Sequencing, Classification, and Data Analysis

V1V2 PCR products were purified using AMPure magnetic beads (Beckman Coulter, United States) and quantified with a Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Fisher Scientific, United States). Samples were equally pooled at a concentration of 30ng/μl, loaded on 2% E-Gel (Thermo Fisher Scientific, United States) and purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Germany). Purified products were sequenced on the Illumina MiSeq platform (Genomic Center, Faculty of Medicine, BIU, Israel). V3V4 amplicons were plate-purified using a SequalPrep™ Normalization Plate (96) Kit (Invitrogen). Equal amounts of the PCR product from each sample were pooled and MiSeq platform compatible adapters were ligated using KAPA HyperPlus Kit (Roche, United States). The library was quantified using KAPA Library Quantification Kit (Illumina, United States) and sequenced on the MiSeq platform using 2 × 300 bp kit at the CEITEC Genomics Core Facility (Brno, Czechia).

Sequencing data were processed using QIIME version 1.9.1 (Caporaso et al., 2010). The raw sequence data are available in the Sequence Read Archive (SRA) under the accession number SUB4321198.

To allow analysis of both datasets together, closed-reference operational taxonomic unit (OTU) picking was used due to non-overlapping regions employed (Navas-Molina et al., 2013; Rideout et al., 2014). Standard procedure involved quality filtering, chimera detection and removal and demultiplexing based on default criteria. Altogether, 522 individual amplicon

libraries were found consisting of a total of 17.26 million paired-end reads (reads per sample after all filtering steps, including chimera and quality filtering, averaged 22,092). Read clustering was performed using a 97% identity threshold. Taxonomic classification was performed based on the bacterial 16S database GREENGENES 13.8 (DeSantis et al., 2006).

In the fungal dataset, 212 individual amplicon libraries consisted of a total of 1,799,822 paired-end reads (reads per sample after all filtering steps, including chimera and quality filtering and ITS extraction, averaged 2,351). Read clustering was performed using a 97% identity threshold against the UNITE database version 7.2 (Koljalg et al., 2013) and identification was conducted using RDP classifier up to the species level as in the case of bacterial datasets with default confidence value (Wang et al., 2007).

Alpha and beta diversity were calculated based on rarefied datasets (220 sequences for bacteria, 200 sequences for fungi per sample) to deal with unequal sequencing output per sample. Normalization was done on the level that was sufficient based on the rarefaction curves that were approaching plateau with selected number of reads. Several alpha diversity indices were calculated, including Chao1, Shannon, Gini-Simpson and observed species. Statistical significance was confirmed using Kruskal–Wallis test with Dunn's multiple comparison test or Mann–Whitney test. 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. Statistical significance was confirmed using PERMANOVA. To identify the main differences in bacterial taxa between the V1V2 and V3V4 regions, we detected differential features using non-parametric factorial Kruskal–Wallis (KW) sum-rank test, compared between-group consistency using Wilcoxon rank-sum test and performed an LDA analysis to assess the effect size summarized using LEfSe (Linear discriminant analysis Effect Size) (Segata et al., 2011). Functional profiling of the recovered communities was done by PICRUST analysis (Langille et al., 2013). To determine the discriminative features for both taxonomic and metabolic profiles of communities, LEfSe analysis tool was employed (Segata et al., 2011). Predicted functional profiles were further analyzed with HUMAnN using KEGG orthology (Abubucker et al., 2012).

Bacteria–Fungi Correlation

Relative abundances of bacteria and fungi were correlated in both back and elbow skin samples. 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.

Enzyme-Linked Immunosorbent Assay

Serum samples were taken from psoriatic patients (n = 28) and healthy controls (n = 27) and stored at −20°C until analysis. Serum level of intestinal fatty acid binding protein (I-FABP) was determined by commercially available ELISA kit (HBT, Hycult Biotech, Netherlands). Serum levels of caspase-cleaved cytokeratin 18 fragment (ccCK 18) and total cytokeratin 18 (CK18) were determined by commercially available ELISA kits M30 and M65, respectively (Apoptosense, Peviva, Sweden), and apoptotic index was counted as their ration (M30/M65). The concentration of ccCK18 and total CK18 in serum was detected in U/L. All assays were performed according to the manufacturer's instructions.

RESULTS

The V3V4 Region Captures Wider Microbial Diversity Than the V1V2 Region

To point out differences in observed bacterial diversity caused by sequencing of different 16S rRNA variable regions, we sequenced the V1V2 and V3V4 regions in skin samples from psoriatic patients and healthy controls. Identical skin samples obtained from the back were sequenced for both regions and were included in the analysis comparing V1V2 to V3V4 marker regions. Observed and estimated richness was significantly higher when using the V3V4 region compared to the V1V2 region (**Figure 1A**). There were no significant differences in Shannon diversity index and when using the Gini-Simpson index, a significant increase in V1V2 region was observed (**Figure 1A**). PCoA of unweighted UniFrac distances revealed a significant difference in beta diversity between the V1V2 and V3V4 regions ($p \leq 0.001$) (**Figure 1B**). In addition, PCoA of weighted and unweighted UniFrac distances between the respective regions and type of sampling showed also significant changes (**Supplementary Figure S1**). Relative proportional differences in bacterial abundances between the V1V2 and V3V4 region are shown in **Figure 1C**. LEfSe analysis revealed the class Gammaproteobacteria, order Pseudomonadales, families Moraxellaceae and Staphylococcaceae, and genera *Staphylococcus* and *Micrococcus* as the most discriminatory for V3V4 region; for the V1V2 region it was the family Planococcaceae which was not detected at all using the V3V4 primer set (**Figures 1D,E**).

Various Sampling Approaches Result in Similar Bacterial Diversity but Different Genera Abundance When Analyzing the V3V4 Region

To evaluate the relative diversity associated with each sampling approach (swab, scraping, and biopsy) in affected and unaffected skin of psoriatic patients and in healthy control skin, we calculated richness and evenness using several diversity indices to account for their specific biases. In psoriatic lesions on the back and elbow, we found that swabs and scrapings result in similar alpha diversity profiles, with emphasis on the consistency of species abundance and diversity. In unaffected back and elbow skin, the observed microbial diversity was similar in swabs and scrapings, in contrast to a slightly higher diversity in back biopsies. In healthy back and elbow skin, we observed similar alpha diversity measures. Weighted and unweighted UniFrac analysis showed significant clustering of back samples in diseased

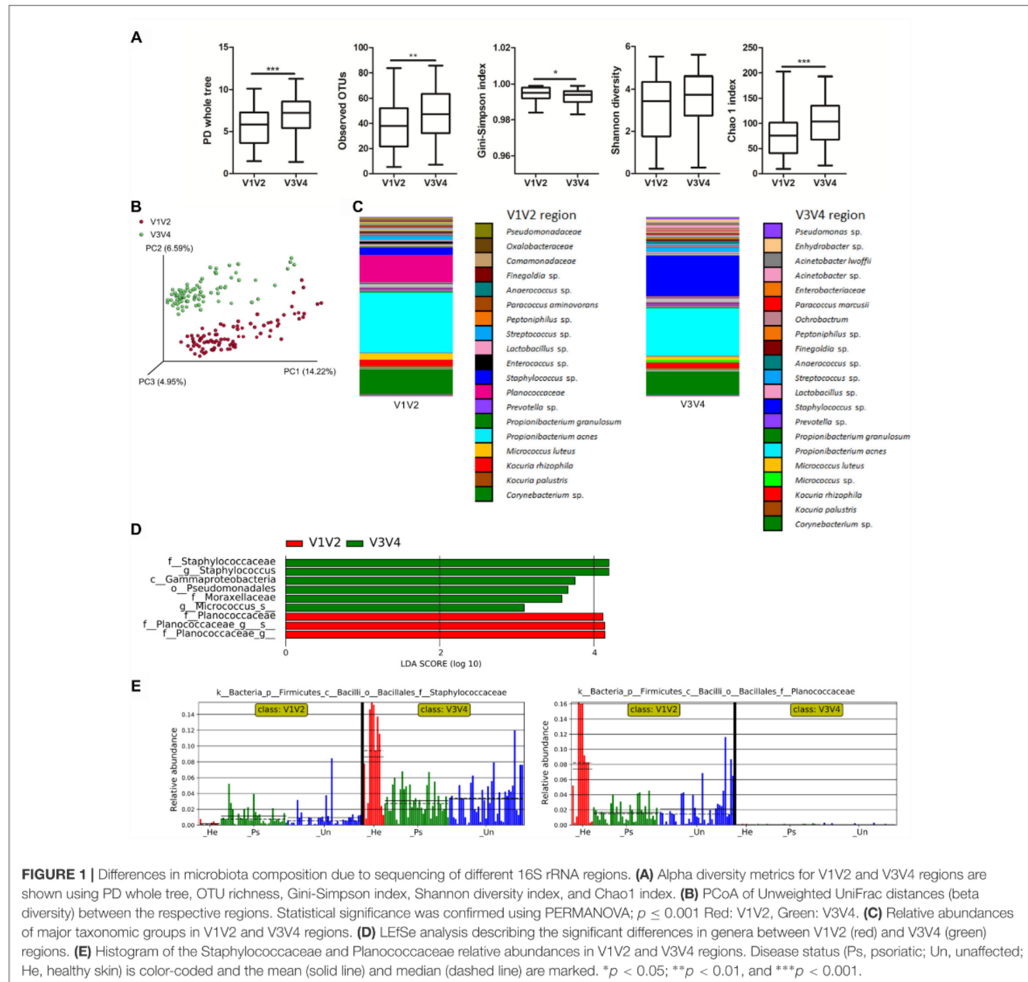

FIGURE 1 | Differences in microbiota composition due to sequencing of different 16S rRNA regions. **(A)** Alpha diversity metrics for V1V2 and V3V4 regions are shown using PD whole tree, OTU richness, Gini-Simpson index, Shannon diversity index, and Chao1 index. **(B)** PCoA of Unweighted UniFrac distances (beta diversity) between the respective regions. Statistical significance was confirmed using PERMANOVA; $p \leq 0.001$ Red: V1V2, Green: V3V4. **(C)** Relative abundances of major taxonomic groups in V1V2 and V3V4 regions. **(D)** LEfSe analysis describing the significant differences in genera between V1V2 (red) and V3V4 (green) regions. **(E)** Histogram of the Staphylococcaceae and Planococcaceae relative abundances in V1V2 and V3V4 regions. Disease status (Ps, psoriatic; Un, unaffected; He, healthy skin) is color-coded and the mean (solid line) and median (dashed line) are marked. $^{*}p < 0.05$; $^{**}p < 0.01$, and $^{***}p < 0.001$.

or unaffected psoriatic skin. In healthy back skin, significant changes were observed only when weighted PCoA analysis was applied (**Supplementary Figures S2, S3**). There was no clustering in beta diversity of diseased or unaffected psoriatic samples isolated from the elbow (**Supplementary Figure S3**). LEfSe analysis of back samples revealed several differentially abundant taxa in biopsies, scrapings and swabs. Elbow samples showed the most differentially abundant taxa in swabs. The main features revealed by LEfSe analysis of the V3V4 region are summarized in **Table 1**. Moreover, relative abundances of selected phylum and genus are displayed in **Supplementary Table S4**. Representative differences in bacterial abundance between sampling methods for the V1V2 region are summarized in **Supplementary Table S5**.

Descriptive and Functional Analysis of Bacterial Microbiota Isolated From Swabs

For a more detailed analysis of the microbial composition related to sample site, we further proceeded with analysis of the V3V4 region in swab samples. This sampling approach yielded comparable richness and evenness to other sampling techniques. We did not observe any significant differences in overall microbial diversity or richness and evenness of microbial populations in either back or elbow samples (**Supplementary Figure S3**), but each sampling site had some taxa significantly associated with it. In back samples, we found *Brevibacterium* to

TABLE 1 | The main discriminative bacterial features related to sampling approaches.

Representative bacterial biomarkers related to sampling approaches				
		Sample type		
Sample site		Swabs	Scrapings	Biopsies
Back	Psoriatic	*Peptoniphilus*	*Streptococcus*	Aeromonadaceae
			Anaerococcus	Microbacteriaceae
			Veillonella	*Lactobacillus*
				Bacillus flexus
				Lachnospiraceae
				Allobaculum
				Parabacteroides
				Bacillus megaterium
				Acinetobacter guillouiae
				Corynebacterium durum
				Mollicutes
	Unaffected	*Staphylococcus*	Moraxellaceae	Enterobacteriaceae
			Comamonadaceae	*Lactobacillus*
				Bacteroides
				Bacillus flexus
				Clostridiales
				Prevotella
				Parabacteroides distasonis
	Healthy	*Finegoldia*	Micrococcaceae	Enterobacteriales
		Peptoniphilus	Xanthomonadales	*Facklamia*
				Cloacibacterium
				Bacillus flexus
				Dermacoccus
				Parabacteroides distasonis
				Mycobacterium
Elbow	Psoriatic	Tissierellaceae	*Pseudomonas*	N/A
		Kocuria rhizophila		
		Kocuria palustris		
	Unaffected	Ruminococcaceae	Enterobacteriaceae	N/A
	Healthy	*Chryseobacterium*	*Pseudomonas*	N/A

be associated with psoriatic skin, and families Coriobacteriaceae and Xanthobacteraceae with healthy skin (**Figure 2A**). In elbow samples, we identified only the species *Kocuria palustris* and genus *Gordonia* as differentially abundant for psoriatic skin (**Figure 2B**). Next, we predicted functional profiles and gene content of bacterial community samples. Many metabolic pathways described in our analyses were similar, since the pathways were common to all samples. Heat map representation of the results from the HUMAnN analysis shows six metabolic pathways common for the back and the elbow: ko00670: One carbon pool by folate; ko00290: Valine leucine and isoleucine biosynthesis, ko00471: D-Glutamine and D-glutamate; ko00660: C5-Branched dibasic acid metabolism; ko00473: D-Alanine metabolism; ko01051: Biosynthesis of ansamycins. However, these discriminative pathways were represented with different abundance in the back (**Figure 2C**) and in the elbow (**Figure 2D**). We then used the LEfSE tool to segregate only those pathways that were discriminative for each site (back, elbow) and disease status (psoriatic, unaffected or healthy) (Segata et al., 2011). Using LEfSe, we showed that the pathway ko00642: Ethylbenzene degradation is significantly associated with unaffected skin both on the back (**Figure 2E**) and the elbow (**Figure 2F**), while only elbow samples showed further association with pathways ko04330: Notch signaling and ko04115: p53 signaling (**Figure 2F**). For descriptive and functional analysis of swab samples done by sequencing the V1V2 region, see **Supplementary Figure S4**.

Psoriatic Back Skin Displays Increased Fungal Diversity With Higher Taxa Similarity Than Psoriatic Elbow Skin

Apart from the analysis of bacterial communities, we also characterized the skin fungal communities in order to detect possible interference of different niche competitors. Analysis of alpha diversity revealed significant differences in fungal richness in swabs from psoriatic and unaffected back skin (Chao1 index) but not in healthy controls. Gini-Simpson and Shannon diversity indices were both non-significant in all sampling approaches and all sampled sites, indicating more or less stable evenness of fungal taxa across back samples (**Supplementary Figure S6**). In elbow samples, all alpha diversity measures were non-significant, suggesting a similar fungal distribution regardless of the type or site of sampling (**Supplementary Figure S7**). Binary-Jaccard metrics revealed significantly different clustering in psoriatic back lesions for different sampling approaches (**Supplementary Figure S6**). Beta diversity in unaffected and healthy skin on the back and the elbow and in psoriatic lesions on the elbow remains unchanged (**Supplementary Figures S5, S7**). To uncover specific fungal biomarkers related to each sampling method to a greater extent, we applied LEfSe analysis focused on different sampling methods, i.e., swabs, scrapings, and biopsies. For better clarity, these results are summarized in **Table 2**.

Descriptive and Functional Analysis of Fungal Microbiota Isolated From Swabs

Following the bacterial analysis, we chose swabs as the most effective sampling approach to accurately characterize the main features of fungal composition related to sampling method. Alpha diversity indices did not show significant differences in fungal richness or distribution of taxa among samples (**Supplementary Figure S8**). Bray–Curtis diversity metrics was significantly different in back skin but not in elbow skin. Heat map representation of fungal abundances (the cut-off abundance was set at 1%) showed the strongest association with psoriatic back skin of the genus *Rhodotorula* followed by the genus *Penicillium*, order Capnodiales and species *Malassezia restricta* (**Figure 3A**). The species *Malassezia sympodialis* seemed to be more associated with unaffected skin of psoriatic patients, whereas the species *Debaryomyces hansenii* and *Malassezia*

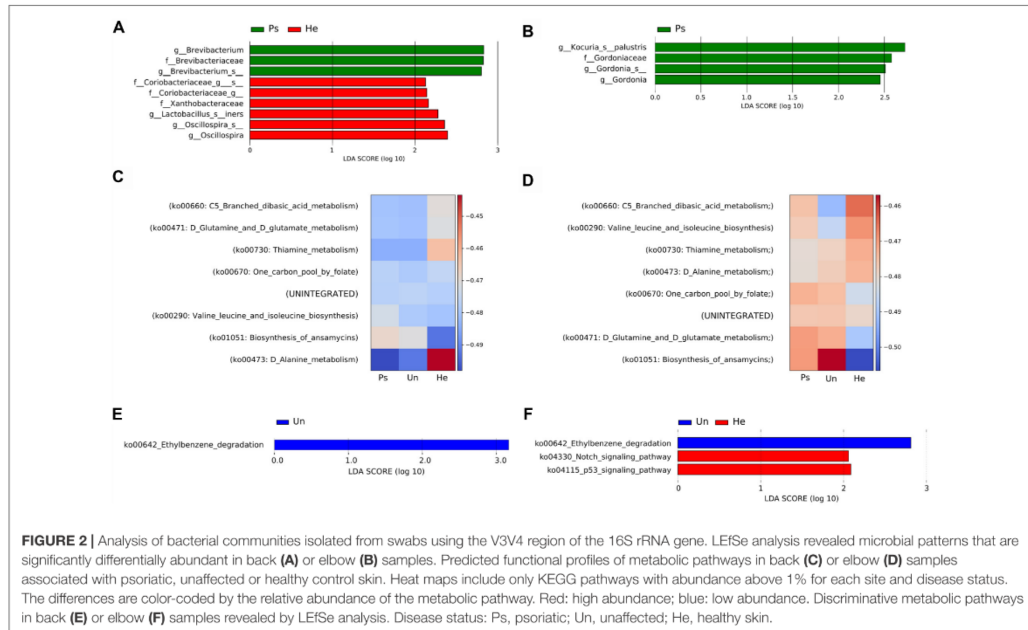

FIGURE 2 | Analysis of bacterial communities isolated from swabs using the V3V4 region of the 16S rRNA gene. LEfSe analysis revealed microbial patterns that are significantly differentially abundant in back **(A)** or elbow **(B)** samples. Predicted functional profiles of metabolic pathways in back **(C)** or elbow **(D)** samples associated with psoriatic, unaffected or healthy control skin. Heat maps include only KEGG pathways with abundance above 1% for each site and disease status. The differences are color-coded by the relative abundance of the metabolic pathway. Red: high abundance; blue: low abundance. Discriminative metabolic pathways in back **(E)** or elbow **(F)** samples revealed by LEfSe analysis. Disease status: Ps, psoriatic; Un, unaffected; He, healthy skin.

TABLE 2 | The main discriminative fungal features related to the sampling approaches.

Representative fungal biomarkers in psoriatic and healthy skin

Sample site		Sample type		
		Swabs	**Scrapings**	**Biopsies**
Back	Psoriatic	*Malassezia* Cystobasidiomycetes *Cladosporium sphaerospermum*	None	*Vermiconia* *Venturia* *Cryptococcus* *Malassezia slooffiae* *Candida parapsilosis* *Candida tropicalis* *Cyberlindnera jadinii*
	Unaffected	None	*Intersonilia*	*Cephalotrichum* *Penicillium*
	Healthy	None	None	*Melampsora*
Elbow	Psoriatic	*Malassezia sympodialis* *Penicillium* Leotiomycetes	*Malassezia slooffiae*	N/A
	Unaffected	*Malassezia slooffiae*	Dothideomycetes	N/A
	Healthy	None	*Debaryomyces hansenii* *Saccharomyces cerevisiae* *Penicillium miczynskii*	N/A

globosa were more associated with healthy control skin. Psoriatic elbow skin was characterized by the presence of *Candida railenensis*, whereas unaffected elbow skin by the genus *Rhodotorula*, species *Malassezia slooffiae*, *Naganishia diffluens* and the genus *Aspergillus*. Healthy elbow skin was associated with the genus *Penicillium* and the order Malasseziales (**Figure 3B**). LEfSe analysis focused on disease status (psoriatic, unaffected or healthy control skin) revealed a discriminative association of

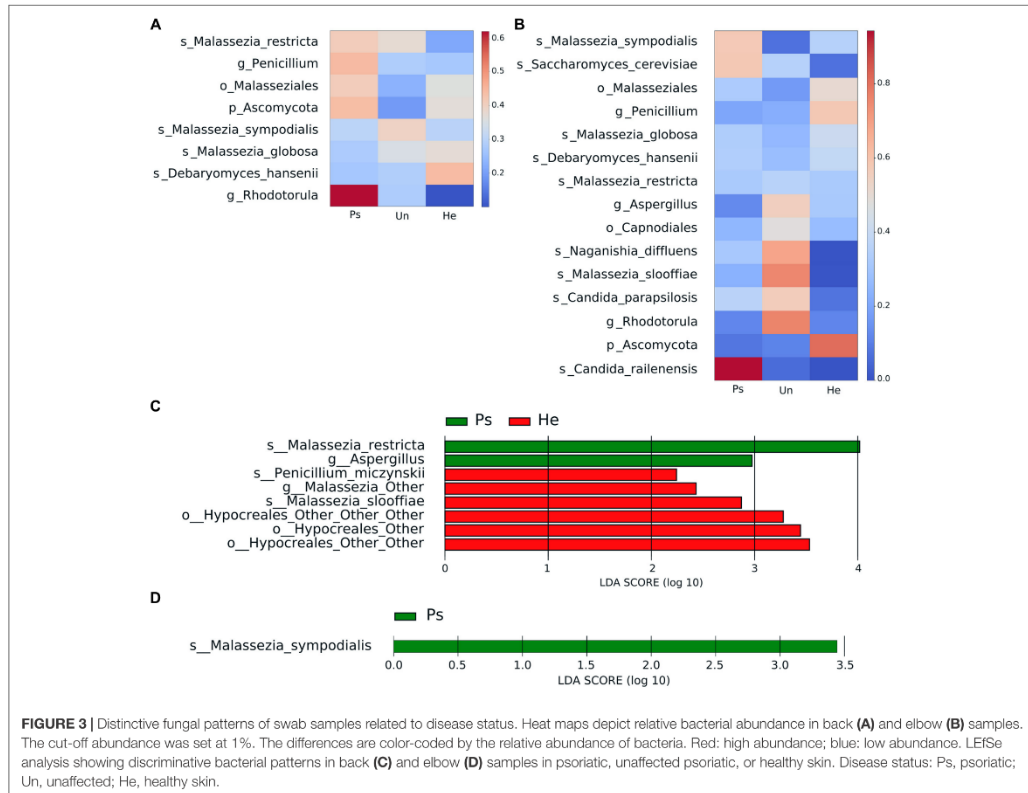

FIGURE 3 | Distinctive fungal patterns of swab samples related to disease status. Heat maps depict relative bacterial abundance in back **(A)** and elbow **(B)** samples. The cut-off abundance was set at 1%. The differences are color-coded by the relative abundance of bacteria. Red: high abundance; blue: low abundance. LEfSe analysis showing discriminative bacterial patterns in back **(C)** and elbow **(D)** samples in psoriatic, unaffected psoriatic, or healthy skin. Disease status: Ps, psoriatic; Un, unaffected; He, healthy skin.

the species *Malassezia restricta* and the genus *Aspergillus* with psoriatic back skin, whereas the species *Penicillium miczynskii*, *Malassezia slooffiae* and the order Hypocreales were significantly associated with healthy back skin (**Figure 3C**). For elbow skin, the only species associated with psoriatic skin was *Malassezia sympodialis* (**Figure 3D**).

Correlation Between Bacteria and Fungi Shows a Specific Pattern Related to Skin Condition and Sampling Site

Next, we investigated whether there is a correlation between fungal and bacterial constituents of the skin microbiome. We analyzed taxonomic correlations with body site and skin condition at genus and order levels. In psoriatic elbow skin, we found statistically significant positive correlation between occurrence of *Kocuria*, *Lactobacillus*, and *Streptococcus* with *Saccharomyces* (r = 0.73; p = 0.01 and r = 0.75; p = 0.01, respectively), which was not observed in healthy skin (r = 0.08; p = 0.84 and r = 0.03; p = 0.94, respectively). Interestingly, the genus *Micrococcus* was negatively correlated with *Capnodiales* in psoriatic skin (r = -0.69), in contrast to a positive correlation of these taxa in healthy control skin (r = 0.91). Other negative correlations were found to be specific for each skin condition and were not supported by positive correlation on the remaining sites (**Figure 4A**). On psoriatic back skin, we found negative correlation between the yeast *Malassezia* and three bacterial genera *Acinetobacter*, *Enhydrobacter,* and *Pseudomonas* (r = −0.59, r = −0.41 and r = −0.54). Similar to elbow skin, these negative correlations were not supported with positive associations, neither in unaffected nor in healthy skin (**Figure 4B**).

Analysis of Intestinal Barrier Integrity Markers Shows Differences in I-FABP Levels

To test the hypothesis that enhanced epithelial disruption in the intestine is a present marker in patients with psoriasis, we measured intestinal fatty acid binding protein (I-FABP) and apoptotic index, the ratio of caspase-cleaved cytokeratin 18 fragment (ccCK 18) and total cytokeratin 18 (CK18) in the sera. The levels of I-FABP were significantly increased in patients with

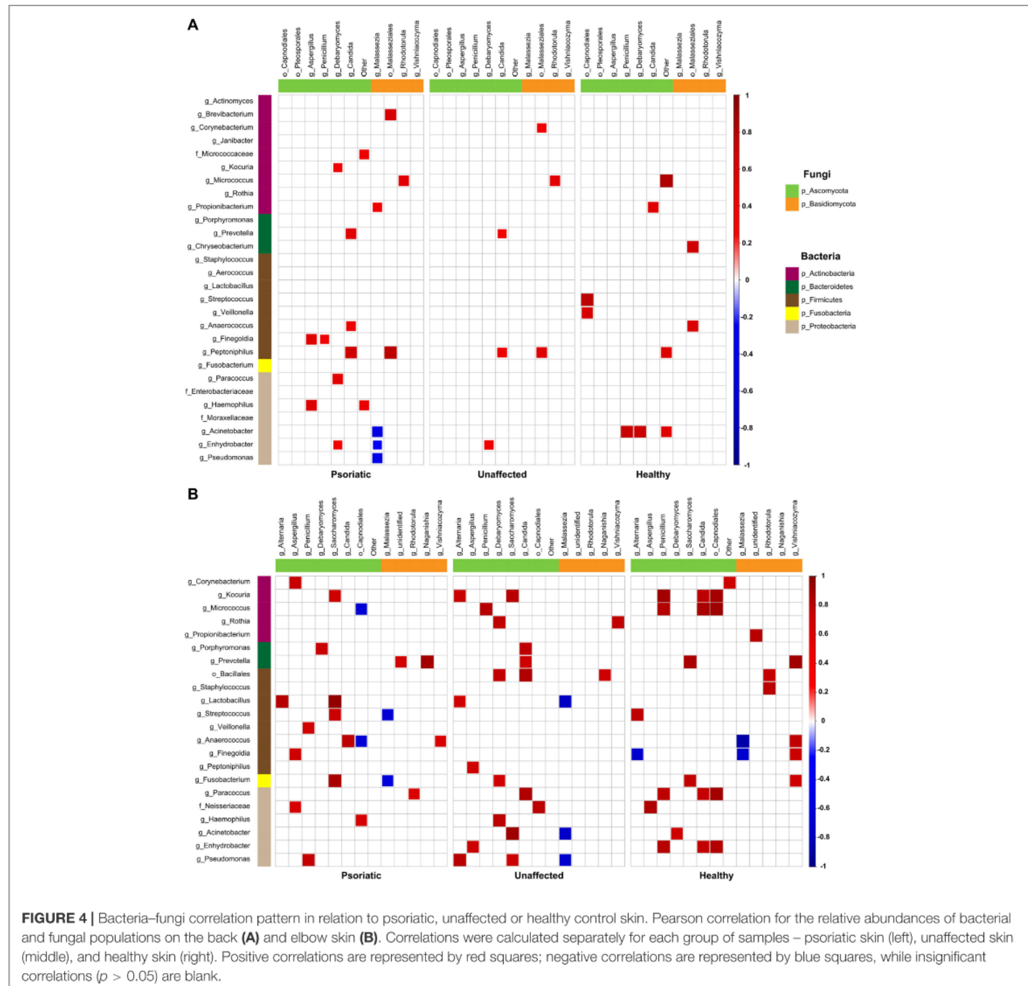

FIGURE 4 | Bacteria–fungi correlation pattern in relation to psoriatic, unaffected or healthy control skin. Pearson correlation for the relative abundances of bacterial and fungal populations on the back **(A)** and elbow skin **(B)**. Correlations were calculated separately for each group of samples – psoriatic skin (left), unaffected skin (middle), and healthy skin (right). Positive correlations are represented by red squares; negative correlations are represented by blue squares, while insignificant correlations ($p > 0.05$) are blank.

psoriasis compared to healthy controls ($p = 0.0413$) (**Figure 5A**). Nevertheless, we did not find any significant differences in the ration of ccCK 18/CK18 between psoriatic patients and healthy controls ($p = 0.5034$) (**Figure 5B**).

DISCUSSION

Several studies previously described differences in microbiota composition in psoriatic patients using dissimilar approaches (Tett et al., 2017; Yan et al., 2017). Here, we report a comprehensive analysis comparing all previously described methodological approaches in one data set. Moreover, for the first time, we include correlations between bacteria and fungi in psoriatic skin samples compared to healthy controls.

It is generally accepted that sequencing of various regions achieves different results when applied to the same samples (Hamady and Knight, 2009). Numerous combinations of primer pairs have been previously tested to select the most appropriate one for skin microbiome survey, but standardized methodology is still lacking (Meisel et al., 2016). Apart from appropriate but costly whole genome shotgun sequencing, primers for V1V3 and V3V4 hypervariable regions were described to sufficiently cover the skin bacterial diversity (Grice and Segre, 2011; Human Microbiome Project Consortium [HMPC], 2012a;

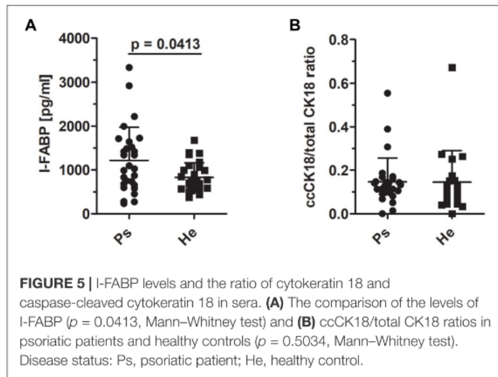

FIGURE 5 | I-FABP levels and the ratio of cytokeratin 18 and caspase-cleaved cytokeratin 18 in sera. **(A)** The comparison of the levels of I-FABP ($p = 0.0413$, Mann–Whitney test) and **(B)** ccCK18/total CK18 ratios in psoriatic patients and healthy controls ($p = 0.5034$, Mann–Whitney test). Disease status: Ps, psoriatic patient; He, healthy control.

Meisel et al., 2016; Castelino et al., 2017). In studies of the microbiome in psoriasis, both V1V3 and V3V4 sets of primers and nearly full length 16S rRNA have been used (Gao et al., 2008; Fahlen et al., 2012; Alekseyenko et al., 2013). Nevertheless, none of the published studies compared the suitability of V1V2 and V3V4 regions for characterizing skin microbial communities in psoriatic patients and healthy controls. For instance, Meisel et al., 2016 showed that using primers amplifying the V4 variable region resulted in a severe underrepresentation of several taxa, especially the genus *Propionibacterium* (Meisel et al., 2016). Our data indicate that this bias is not present when the V3V4 region is sequenced. Moreover, Meisel et al. (2016) were able to classify the majority of *Staphylococcus* using the V1V3 region, but not the V4 region. This is consistent with our finding that the V3V4 region was able to better capture genus *Staphylococcus* but primers for V1V2 region were better in classifying them to the species. However, our data show a marked absence of *Planococcaceae* when using the V3V4 region. In summary, we confirmed that the selection of primers used for studying the skin microbiota has an important impact on the resulting taxonomic coverage and thus for the further interpretation of the data. However, sequencing the V3V4 region resulted in a higher alpha-diversity overall.

Up to now, no common microbiota pattern in psoriatic patients has been identified across all the published work. This could be due to methodological differences, e.g., different sampling methods and sequencing of different variable regions, or due to a high degree of interindividual variation, specific niches of different sampling sites or low abundance of discriminatory taxa (Tett et al., 2017; Yan et al., 2017). For this reason, we sampled two different body sites by three different sampling techniques to be able to assess all of the above described aspects in one comprehensive study. However, not all participants were willing to provide biopsy sample, therefore we disposed of biopsies only from 10 patients. In contrast to other studies that compared various body sites in one study, we focused only on two well defined body sites – the elbow and the back, which are common sites frequently affected by psoriasis (Gao et al., 2008; Fahlen et al., 2012). One similar recent paper compared the elbow and the retroauricular crease as representative sites of psoriasis using shotgun sequencing, but unlike the present study focused only on isolates from swabs (Tett et al., 2017). Similarly as Tett et al. (2017) we tried to reduce the variability that arise from intraindividual and interindividual differences in microbiota composition by using control samples from unaffected skin from the same sampled patient. To deal with intra- and interindividual variation, we included not only samples of both psoriatic and contralateral unaffected skin from the same patient, but also samples from healthy controls.

Several host factors, including gender, age, place of residence, living with animals, hygiene habits, occupation, and ethnicity influence the composition of the skin microbiome (Fierer et al., 2008; Grice et al., 2009; Ying et al., 2015). Though, it seems that the effect of the psoriasis presence is much stronger than the effect of gender, since we have not found any significant changes in alpha diversity in psoriatic patients (**Supplementary Figure S9**). It has been also shown that different skin layers contain different bacterial communities (Grice et al., 2008; Kong et al., 2017). Nevertheless, the majority of previously published studies of the microbiome in psoriasis used only swabs as a single sampling technique (Tett et al., 2017; Yan et al., 2017). Here we compare swabs, scrapings, and biopsies to avoid potential sampling biases. Although due to ethical issues, we were not able to collect the biopsies from most of our tested individuals. In line with data on healthy skin (Grice et al., 2008), we found that the alpha diversity and the presence of main skin bacterial taxa in all sampling techniques are comparable. Moreover, we show that this holds irrespective of disease status. However, we observed higher richness and evenness in psoriatic skin on the elbow compared to psoriatic back skin, which is consistent with previous research and possibly reflects environmental differences between these two microhabitats (Tett et al., 2017). Comparing the microbial heterogeneity, we have not detected significant beta diversity differences between psoriatic lesions and unaffected psoriatic skin nor on the back neither on the elbow. Additionally, our analysis revealed several minor differences depending on the sampling approach. The relative abundance of *Streptococcus* in swabs, scrapings and biopsies was higher in psoriatic lesions compared to controls regardless of the sampling site, which is consistent with previous findings (Fahlen et al., 2012; Alekseyenko et al., 2013). In contrast, the abundance of *Propionibacterium* was lower in psoriatic lesions and unaffected psoriatic skin compared to healthy skin only on the elbow but not on the back. This highlights the importance of site-specific niches, e.g., different microbiota composition at oily and dry skin sites (Grice et al., 2008; Fahlen et al., 2012; Belkaid and Segre, 2014). In agreement with the findings of Fahlen et al. (2012), we described lower abundances of *Staphylococcus* in psoriatic skin biopsies (Fahlen et al., 2012). On the other hand, *Actinobacteria* and *Propionibacterium* were lower in biopsies of healthy skin.

As might be expected, we found that many of the identified metabolic pathways are common to all samples and can

be assigned to "core" pathways. Most metabolic pathways of elbow skin microbiota uncovered in our metagenomic study overlap with those previously described (Tett et al., 2017). We did not observe any niche-specific variations in the distribution of the most abundant KEGG pathways in the elbow and back samples. Interestingly, we identified the ethylbenzene degradation pathway as the only discriminative pathway common for unaffected psoriatic skin on both sites. This could be connected to the increase in abundance of *Pseudomonas*, which are known to utilize ethylbenzene as a source of energy (Utkin et al., 1991). We also found a significantly lower abundance of Notch signaling pathway in psoriatic skin compared to healthy skin. This is in concordance with previously reported data about Notch signaling, showing that alterations in this pathway, together with aberrant expression of keratin 10 and keratin 14, is associated with abnormal keratinocyte differentiation leading to unorganized suprabasal epidermal strata (Thelu et al., 2002; Ota et al., 2014).

Studies concerning the mycobiome composition in psoriasis are still scarce and no prior studies assessed the impact of different sampling methods on the recovered mycobiome composition. We observed comparable alpha diversity and microbial patterns in the skin mycobiome regardless of the sampling technique used. In comparison to study by Findley et al. (2013), we detected higher number of genera. This dissimilarity could be caused by different strategy in clustering procedure. Our study substantiates previous findings that *Malassezia* is the dominant fungal genus occurring on the human skin and that psoriatic lesions display greater fungal diversity than healthy skin (Findley et al., 2013). Moreover, we detected that psoriatic lesions on the back are predominated by *M. restricta* as previously described by Paulino et al. (2006), followed by *M. globosa* and *M. sympodialis,* and we found no consistent dichotomous differences between the tested groups. In agreement with Takemoto et al. (2015), we observed that the ratio of *M. globosa* to *M. restricta* is lower in psoriatic lesions on the back compared to healthy skin (Takemoto et al., 2015). The same pattern is evident on the elbow, where the ratio was lower in psoriasis patients, both in lesions and in unaffected skin. Psoriatic lesions on the elbow were further characterized by a significantly higher abundance of *M. sympodialis* compared to healthy skin. However, this was not true for back skin, which emphasize the need to keep in mind the differences in mycobiome composition in different skin niches, for example when comparing oily and dry sites. Moreover, not only skin niches but also ethnicity probably plays an important role in *Malassezia* presence in the psoriatic lesions as reviewed by Prohic et al. (2016). For example, in a study cohort of Polish patients, *M. sympodialis* was the predominant species, whereas in the psoriatic skin of Canadian patients *M. globosa* was the most common (Gupta et al., 2001; Jagielski et al., 2014). In contrast with these studies, Japanese patients mainly harbored *M. restricta* (Amaya et al., 2007). The mechanism by which *Malassezia* could contribute to the pathogenesis of psoriasis is not yet fully described, but it is known that *M. sympodialis* can enhance the production of pro-inflammatory cytokines IL-1 (interleukin 1), TNF-α (tumor necrosis factor alpha), IL-8 (interleukin 8), and IL-6 (interleukin 6) in keratinocytes (Watanabe et al., 2001). Moreover, *M. sympodialis* can induce uncontrolled pro-inflammatory maturation of dendritic cells and activation of mast cells, which release leukotrienes, which are increased in patients with psoriasis (Fauler et al., 1992; Buentke et al., 2002; Selander et al., 2009).

Our study is the first to conduct a simultaneous analysis of both bacterial and fungal microbiota to clarify the disease-specific inter-kingdom differences between patients with psoriasis and healthy controls. Consistently with a study on healthy skin (Findley et al., 2013), we observed more frequent bacteria–fungi equilibrium on the elbow than on the back, which reflects the different microenvironments. This suggests a greater importance of such equilibrium in mutual relationships or competition in dry and more exposed skin compared to sebaceous skin sites. Recently, an important interaction between *Lactobacillus* and *Streptococcus* has been described (Saroj et al., 2016). Here, we notice its importance in psoriatic patients together with concurrent abundance of *Saccharomyces.* Further studies are needed to achieve a more thorough understanding of potential inter-kingdom interactions in the skin microbiome with emphasis on their role in the pathogenesis of psoriasis.

There is growing evidence which emphasize the importance of the gut-skin axis in the pathogenesis of psoriasis (Mattozzi et al., 2012; Yan et al., 2017). Fox example, a study showing that medical treatment of the so-called small intestinal bacterial overgrowth syndrome could mitigate psoriasis (Drago et al., 2018), suggests an undeniable importance of microbiome in psoriasis pathogenesis. Moreover, recent study by Tan et al. (2018) described decreased abundance of *Akkermansia muciniphila*, an important producer of short fatty acid binding protein and mucin degrading bacteria, in the gut of psoriatic patients (Tan et al., 2018). We found that levels of I-FABP, a marker of cell epithelial damage, were increased in psoriatic patients in comparison to healthy controls which is in agreement with study by Sikora et al. (2018). Since the disruption of intestinal barrier seems to play a role in disease pathogenesis we searched for another marker of enterocyte damage – the ratio of ccCK18/CK18, but we did not find any significant differences between psoriatic patients and healthy controls. Nevertheless, more studies are needed to describe other markers of intestinal barrier disruption and their effects on psoriasis pathogenesis with the final goal to develop a new preventive or treatment options for psoriatic patients.

CONCLUSION

In conclusion, the data reported here extend our understanding of microbiota composition in psoriatic patients. We provide a unique insight into disease-specific inter-kingdom network

alterations and highlight the importance of viewing bacteria and fungi as important interconnected players in disease pathogenesis. A deeper understanding of the complex microbial ecosystem is needed to be able to modulate the equilibrium therapeutically by using probiotics, antimicrobials and even topical microbiota transplantation (Langan et al., 2018; Myles et al., 2018).

AUTHOR CONTRIBUTIONS

HT-H, JH, KJ, OK, and ZJ conceived and designed the research. KJ, FR, and PB examined the patients and collected the samples. ZS performed the experiments. ZS, MKo, MKv, KK, YP, AU, and ZJ analyzed and interpreted the data. ZS, KK, OK, and ZJ wrote the manuscript. All authors revised and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.00438/full#supplementary-material

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5. DISCUSSION

The skin is a multilayer barrier between the inner and outer environment. It is an active immunological environment which, besides other functions, maintains the homeostasis between the resident cutaneous commensal microbiota and the host.

Constant interactions between microbiota and the immune system are essential for priming the immune system since birth. Microbiota composition as well as the related homeostasis are mainly being adjusted during the first three years of life and thus significantly impact the sensitivity to diseases later in life (Hansen et al. 2012, Rodriguez et al. 2015, Zanvit et al. 2015). An imbalance in cutaneous or intestinal microbiota communities, referred to as dysbiosis, can lead to a shift in the immune system reactivity and to inflammatory diseases (Tlaskalová-Hogenová et al. 2011). 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 (Hidalgo-Cantabrana et al. 2019, Stehlikova et al. 2019a). 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.

Despite the differences between animals and humans, mouse models are successfully used to mimic human skin diseases (Avci et al. 2013). In our study we used a mouse model of experimental psoriasis induced by IMQ (IISI), which is a well-established model of acute skin inflammation, with symptoms resembling human psoriasis (Van der Fits et al. 2009). To have deeper insight into the role of microbiota in the pathogenesis of psoriasis, we used germ-free (GF) mice in our experiments. GF mice are an indispensable research tool for understanding the role of microbiota in pathogenesis of many diseases, as documented by the work of my colleagues (Hrncir et al. 2008, Kozakova et al. 2016, Schwarzer et al. 2011). Last but not least, antibiotics are a powerful tool for manipulating microbiota composition in conventional mice, to experimentally assess its involvement in various diseases. In contrast to GF mouse models, antibiotics are useful for studying the role of bacteria while maintaining normal physiological functions (Kennedy et al. 2018, Schwarzer et al. 2019, Štěpánková et al. 1998). Even though the bacterial load after ATB administration does not change, the bacterial community composition can be altered (Stehlikova et al. 2019b, Zakostelska et al. 2016).

Using IISI, we have analyzed the effect of microbiota on the development of psoriatic skin inflammation in CV, as well as in GF mice (Zakostelska et al. 2016). 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. Furthermore, MIX-treated CV mice and GF mice had lower skin and systemic inflammation, as manifested by reduced frequencies of γδ-T cells and Th17 cells in spleen and lymph nodes, in comparison to CV mice treated by water (Zakostelska et al. 2016). This is in agreement with Zanvit et al. (2015), who used adult CV mice treated with a mixture of only vancomycin and polymixin B. In contrast to our study, Zanvit et al. (2015) 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 (Zanvit et al. 2015). In line with Zanvit et al. (2015), our fecal microbiota analysis revealed a significantly lower Shannon diversity index in MIX-treated mice, as well as discrete clusters of samples collected before and after antibiotic treatment (Zakostelska et al. 2016). Our data are in agreement also regarding the observed microbiota abundance, e.g. increase of Lactobacillales in MIX-treated mice (Zakostelska et al. 2016, Zanvit et al. 2015). Many studies have reported a beneficial effect of lactobacilli on cutaneous health (Baba et al. 2010, Gueniche et al. 2014, Ogawa et al. 2016, Zhao et al. 2018), as well as their beneficial role in improving intestinal barrier, leading to decreased sensitization to allergens (Kozakova et al. 2016). In addition, even the administration of lysate of *Lactobacillus casei* DN-114 001 reduced the severity of experimental colitis in mice by increasing numbers of Treg cells and decreasing the production of proinflammatory cytokines, while also changing the gut microbiota composition (Zakostelska et al. 2011). This combined evidence led us to conclude that specific composition of intestinal microbiota, e.g. a lack of lactobacilli, could be responsible for enhancing the Th17 response and thus contribute to IISI in mice (Zakostelska et al. 2016).

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. The proinflammatory cytokine expression profile in the skin was most affected by streptomycin and MET (Stehlikova et al. 2019b).

Some antibiotics may have immunomodulatory anti-inflammatory properties, which are highlighted by their potential to treat non-infectious conditions (Al-Banna et al. 2013, Mencarelli et al. 2011, Miner et al. 2005). Because MET is one of the antibiotics often used for its anti-inflammatory properties and since MET treatment significantly decreased the IISI inflammation in CV mice, by using GF mice we aimed to find out whether MET influences the IISI in a microbiota-dependent or independent manner (Stehlikova et al. 2019b). There might be a minor immunomodulatory effect of MET that is microbiota independent, because in GF mice treated with MET we observed higher Nfkbiz expression than in controls, while another study reported that deficiency of Nfkbiz caused atopic dermatitis in mice (Shiina et al. 2004). Nevertheless, it has been postulated that Nfkbiz might also function independently of the NF-κB pathway (Ishiguro-Oonuma et al. 2015), so the actual role of Nfkbiz in IISI and skin homeostasis remains to be investigated. 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 (Stehlikova et al. 2019b). 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 (Heissigerova et al. 2016) or in improving the SIBO syndrome, which is primarily caused by small intestinal dysbiosis (García-Collinot et al. 2020, Soifer et al. 2010).

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 (Stehlikova et al. 2019b). VAN- and MET-induced shifts in microbiota composition remained stable after subsequent IISI induction and lasted until the end of the experiment. The observed effect of MIX was probably only the combined effect of the co-administration of VAN and MET (Stehlikova et al. 2019b).

The decrease in skin microbiota diversity in VAN-treated mice before IISI is consistent with previous findings of Zanvit et al. (2015), 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 (Zhang et al. 2015). The authors revealed altered skin

microbial density and composition after oral VAN treatment, in particular a reduced proportion of *Staphylococcus*-related sequences, as well as lower IL17 expression in mouse wounded skin (Zhang et al. 2015). These findings are in line with the results of our study and the study of Zanvit et al. (2015).

Interestingly, we discovered that in the control group the cutaneous, but not intestinal microbiota diversity markedly decreased after IISI induction. This might be a consequence of the severity of skin inflammation in CV mice compared to the ATB-treated groups of mice. Decreased skin microbial diversity in control mice after IISI induction, however, contrasts with previous data published by Zanvit and coauthors, who reported no differences between ATB and control groups after IISI induction (Zanvit et al. 2015). This could be the result of sequencing a different 16S rRNA region (i.e. V3V4 versus V4) or using a different ATB mix (Zakostelska et al. 2016, Zanvit et al. 2015).

It was recently observed that staphylococci and streptococci found in mouse fecal samples worsened experimental psoriasis manifestation (Okada et al. 2020). In our study, in MET-treated group of mice which had mild IISI, there was lower abundance of staphylococci and streptococci in IISI lesions compared to controls. Moreover, we detected no staphylococci or streptococci in the feces of those MET-treated mice, both before and after IISI induction. In contrast to that, we found streptococci species in the feces of control mice before IISI induction and increase of staphylococci after IISI induction (Stehlikova et al. 2019b). When Okada et al. (2020) administered *Staphylococcus aureus* and *Streptococcus delineate* orally to ATB-treated mice with IISI, it exacerbated skin lesions and elevated the levels of proinflammatory cytokines TNFα, IL17 and IL22 (Okada et al. 2020). 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 (Okada et al. 2020).

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. Taken together, MET-treatment can alleviate IISI symptoms and is associated with increased lactobacilli 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 (Jang et al. 2014, Kim et al. 2015, Kim et al. 2020, Kong et al. 2012, Mariman et al. 2016, Prakoeswa et al. 2020). Furthermore, certain microbial species were shown to populate

Th17 or Treg cells (Atarashi et al. 2011, Round and Mazmanian 2010, Wang et al. 2019). Taking all those information into consideration, we decided to monocolonize IISI mice with either *L. plantarum* WCFS1 (LP) or segmented filamentous bacteria (SFB). We expected a relief of IISI symptoms after monocolonization with LP, and their worsening during monocolonization with SFB (Górska et al. 2014, Ivanov et al. 2009). 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 (Stehlikova et al. 2019b). It may seem that this is partially inconsistent with other mice studies reporting the role of SFB bacteria in inducing the proinflammatory response (Koga et al. 2019, Kwon et al. 2018). 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 (Stepankova et al. 2007a).

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 (Kong et al. 2017, Naik et al. 2012, Salter et al. 2014). Many host factors, such as gender, ethnicity, handedness, living with animals, hygiene and cosmetics habits, can impact the composition of skin microbiota (Fierer et al. 2008, Kim et al. 2019). Results from our human study suggest that psoriasis is a stronger factor than gender in determining skin microbiota composition, since we have found no differences in alpha diversity between males and females affected by psoriasis (Stehlikova et al. 2019a).

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. For example, Gao et al. (2008) studied the cutaneous psoriatic microbiome in only 6 patients, sampling the forearm by swabs; Fahlén et al. (2012) examined skin biopsies from the trunk, arms and limbs; Tett et al. (2017) sampled skin on the elbow and behind the ear by swabs, and Ogai et al. (2018) performed tape-stripping to reveal the skin microbiome composition on the back. Ethnicity, i.e. a group of people with similar culture and habits, although considered a secondary factor affecting microbiota composition, might also be behind the discrepancies reflected in the published results. Microbiota

composition can differ among, for example, the Irish (Fahlén et al. 2012), Americans (Alekseyenko et al. 2013, Brooks et al. 2018, Gao et al. 2008), Czechs (Stehlikova et al. 2019a) or Indians (Gupta et al. 2017).

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 (Bukin et al. 2019, Castelino et al. 2017, Willis et al. 2019, Yang et al. 2016). While some researchers claim that the DNA extraction strategy is paramount and that the choice of hypervariable regions has only a minor influence (Teng et al. 2018), others maintain that the selection of 16S rRNA region plays an important role (Jo et al. 2016, Kerrigan et al. 2019). For example, the V1V3 region could better distinguish among *Staphylococcus* species (Conlan et al. 2012, Jo et al. 2016), and using primers for the V4 region results in underrepresentation of *Cutibacterium* species (Meisel et al. 2016). The V3V4 region has been described to sufficiently cover the skin microbial diversity (Castelino et al. 2017, Grice and Segre 2011, Group 2012) and Teng et al. (2018) 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 (Bukin et al. 2019). Therefore, we have compared the sets of primers for V1V2 and V3V4 regions on identical samples from psoriatic patients and healthy controls (Stehlikova et al. 2019a). 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. (2013) whose finding was further supported by Meisel et al. (2016). Nevertheless, although primers for V1V2 regions were better in classifying *Staphylococcus* to the species level, sequencing the V3V4 region recovered greater overall diversity (Stehlikova et al. 2019a). This is in line with Graspeuntner and colleagues, who also confirmed greater number of taxa identified using the V3V4 region of 16S rRNA (Graspeuntner et al. 2018).

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 (Fahlén et al. 2012, Gao et al. 2008), 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 (Stehlikova et al. 2019a). Similarly to Tett et al. (2017), 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 (Stehlikova et al. 2019a).

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. (2020), who researched the moist and dry areas of the skin, and consistent with other data on healthy skin (Grice et al. 2008), 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. (2017) and possibly underscores the microbiota changes caused by the disease and reflects the conditions of distinct microenvironments as well. However, Tett et al. (2017) 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 (Stehlikova et al. 2019a). 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 (Bay et al. 2020). Therefore, this could be the reason why we have detected so many distinguishing species in biopsy samples in contrast to swabs and scrapings (Stehlikova et al. 2019a). Unfortunately, most of our participants were not willing to provide a biopsy sample and none of them from the elbow, as this harsh intervention might flare up the psoriasis symptoms at the site of injury, which is known as the Koebner phenomenon (Ji and Liu 2019). Due to this reason, we had back biopsies from only 10 patients and, therefore, we focused more

on the skin microbiota analysis from the most common and best tolerated swab sampling. Nevertheless, in this scarce set of biopsy samples we observed that biopsies from healthy controls are characterized by almost absolute predominance of *Staphylococcus* species (90.9 %), whereas biopsies from psoriatic patients had variable microbiota composition (unpublished data). This partially contrasts with Fahlén et al. (2012), who also investigated the microbiota composition of psoriatic patients in biopsy samples, since they 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 (Fahlén et al. 2012). However, the data are not directly comparable, since Fahlén et al. (2012) 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 (Alekseyenko et al. 2013, Gao et al. 2008). On the other hand, despite the differences in sampling strategy, our data are mostly consistent with other studies that investigated human psoriasis (Drago et al. 2016, Fahlén et al. 2012). Given the differing *Staphylococcus* abundances between our human study and the study of Alekseyenko et al. (2013) and Gao et al. (2008), who used the same sampling approach (i.e. swabs), we suspect that there must be other reasons for the inconsistent results, besides sampling methodology. The reasons might be the already discussed sequencing approaches, high inter-individual variation, specific niches of different body sites, or low abundance of discriminatory taxa (Kverka and Tlaskalová-Hogenová 2017, Tett et al. 2017, Yan et al. 2017).

When oily and dry skin were compared in their beta-diversities, the oily skin showed larger beta diversity than the dry skin (Stehlikova et al. 2019a, Tett et al. 2017). This possibly reflects the differences of oily and dry microenvironments (Grice et al. 2008). 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. (2017), where the authors found larger beta diversity in oily skin, particularly in psoriatic compared to unaffected skin. However, since Tett et al. (2017) used shotgun metagenomics in their study, it is difficult to compare the results, since different methodological strategies vary in their outcomes (Jarrin et al. 2015).

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 (Hurabielle et al. 2020). 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 (Amaya et al. 2007, Findley et al. 2013, Jagielski et al. 2014, Takemoto et al. 2015). 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 (Findley et al. 2013). For instance, *M. sympodialis* is known to enhance the production of proinflammatory cytokines in keratinocytes (Watanabe et al. 2001) and induce the activation of mast cells, which then release leukotriens, increased in atopic dermatitis and psoriasis patients (Buentke et al. 2002, Selander et al. 2009). 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 (Stehlikova et al. 2019a). This contrasts with the study of Paulino et al. (2006) 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. (2006) 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 (Takemoto et al. 2015).

An intriguing aspect is the already mentioned ethnicity, which could confound the results. For example, *M. sympodialis* was the predominant species in the Polish cohort of patients (Jagielski et al. 2014), whereas in Canadian patients it was *M. globosa* (Gupta et al. 2001) and in Japanese patients it was *M. restricta* (Amaya et al. 2007).

Consistently with our observation, the predominance of *M. restricta* on psoriatic oily skin was confirmed in another Japanese study (Koike et al. 2020). Moreover, the authors revealed that the psoriatic skin mycobiome composition is retained even after systemic anti-TNF or anti-IL17 treatment (Koike et al. 2020). Interestingly, another study showed gut microbial changes after

anti-IL17, but not anti-IL12/23 treatment (Yeh et al. 2019). However, the baseline microbiota composition of those patients differed markedly between responders and non-responders to anti-IL17 treatment, which might be useful as a potential microbiota biomarker of response to anti-IL17 treatment (Yeh et al. 2019). Since Koike et al. (2020) deal with fungi on psoriatic skin and Yeh et al. (2019) with bacteria in the gut, it remains to be elucidated whether the treatment affecting intestinal bacteria also affects the intestinal fungi, and whether this treatment could also influence the skin bacterial composition. In our study, the majority of tested patients were under various kinds of therapy (i.e. local, systemic or under biological drugs), depending on the disease severity.

Regarding the published literature it seems that some medication could actually impact the microbiota composition (Koike et al. 2020, Yeh et al. 2019), and that some patients might not respond to the treatment due to possible differences in microbiota composition (Dei-Cas et al. 2020, Yeh et al. 2019). Taking this into account, the altered microbiota composition inevitably leads to altered microbiota interactions and this might consequently influence the overall dynamics of the immune system and vital functions of the human body.

To better understand the disease-specific differences, we analyzed the potential interactions between bacterial and fungal species in psoriatic patients versus healthy controls. The correlation analysis was based on the taxonomic abundances of particular species. We noticed many more interactions on the dry elbow skin when compared to the back skin, which corresponds to the study of Findley et al. (2013). This observation probably reflects the ecological conditions in both microenvironments. It could also reflect the potential higher competition in the more exposed dry skin in comparison to the less exposed oily back skin (Li et al. 2017).

Chang et al. (2018) 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. (2018) and both genera were positively correlated with Malasseziales in unaffected psoriatic skin on the back in our study (Stehlikova et al. 2019a). Furthermore, *Corynebacterium* clustered with *Finegoldia* (Chang et al. 2018) and both genera were positively correlated with *Aspergillus* on elbow psoriatic lesions (Stehlikova et al. 2019a). 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. Disturbances in biodiversity and composition of gut microbiota, even in less abundant species, have been linked to many diseases. New growing evidence suggests that psoriatic patients also suffer from intestinal dysbiosis (Codoñer et al. 2018, Dei-Cas et al. 2020, Hidalgo-Cantabrana et al. 2019, Chen et al. 2018, Shapiro et al. 2019, Yegorov et al. 2020). 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* (Shapiro et al. 2019). Recently, it has also been shown that psoriatic patients have a lower abundance of *Akkermansia muciniphila*, an important producer of SCFA-binding protein, in the gut (Tan et al. 2018). We did not sample intestinal microbiota of psoriatic patients, but in our mouse study we did not detect any significant changes in *A. muciniphila* abundance during IISI development. Other human studies reported a lack of *A. muciniphila* in other diseases, such as allergic asthma or ulcerative colitis (Demirci et al. 2019, Rosso et al. 2020).

Enterotype 2 classified by Codoñer et al. (2018), characterized by the predominance of *Prevotella* species, is susceptible to more frequent bacterial translocations, promoting inflammation. Furthermore, our results together with others consistently show an increased concentration of serum biomarkers indicating intestinal barrier damage in psoriatic patients, such as Claudin-3 or I-FABP (Sikora et al. 2019a, Sikora et al. 2019b, Stehlikova et al. 2019a). In addition, levels of I-FABP positively correlate with increased values of BMI, PASI, and NLR (neutrophil to lymphocyte ratio) pointing to the fact that intestinal integrity is affected by obesity, severity of the disease and systemic inflammation (Sikora et al. 2019b). Other markers of enterocyte damage, i.e. the ratio of ccCK18/CK18 did not show significant differences between psoriasis patients and healthy controls (Stehlikova et al. 2019a).

Conducting a human microbiome research has its benefits and drawbacks. Apart from the costly experiments, researchers have to deal with difficult-to-control sample collection, generation and analysis of the data. Furthermore, human microbiota studies often do not deal with causality and provide mostly correlative results. Experimental animal models, although often expensive

and difficult to maintain, offer great opportunities and could help us gain insight into different aspects of the balanced and dysbiotic human microbiota (Fritz et al. 2013).

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 (Eckburg et al. 2005). However, this hypothetical assumption, although based on many observations, does not have to be true in all cases (Fredricks et al. 2005, Chang et al. 2018, Srinivasan et al. 2012).

6. CONCLUSIONS

I.

CV mice treated with a mixture of broad-spectrum ATBs were more resistant to IISI, similarly to GF mice. ATB treatment profoundly changed the gut microbiota profile of CV mice, which resulted in a lower degree of local and systemic Th17 activation (Zakostelska et al. 2016). MET was the most effective antibiotic in mitigating the IISI symptoms, due to its antimicrobial activity and not its immunomodulatory effect, as we showed in GF mice. Furthermore, monocolonization of mice with single bacteria species was not sufficient to change the course of IISI (Stehlikova et al. 2019b).

II.

Different techniques of sample collection uncovered similar richness, evenness and genera abundance of the present taxa, but each technique highlighted some specific bacterial or fungal taxa associated with the particular method (i.e. swab, scraping, and biopsy). Each sampling site (psoriatic, unaffected, healthy), as well as body location (elbow, back) was also characterized by specific microbial communities. The bacteria-fungi co-occurrence pattern, distinct in psoriatic, psoriatic-unaffected and healthy skin, suggests a link between niche occupancy and psoriatic changes on the skin (Stehlikova et al. 2019a).

III.

Elevated serum levels of I-FABP were found in patients with psoriasis, pointing at intestinal barrier disruption. Although we did not find an increase in serum levels of ccCK18, another marker of intestinal barrier impairment, intestinal integrity certainly plays an important role in the pathogenesis of psoriasis (Stehlikova et al. 2019a).

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

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