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Characteristic of decreased bacterial diversity in psoriasis

[*Característica da diminuição da diversidade bacteriana na psoríase*]

Tong *Su*[1#], Fang *Liu*[2#],Cai-Xia *Kou*[3], Hai-Bo *Liu*[2], Sheng-Jing *Xu*[1], Dong-Yan *Zhang*[1], Fan *Wei-Xin*[4]*, Chao *Fang*[5]*, Min *Zhang*[1]*

[1]Jiangning Hospital of Nanjing Medical University, Department of Dermatology, Nanjing, Jiangsu, 211100, China
[2] Hospital affliated to Nanjing University, Department of Dermatology, Nanjing, China Nanjing, Jiangsu, China
[3]Hospital of Nanchang University, Department of Dermatology, Nanchang, Jiangxi, 330006, China.
[4]Hospital of Nanjing Medical Uniuersity, department of dermatology and Venereology, Nanjing 210029, China
[5]Central Laboratory of The Affiliated Jiangning Hospital with Nanjing Medical University, Nanjing, Jiangsu, 211100, China

ABSTRACT

Disturbance of commensal intestinal microbiota is related to chronic inflammatory dermatosis. We analyzed the diversity of the gut microbiota to characterize the biological variation of psoriasis (Ps). Significant differences of gut microbiome profiles were revealed in murine model with psoriasis by sequencing 16S rRNA V3-V4 variable region. Group comparisons included the imiquimod cream (IMQ group, n=8), the imiquimod cream and antibiotics (ATB) (PC+IMQ group, n=8) and the healthy control (CTRL group, n=8). The gut microbiota existed in Ps groups including IMQ group and PC+IMQ group encompassed less diversity than controls, which were attributed to decreased presence of several taxa. The two Ps groups were characterized by significant reduction in firmicutes. In this study, microbiota of psoriasis was defined by an increase presence of Bacteroides. After treated with ATB, we found substantial increase of Lactobacillales but significant decrease of Clostridiales and Coriobacteriales. Relative lower abundance of multiple intestinal bacteria was observed in Ps groups. Although part of genera were concomitantly reduced in both IMQ and PC+IMQ conditions, we discovered the specialty of PC+IMQ group samples was that contained lower abundance of beneficial taxa. Characteristics of gut microbiota profiles in Ps mice were comparable to profiles in patients with Ps, which were related to alteration of specific inflammatory proteins in disease groups but were significantly different from control group. Thus, this study emphasizes the role of intestinal microbiota in the pathogenesis of Ps and provides new insight for investigating association between intestinal microbes and immune inflammation.

Keywords: intestinal microbiota, chronic inflammatory dermatosis,psoriasis, microbiome

RESUMO

A perturbação da microbiota intestinal comensal está relacionada à dermatose inflamatória crônica. Analisamos a diversidade da microbiota intestinal para caracterizar a variação biológica da psoríase (Ps). Diferenças significativas do perfil microbiológico intestinal foram reveladas no modelo murino com psoríase pelo sequenciamento da região variável 16S rRNA V3-V4. As comparações de grupo incluíram o creme imiquimod (grupo IMQ, n=8), o creme imiquimod e antibióticos (ATB) (grupo PC+IMQ, n=8) e o controle saudável (grupo CTRL, n=8). A microbiota intestinal existia nos grupos Ps, incluindo o grupo

*Corresponding author: Zhangmin201508@163.com Chaofang025@163.com fanweixin@medmaii.com.cn
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# Theses authors contributed equally to this work as the co-first authors

IMQ e o grupo PC+IMQ englobava menos diversidade do que os controles, que foram atribuídos à diminuição da presença de vários taxa. Os dois grupos de Ps caracterizavam-se por uma redução significativa nos firicutes. Neste estudo, a microbiota da psoríase foi definida por um aumento da presença de bacteroides. Após o tratamento com ATB, encontramos um aumento substancial de Lactobacillales mas uma diminuição significativa de Clostridiales e Coriobacteriales. Uma menor abundância relativa de bactérias intestinais múltiplas foi observada nos grupos de Ps. Embora parte dos gêneros tenha sido concomitantemente reduzida tanto em condições IMQ como PC+IMQ, descobrimos que a especialidade das amostras do grupo PC+IMQ era que continham menor abundância de taxas benéficas. As características dos perfis de microbiota intestinal em ratos de Ps eram comparáveis aos perfis em pacientes com Ps, que estavam relacionados à alteração de proteínas inflamatórias específicas em grupos de doenças, mas eram significativamente diferentes do grupo controle. Assim, este estudo enfatiza o papel da microbiota intestinal na patogênese do Ps e fornece novos conhecimentos para investigar a associação entre micróbios intestinais e inflamação imunológica.

Palavras-chave: psoríase, microbioma

INTRODUCTION

Psoriasis is an inflammatory dermatosis mediated by immune response which impacts 1% to 3% of the world's population (Deng *et al*., 2016), and its pathogenesis has not been well clarified. It has been proposed to be multi-factorial, affected by various mechanisms including genetics and immunological factors (Afifi *et al*., 2017; Roberson and Bowcock 2010). The emerging data indicated that the changes in microbiome composition were linked to the development of multiple diseases, such as AIDS (Koay *et al*., 2018), type 2 diabetes (Sircana *et al*., 2018) and inflammatory bowel disease (DeGruttola *et al*., 2016). Currently, etiopathogenesis of psoriasis was found to be prominently implicated in variation of microbiota composition and diversity (Yan *et al*., 2017). Significant alteration of gut microbiome in psoriasis was revealed and Staphylococcus aureus was identified as a potential contributor to psoriasis pathogenesis (Chang *et al*., 2018). Fecal samples of mice with psoriasis tended to enrich in bacteria of phyla Firmicutes and Proteobacteria which both contributed to over one-third relative abundance, while the content of such two microbes in control samples were relatively low (17.95% and 3.81%, respectively). In this study, we attempt to characterize intestinal microbial composition of psoriasis by BALB/c mice.

METHOD

Female BALB/c mice at five weeks old reared in conventional conditions were applied in this study. The BALB/c mice were placed in plastic isolators for one week before being treated for experiment, which were kept at ventilated and dry, temperature 22-25°C, humidity 50%~70%, single mouse single isolators, free drinking water and diet. All experiments were approved by the Animal Care and Use Committee of The Affiliated Jiangning Hospital of Nanjing Medical University (Number: 2020-03-084K01).

The mice in the experimental group were daily injected with 62.5mg of imiquimod (IMQ) cream (US, 3M Medical Products) on the shaved back, and the control mice were daily injected with similar amount of control cream (Vaseline, Aromatica CZ, Czech Republic), both for three consecutive days. By assessing clinical Psoriasis Area and Severity Index (PASI), the severity of erythema and scaling were monitored daily.

Mice were treated with Penicillin and the treatment continued until the end of the experiment. 80 units of penicillin sodium were prepared at a concentration of 200mg/mL using 0.9% physiological saline. Penicillin solution was intraperitoneally injected with 0.1ml (d.d) and the amount of antibiotics reached 1000mg/(kg.d). 8 mice of control group (CTRL group) were intraperitoneally injection of 0.1 ml (d) once daily with 0.9% saline, continued injection of normal saline until the end of the experiment.

The dorsal skin was fixed in 5% formalin solution,subsequently dehydrated, and embedded in paraffin. Next, samples were sliced in 6μM sections and stained with hematoxylin & eosin. Samples were sent for histopathological examination by an experienced pathologist (PR) blinded to all treatment information of the mice.

Fecal samples were obtained for all BALB/c mice within 24 hours of production on the 11th day, the mouse feces microbiological analysis of the mouse gastrointestinal flora 16s microbiome sequencing (Shanghai Meiji Culture Communications, Shanghai, China). The power Soil DNA Isolation Kit (MO BIO Laboratories) was used for extracting total bacterial DNA from samples according to the manufacturer's protocol. Quality and quantity of purified DNA were measured by the ratio of OD260/OD280 and OD260/OD230. The qualified DNA extraction products were then stored at -80°C for further processing. Amplification of V3-V4 region of bacterial 16S rRNA gene was performed by using universal primer pair (Forward: 5'-ACTCCTACGGGAGGCAGCA-3'; reverse: 5'-GGACTACHVGGGTWTCTAAT-3') combined with adapter sequences and barcode sequences. Each PCR amplification reaction has a total volume of 50μL, which included Buffer (10 μL), High GC Enhancer (10μL), Q5 High-Fidelity DNA Polymerase (0.2μL), dNTP (1μL), primers (each with10μM) and genome DNA (60ng). The thermal cycling conditions of amplification reaction were set as: firstly, initial denaturation at 95°C for 5min; secondly, run 15 cycles at 95°C for 1 min, 50°C for 1 min and 72°C for 1 min; finally, extension at 72°C for 7 min. The products generated from first PCR step were purified through VAHTSTM DNA Clean Beads and were used as template DNA for second round of PCR reaction, which was consisted of 10 μM of each primer, 10μL template DNA, 20 μL 2 × Phusion HF MM and 8μL ddH2O. The corresponding thermal cycling conditions were set as: initial denaturation at 98°C for 30s; then 10 cycles at 98°C for 10s, 65°C for 30s min and 72°C for 30s; finally, extension at 72°C for 5min. The final quantification of PCR products was performed by Quant-iT™ dsDNA HS Reagent. All prepared samples containing bacterial rRNA genes were pooled together to perform in High-throughput sequencing in Illumina HiSeq 2500 platform (2×250 bp paired ends) at Biomarker Technologies Corporation, Beijing, China.

To isolate total RNA, RNeasy Mini Kit was applied for approximate 50 mg of mouse skin tissue following the manufacturer's protocol. 1μL of total RNA was reverse transcribed using 4μL 5 × PrimeScript RT Master Mix (RR036A, takara) and 15μL DEPC water (KeyGEN BioTECH, Nanjing, China). The product cDNA was used as template for quantitative PCR analysis with the Fluorescence quantitative PCR instrument (ABI, steponeplus). Each PCR reaction with a total volume of 20μL containing 2μL of a 1:10 dilution of each cDNA preparation, 10μL of 2 × qPCR Master Mix, and 0.2 μL of each primer. The reaction procedure was set as follows: 95°C for 5 min, followed by 40 cycles at 95°C for 5s, 60°C for 31s. Alterations of gene expression were measured by $2^{-\Delta\Delta CT}$ (Livak) method. In this study, GAPDH served as reference gene, which expression levels were used for quantitative normalization. The mRNA expression levels of mice in three groups were shown as fold change. Data were presented as mean ± SEM of the values measured in all samples.

The mRNA expressions of cytokines IL-23, IL-17A, IL-17F, IL-4, IL-22 and IL-10 in lesions were detected by Real-Time PCR on 5, 7, 9, 11, and 14 days. Primers were listed in Supplementary Table S1.

Two experimental groups were compared by unpaired Student's t-test. The comparisons of multiple experimental groups with control group were performed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison tests. Data were presented as the mean ± standard deviation (SD). The differences with p value < 0.05 were considered as statistically significant. All statistical analyses were performed on GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA).

Table S1. Primers for lesion cytokines determination

Cytokines	Primers	bp
IL-4	Forward: 5'- CAAACGTCCTCACAGCAACG-3' Reverse: 5'- CCTTGGAAGCCCTACAGACG-3'	63
IL-17A	Forward: 5'- ACTACCTCAACCGTTCCACG-3' Reverse: 5'- TCAGGGTCTTCATTGCGGTG-3'	55
IL-22	Forward: 5'-GTGCCTTTCCTGACCAAACT-3' Reverse: 5'-ACTGTCTCCTTCAGCCTTCT-3'	101
IL-23	Forward: 5'-CCTGCTTGACTCTGACATCTT-3' Reverse: 5'-TCCTAGTAGGGAGGTGTGAAG-3'	85
IL-17F	Forward: 5'- ACGTGAATTCCAGAACCGCT-3' Reverse: 5'- TGATGCAGCCTGAGTGTCTG-3'	113
IL-10	Forward: 5'-AGAGCCACATGCTCCTAGA-3' Reverse: 5'-CCTGCATTAAGGAGTCGGTTAG-3'	108
GAPDH	Forward: 5'-GGGTGTGAACCACGAGAAATA-3' Reverse: 5'-GTCATGAGCCCTTCCACAAT-3'	129

Cytokines symbols, sequences of primers (forward and reverse) and product length were listed.

RESULTS AND DISCUSSION

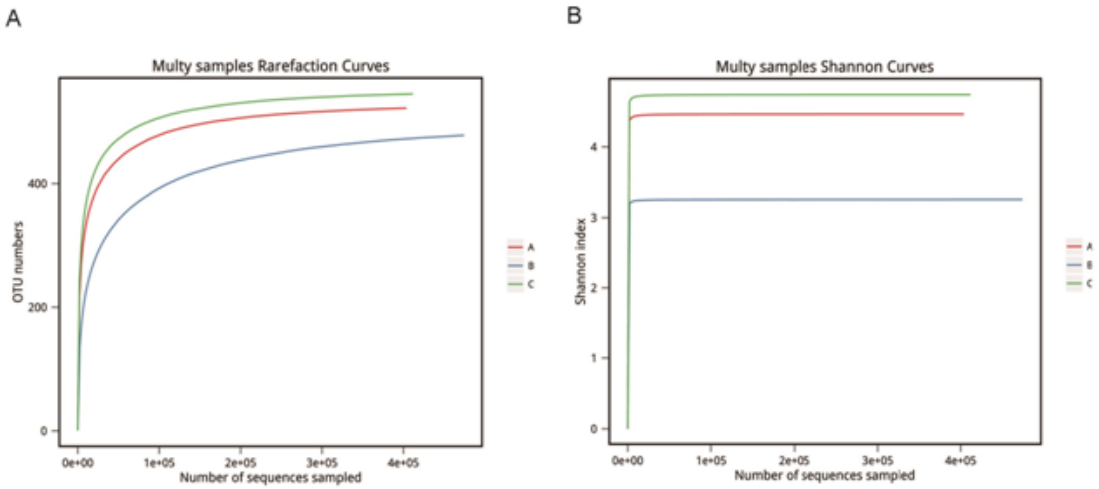
To identify association of psoriasis with intestinal microbiota, we employed microbiome sequencing on 16S rRNA V3-V4 genes of fecal bacterial from BALB/c mice. We counted the various indicators of sequencing output inspecting a mean of 68492 sequences per sample (PE Reads, raw tags, clean tags, effective tags, average sequencing depth (bp), Q20 (%), Q30 (%) and Effective (%) per sample, overview were listed in Supplementary Table S2).

Table S2. Microbiome sequencing information of samples

Sample ID	PE Reads	Raw Tags	Clean Tags	Effective Tags	AvgLen(bp)	GC(%)	Q20(%)	Q30(%)	Effective(%)
A1	80027	74100	68345	67353	417	54.1	96.12	92.58	84.16
A2	80016	73974	68490	67056	414	54.35	96.1	92.53	83.8
A3	79743	72788	66513	65280	419	53.91	95.86	92.11	81.86
A4	80210	72457	66424	65364	418	54.35	96.03	92.36	81.49
A5	80105	73163	67187	66426	420	54.2	96.07	92.42	82.92
A6	80027	73329	67352	66036	417	54.05	96.02	92.36	82.52
A7	79967	73863	68370	67421	416	54.59	96.2	92.65	84.31
A8	80031	72958	67007	65673	417	54.05	95.97	92.3	82.06
B1	79923	73523	67696	65542	423	49.8	95.89	92.28	82.01
B2	79614	74144	68688	66027	416	52.15	96.08	92.6	82.93
B3	79852	74767	69606	68473	419	50.98	96.15	92.72	85.75
B4	79967	74595	69280	66900	418	50.93	96.07	92.59	83.66
B5	79949	75154	68518	67647	427	54.44	95.9	92.27	84.61
B6	80349	76297	70248	68943	426	54.14	96.04	92.54	85.8
B7	80299	75896	69439	68119	426	54.4	95.94	92.36	84.83
B8	80116	75122	70178	68371	415	53.38	96.26	92.84	85.34
C1	80226	73794	67966	66679	417	54.08	96.05	92.47	83.11
C2	80084	73861	68300	66590	416	54.04	96.08	92.5	83.15
C3	79658	73620	68138	67022	416	54.14	96.03	92.42	84.14
C4	80670	74425	68738	67034	416	54.07	95.97	92.33	83.1
C5	80184	74025	68444	66805	416	53.73	95.96	92.31	83.31
C6	80019	74897	69849	68356	413	54.74	96.18	92.7	85.42
C7	79960	74934	70109	68873	411	54.02	96.07	92.55	86.13
C8	79979	74354	68924	67377	419	53.84	96.13	92.58	84.24

Per sample (Sample ID) with Paired end reads (PE Reads), raw tags (Raw Tags), clean tags (Clean Tags), effective tags (Effective Tags), average length (AvgLen), percentage of GC content (GC), percentage of Quality>20 (Q20), percentage of quality>30 (Q30), and percentage of effective was listed.

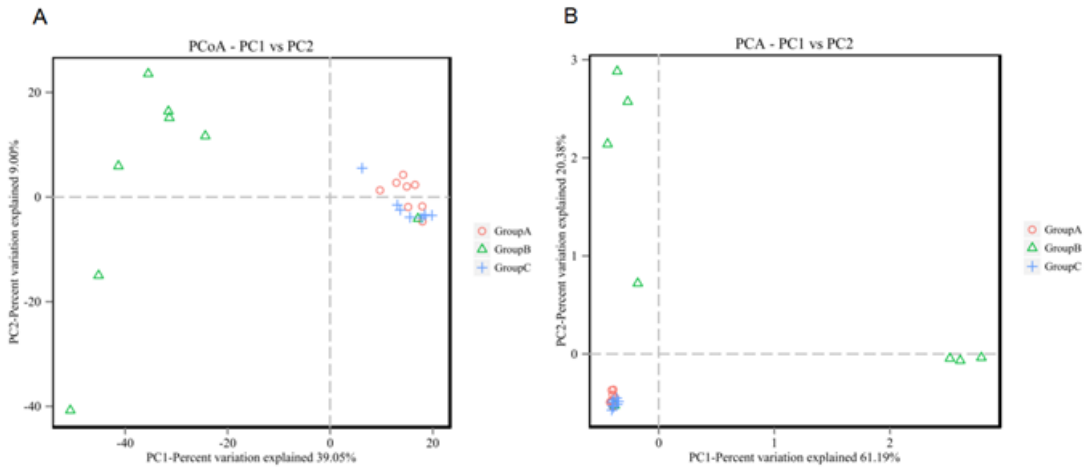
We assessed richness of fecal microbiome using operational taxonomic unit (OTU) counts in per sample. Amount of OTUs observed in psoriasis samples including group A (IMQ group) was less than that in control samples group C (CTZL group) (Figure 1A). Each individual sample was calculated microbial diversity by operational taxonomic unit (OTU) counts. We found a decreased tendency which reflected association between microbial richness and penicillin treatment in psoriasis fecal sample: Amount of OTUs observed in PC+IMQ group was lower than that of control group (Figure 1A). From the comparison of sample type, OTUs of samples in group A and group B ((PC+IMQ group) were lower than group C. Then we calculated Shannon index to evaluate bacterial diversity within samples finding group C also higher than the other two groups (Figure 1B). Both metrics revealed reduced diversity of microbiota in psoriasis samples compared to controls.



Group A (red) and group B (blue) samples have decreased diversity compared to controls (green). Diversity indices were evaluated by (A) OTU numbers and (B) Shannon index.

Figure 1. Comparison of diversity indices in experimental groups and control group

Principal coordinates analysis (PCoA) was employed in our study to visualize the similarity and difference of microbiome in multiple groups, which applying an unweighted UniFrac analysis on intestinal microbiota. The result revealed microbiota of group B (PC+IMQ group) was profoundly distinguished from the group A (IMQ group) and group C (CTRL group). Although we found several control samples were clustered close with IMQ group (Figure 2A), most of samples formed the clear and separate clusters. Then we performed principal component analysis (PCA) to compare the microbiome composition among different groups. Similar results were found that two experimental groups (group A and B) were clustered together but apart from the control group C (Figure 2B).



(A) Principal coordinates analysis (PCoA) based on an unweighted UniFrac analysis of intestinal microbiota. Each point represented a sample. Samples in group A (IMQ group), group B (PC+IMQ group) and group C (CTRL group) were marked as red circles, green triangles and blue crosses, respectively. The PC1 explained 39.05% of total variability and PC2 explained 9% of total variability. (B) Principal Component Analysis (PCA) of the microbiome composition comparing. PC1explained the 61.19% of the total variability found, and PC2 explained the 20.38% of the total variability.

Figure 2. Clustering analysis of three groups with different experimental treatments.

The fecal microbiome in all groups consisted of three dominant phyla: Firmicutes, Proteobacteria, and Bacteroidetes (Figure 3).

A) Firmicutes and Proteobacteria were decreased while Bacteroidetes was increased in IMQ group (group A) compared with CTRL group (group C). In PC+IMQ group (group B), Firmicutes was decreased but Proteobacteria was increased compared with group C. We found the relative abundances are inconsistent between PC+IMQ group (group B) and CTRL group (group C) at genus level (Figure 3B). Subsequently, linear discriminant analysis (LDA) was performed to identify significantly enriched types of bacterial. The bacterial taxa enrichment in samples from group A (blue), group B (orange), and group C (green) at genus level with LDA score (log 10) higher than 4 were shown as barplot (Figure 3C).

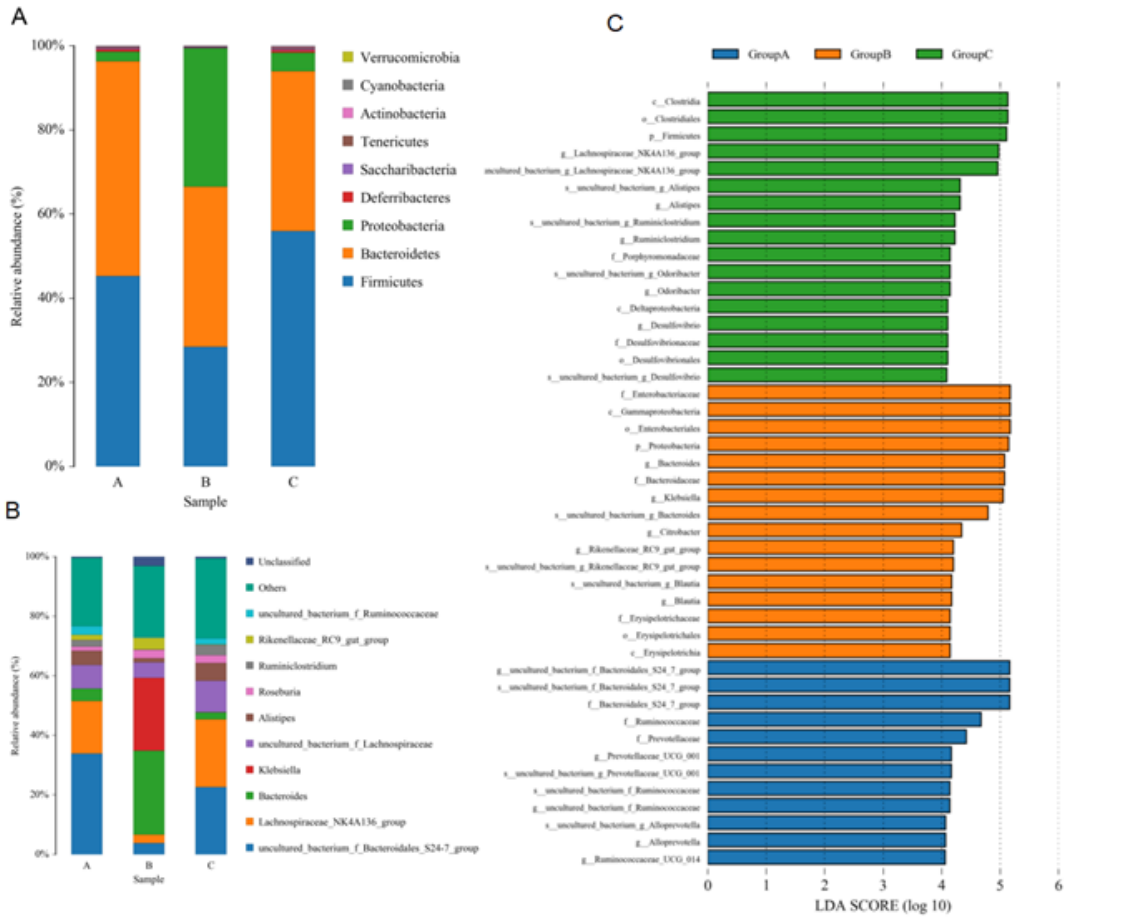
Mice in the experiment group and control group were treated with penicillin and placebo (0.9% saline) respectively till the end of the experiment. During three consecutive days of treatment, we applied IMQ daily on shaved back skin of mice to induce skin inflammation (Supplementary Figure 5). To determine whether reduction of microbiota in adult mice alters disease development, we treated mice with penicillin till the end of the experiment. Mouse skin tissues were collected followed by performing qPCR to detect the expression of inflammatory cytokines including IL-23, IL-17A, IL-17F, IL-4, IL-22 and IL-10. We evaluated the relative expression levels of these five cytokines at 5, 7, 9, 11 and 14 days (Figure 4). We found the expression level changed significantly from day 9. Enhanced expression levels of IL-23, IL-17A and IL-22 were found in IMQ group compared with control group. However, in PC+IMQ group which suffered penicillin treatment, the increase of such three genes expression was reduced.

We applied imiquimod on shaved back skin of mice and the obvious symptoms of psoriasis dermatitis were observed.

DISCUSSION

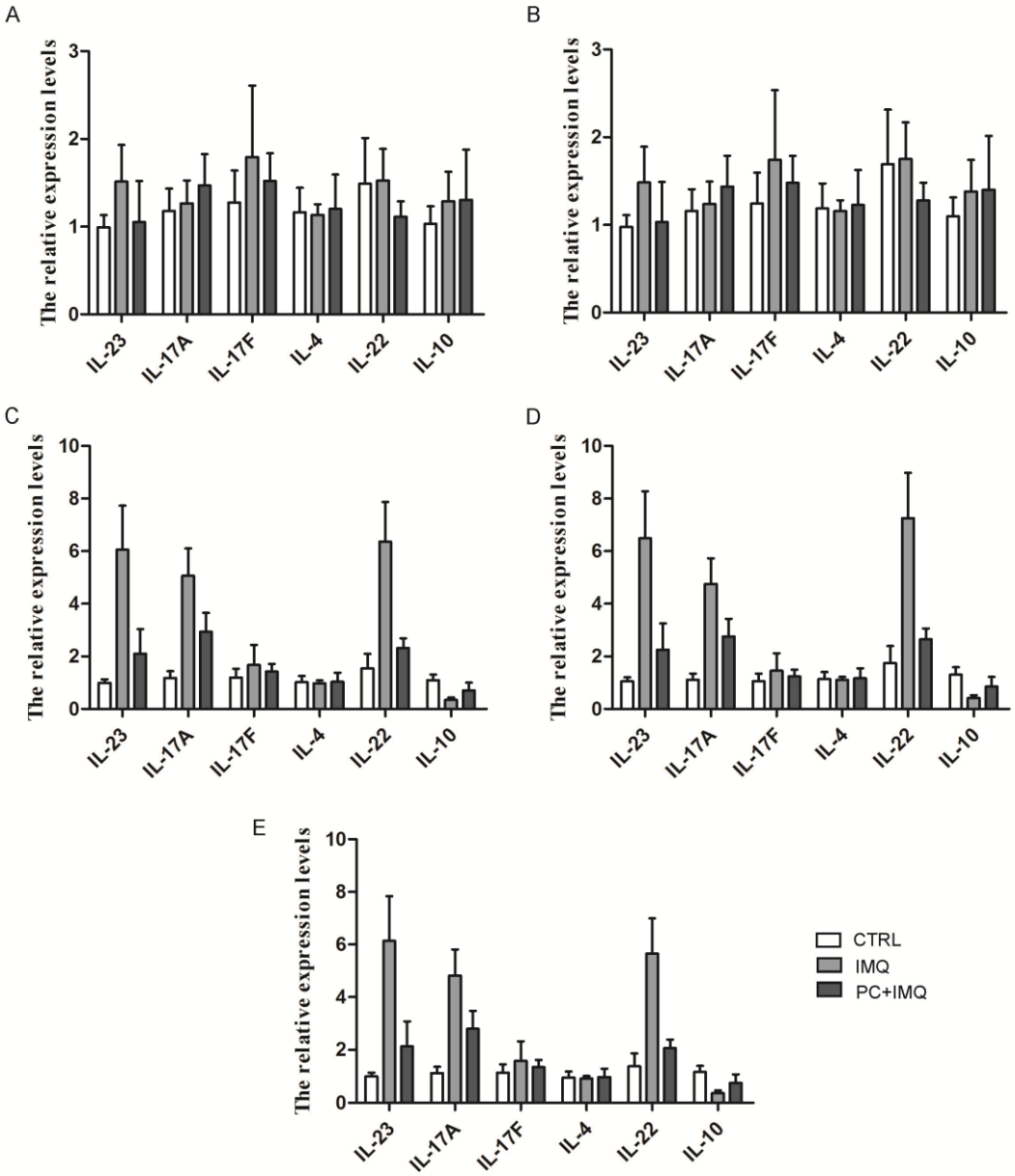
Psoriasis is considered a systemic autoimmune inflammatory and proliferative chronic disease (Deng *et al*., 2016). Although the pathogenesis of psoriasis has not been clearly elucidated, it is hypothesized to be multifactorial and affected by genetic and immune factors. The microbiota in skin and intestines were significantly altered in patients with psoriasis (Stehlikova *et al*., 2019). Previous research observed that microbial infections were commonly related to development and/or aggravation of psoriasis further highlighted the importance of microbiota

in psoriasis induction and pathogenesis (McFadden *et al*., 2009). Infection of streptococcal and colonization of Staphylococcus aureus, Malassezia, or Candida albicans on skin or mucosa were able to accelerate skin disease exacerbation (Weisenseel *et al*., 2002). Altered composition of intestinal flora changes the host's systemic proinflammatory status (Tlaskalová-Hogenová *et al*., 2011). Gut-skin axis takes meaningful role in pathogenesis of psoriasis, which has been recently documented in patients and animal models of psoriasis (Drago *et al*., 2018). Bacterial translocation into bloodstream caused by increased intestinal permeability in psoriatic patients may be connected to outbreaks of plague psoriasis (Ramírez-Boscá *et al*., 2015). Besides, alteration in intestinal microbial diversity happened in obesity patients, particularly reduced abundance of Akkermansia muciniphila has also been monitored in patients with psoriasis (Shen *et al*., 2017). One study demonstrated that mice who ingested probiotic bacterium Lactobacillus reuteri grew thicker skin, denser and shinier fur, and attain better reproductive fitness, which highlighted the importance role of gut-skin axis (Levkovich *et al*., 2013). Another previous work declared that broad spectrum antibiotic treatment (MIX) afforded both conventional and GF mice better resistance to imiquimod (IMQ)-induced skin inflammation (IISI) (Zákostelská *et al*., 2016). Similarly, it was proposed that antibiotic treatment in adult other than newborn mice lead to amelioration of IISI (Zanvit *et al*., 2015).



(A) Phylum and (B) genus level compositions of skin microbiome in group A (IMQ group), group B (PC+IMQ group) and group C (CTRL group). Only the predominant taxa are shown. Other relative lower abundant taxa are not plotted. (C) Bacterial taxa enrichment at genus level was drew by barplot. Terms of group A, group B and group C were colored by blue, orange and green, respectively. Significantly enrichments were showed with LDA score (log 10) > 4.

Figure 3. Taxonomical compositions and microbial signatures associated with each group.



The expression levels of IL-23, IL-17A, IL-17F, IL-4, IL-22 and IL-10 were detected by Real-Time PCR. Barplots showed the dynamic changes at (A) 5 days, (B) 7 days, (C) 9 days, (D) 11 days, and (E) 14 days. White bars represented CTRL groups, grey bars represented IMQ groups, and black bars represented PC+IMQ groups.

Figure 4. Alteration of five cytokine expression levels in different days and groups.

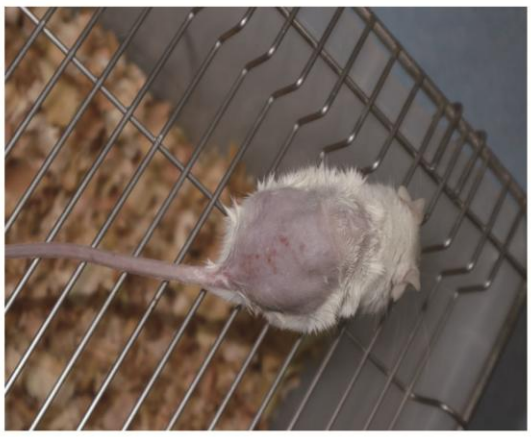


Figure 5. Mice model with induced skin inflammation.

In our study, imiquimod (IMQ)-induced psoriasis model was employed as experimental mouse. The diversity of gut microbiota observed in BALB/c mice with psoriasis was lower than controls, which may result from decreased existence of several taxa. Moreover, compared to controls, mice treated with IMQ Firmicutes and Proteobacteria were significantly decreased while Bacteroidetes were increased. There was decreased Firmicutes but increased of Proteobacteria found in PC+IMQ group in the intestine. The alteration of microbial community varies with different antibiotics. Mice treated with broad spectrum of antibiotics (MIX) had distinctive microbiota components in the intestine, including significantly enriched abundance of genus Lactobacillus. A significantly increased abundance of genera Bifidobacterium and Enterococcus both on the skin and in the intestine were observed in MET (metronidazole)-treated mice, and abundance of Parabacteroides distasonis was found to be the most significant variation in the intestine. Our findings provide a clue for investigating regulation relationship of intestinal and skin microbiota with IISI and underlines indispensable role of microbiota diversity in pathogenesis and development of psoriasis (Stehlikova *et al*., 2019).

Alterations of the microbiota (dysbiosis) are factors associated with the development of inflammatory and systemic autoimmune diseases (Longman and Littman 2015). During the individual's development, the interaction between host and microbe could change. Previously research identified that treatment of antibiotics (mixture of vancomycin and polymyxin B) reduces the harshness of skin inflammation in adult mice with psoriasis-like symptom, while offspring of these parents exposed to antibiotics developed exacerbated psoriasis (Zanvit *et al*., 2015). Although the effect on load of the intestinal flora is usually negligible, such antibiotic treatment can significantly change the composition of microbiota. Given the development and reactivity of immune cells were influenced by indigenous gut microbiota, we inspected the connection among microbiota diversity, T cell response and psoriatic lesion formation using imiquimod-induced murine model of psoriasis. Subsequently, skin inflammation was induced in BALB/c mice to explore the role of microbiota, finding that intraperitoneally injection with Penicillin (PC) solution alleviated severity of skin inflammation through down-regulation of IL-17, IL-23 and IL-22. Inflammation response in in BALB/c mice treated with PC exhibits milder signs of IISI supporting the conclusion that curative effect might mediated by altered composition of microbiota.

Dysbiosis of microbiota observed in this study is in concordance with recent proposed evidence that psoriasis patients contain distinct gut microbiota composition in comparison of healthy individuals. It is characterized by lower diversity and several significantly enriched bacterial taxa (Hidalgo-Cantabrana *et al*., 2019). It has been illustrated that psoriasis patients presented microbiota profile characterized by decrease proportion in Firmicutes and Proteobacteria but increased in Bacteroides at phylum level. The reduction in Bacteroides and Bacteroides is also consistent with previously research of intestinal microbiota alteration in psoriatic arthritis (Scher *et al*., 2015) and psoriasis. Furthermore, we detected increase abundance of Ruminococcus, Blautia, Collinsella, Dorea and Bifidobacterium in psoriasis group, in which was also reported by previous research (Codoñer *et al*., 2018). Given differences in sequencing technology and computational analysis methods (such as database applied for bacterial annotation), divergence existed between studies could be considered as a tolerable outcome (Sinha *et al*., 2017).

In summary, this work aims to characterize intestinal microbial composition in psoriasis disease and to explain putative relation between microbiome and psoriasis progression. By using high-throughput sequencing platforms followed by bioinformatics analysis on 16S gene of microbe, we monitored and assessed the changes in the composition of the mouse intestinal flora during penicillin treatment. Our results revealed that, after intraperitoneally injection of penicillin, obvious alterations were raised including substantial decreased microbial diversity. IISI is directly regulated by intestinal microbiota, which emphasizes the significance of microbiota in pathogenesis of psoriasis. Our findings point to an attractive link between skin inflammation and intestinal microbiota, suggesting gut microbiota have a potentially important role in psoriasis disease.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments were approved by the Animal Care and Use Committee of The Affiliated Jiangning Hospital of Nanjing Medical University (Number: 2020-03-084K01).

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