Research Square

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Altered gut microbiota correlate with different immune response to HAART in HIV-infected individual

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Abstract

Background: Although gut microbiota dysbiosis has recently been reported in HIV infected individuals, the relationship between the gut microbiota and immune activation in patients with different immune response to highly active antiretroviral therapy (HAART) is still not well understood. Gut microbiota and immune activation were studied in 36 non-HIV-infected subjects (Health Control) and 58 HIV-infected individuals which included 28 immunological responders (IR) and 30 immunological non-responders (INR) (≥500 and <200 CD4+ T-cell counts/μl after 2 years of HIV-1 viral suppression respectively) without comorbidities.

Results: Metagenome sequencing revealed that the diversity and composition of gut microbiota could not be recovered completely as normal gut environment in HIV-infected individuals although with immunological response after long-term effective ART. At the genus level, predominant genera Fusobacterium, Ruminococcus_gnavus and Megamonas were more abundant, whereas Faecalibacterium, Alistipes, Bifidobacterium, Eubacteriumrectale, and Roseburia were depleted in IR and INR group than health control. Ruminococcaceae and Alistipes were positively correlated to nadir and current CD4+ T-cell, but negatively correlated to CD8+CD57+ T-cell. Inflammation markers and translocation biomarkers (LPS) levels were positively correlated to the abundance of genus Ruminococcus and Fusobacterium, but were negatively correlated to genus Faecalibacterium. Escherichia-Shigella and Blautia were enriched significantly in IR than INR group. Escherichia-Shigella were negatively correlated to CD4/CD8 ratio, but positively correlated to CD8+CD57+ T-cell.

Conclusions: Altogether, the gut microbiota is one of the factors contributing to different immune response to HAART. Fusobacterium, Alistipes, Ruminococcaceae, Faecalibacterium and Escherichia-Shigella maybe the major genus contributed to different immune response in immunodiscordant and immunoconcordant patients on long-term suppressive ART.

Background

Life expectancy of individuals infected with human immunodeficiency virus (HIV) has increased enormously and HIV infection becomes a chronic manageable disease in combination antiretroviral therapy (ART) era. Most patients with ART can achieve distinct reduction of HIV viral load and improvement of CD4 + T-cell. However, the extent of immunologic recovery varied greatly between individuals. The subjects who maintain low increases of CD4 + T cells despite with complete viral suppression are considered as immunological nonresponders (INR) or immunodiscordant patients [1], which contrast with immunological responders (IR) or immunoconcordant patients [2]. As there is no unified definition of INR, the prevalence of INR ranges from 15–30% in the cohorts [3–5]. The INR were considered when absolute CD4 + T cells fail to reach 200cells/μl after years of ART, although 350cells/μl are also used as cutoff values in the literature [6]. In contrast, IR were defined as CD4 + T-cell counts > 500 cells/μl.

Emerging evidence suggest that the gut microbiome of HIV-infected patients were different from those of HIV-uninfected individuals [7]. However, these studies did not consider the immune response to ART and pay little attention to China [7]. Recently, there are two studies reported that gut microbiota were associated with CD4 recovery in HIV-infected patients [8, 9]. The microbiome of chronic HIV infected individuals were studied in China, however, these patients had heterogeneous HIV progression and immune response to ART [10, 11]. Studies showed that HIV-mediated destruction of gut mucosa lead to local and systemic inflammation [12]. Moreover, chronic inflammation is reported to be associated with gut microbiome in non-AIDS populations [13]. These suggested the gut microbiome may take part in immune activation in HIV-infected individuals with ART [12, 14–16]. Despite the many studies related to microbiome in HIV-infected patients, there have been relatively few reports detailing the gut microbiome that occur in patients with different immune response to ART [17, 18]. Therefore, in this study a comparative study of the gut microbiome and immune activation was conducted in HIV patients with different immune response to ART. 16S ribosomal RNA (rRNA) targeted sequencing and flow cytometry were used to character the gut microbiome and their relationship with immune activation in immunodiscordant and immunoconcordant patients on long-term suppressive ART.

Results

Clinical characteristics and pyrosequencing data summary

Characteristics of the 28 IR patients, 30 INR patients and 36 health controls, including demographics, clinical characteristics, and pyrosequencing results were summarized in Table 1. The rate of MSM transmission route was 57.1% vs 51.7%, while the rate of the heterosexual (HTS) transmission route was 20.7 vs 21.4%, and others were missing from their records. There was no significant difference between the rate of the transmission route in IR and INR group ($p = 0.779$). The viral load of all HIV-infected individuals on ART was not detected. Nadir and current CD4 + T cell count were significantly higher in IR group than INR group (Table 2). No differences in duration of ART and ongoing ART regimen were observed between IR and INR group. Other characteristics such as gender, age and body mass index (BMI) were generally matched between the IR group, INR group and control group.

Table 1
Clinical characteristics data summary

		HIV ART(+)		P-value
	Health Control	Immune Responders(IR)	Immune Nonresponders(INR)	
Number of subjects	36	28	30	
Gender male/female	33/3	25/3	29/1	
Age (mean ± SD)	33.11 ± 3.95	36.64 ± 10.2	36.6 ± 7.19	NS
BMI(mean ± SD)	21.42 ± 3.27	21.06 ± 2.37	20.67 ± 2.74	NS
Smoking	1	0	1	NS
Transmission, no.				
Heterosexual	NA	6	6	NS
Homosexual transmission	NA	16	15	NS
Data Missing	NA	6	8	NS
HAART months (mean ± SD)	NA	37.25 ± 13.61	34.00 ± 10.24	NS
Ongoing cART regimen, no. patients (%)				
NNRTI based	NA	25	27	NS
PI based	NA	3	3	NS

NA (not available), NS (no significant) indicates p-value > 0.05, *compared between IR and INR group.
NNRTI: Non-nucleoside reverse transcriptase inhibitors; PI: Protease inhibitor.

Table 2
Cellular immune activation markers sequencing data summary

		HIV ART(+)		P-value
T cell markers	**Health Control**	**Immune Responders(IR)**	**Immune Nonresponders(INR)**	
Nadir $CD4^+$T cells (mean ± SD)	NA	309.89 ± 128.81	95.23 ± 108.92	< 0.0001
Current $CD4^+$T cells (mean ± SD)	NA	608.30 ± 158.25	230.5 ± 87.50	< 0.0001
Current CD4+/CD8 + T-cell ratio	NA	0.8 ± 0.36	0.35 ± 0.20	< 0.0001
HIV RNA	NA	ND	ND	
%CD4 + HLADR + CD38+	NA	7.72 ± 4.30	10.30 ± 10.45	NS
%CD4 + CD25+	NA	1.26 ± 0.99	1.09 ± 1.04	NS
%CD4 + CD57+	NA	2.57 ± 1.92	2.43 ± 3.30	NS
%CD8 + HLADR + CD38+	NA	20.68 ± 11.35	23 ± 12.39	NS
%CD8 + CD57+	NA	14.36 ± 7.11	23.98 ± 12.30	0.001
Cytokines				
IL-2(pg/mL, mean ± SD)	9.47 ± 6.79	120.96 ± 113.96	153.81 ± 118.79	< 0.0001
IL-4(pg/mL, mean ± SD)	13.49 ± 31.01	34.52 ± 43.30	32.51 ± 31.17	0.009
IL-6(pg/mL, mean ± SD)	8.39 ± 12.57	126.90 ± 95.33	130.82 ± 60.62	< 0.0001
IL-9(pg/mL, mean ± SD)	3.68 ± 6.20	94.00 ± 78.07	96.57 ± 48.71	< 0.0001
IL10(pg/mL, mean ± SD)	2.48 ± 2.35	44.69 ± 37.66	51.32 ± 31.65	< 0.0001
IL-13(pg/mL, mean ± SD)	85.45 ± 65.02	96.76 ± 71.54	82.94 ± 62.96	NS
IL-17A(pg/mL, mean ± SD)	26.22 ± 52.89	133.41 ± 161.61	94.80 ± 83.84	< 0.0001

#Indicate the alpha diversity was calculated after the reads number of each sample were equalized. NA (not available), ND (not detected), NS (no significant) indicates p-value > 0.05, *compared between IR and INR group.

		HIV ART(+)		P-value
IL-17F(pg/mL, mean ± SD)	5.45 ± 5.68	19.15 ± 19.48	20.59 ± 20.34	< 0.0001
IL-21(pg/mL, mean ± SD)	41.33 ± 48.87	104.43 ± 88.11	93.84 ± 56.89	0.002
IL-22(pg/mL, mean ± SD)	153.98 ± 97.15	291.60 ± 176.36	293.10 ± 125.37	0.001
IFN-γ(pg/mL, mean ± SD)	12.55 ± 29.52	81.57 ± 84.50	69.36 ± 83.42	< 0.0001
TNF-α(pg/mL, mean ± SD)	8.39 ± 15.57	38.86 ± 44.19	32.22 ± 26.47	< 0.0001
LPS(pg/mL, mean ± SD)	24.22 ± 18.88	104.98 ± 56.15	76.55 ± 40.05	< 0.0001*
sCD14(pg/mL, mean ± SD)	1583.60 ± 292.80	2480.42 ± 999.88	2142.65 ± 496.53	< 0.0001
Pyrosequecing data				
Sobs index#	194.11 ± 47.45	116.89 ± 39.71	118.11 ± 46.96	< 0.0001
Shannon index#	3.14 ± 0.53	2.47 ± 0.54	2.44 ± 0.56	< 0.0001
Simpson index#	0.12 ± 0.09	0.19 ± 0.11	0.19 ± 0.13	< 0.0001
ACE#	223.97 ± 53.59	147.26 ± 39.98	148.41 ± 49.99	< 0.0001
Chao 1 index#	224.29 ± 54.76	144.52 ± 45.25	145.37 ± 51.01	< 0.0001
Good's coverage (%)#	99.82 ± 0.04	99.87 ± 0.04	99.87 ± 0.04	< 0.0001

#Indicate the alpha diversity was calculated after the reads number of each sample were equalized. NA (not available), ND (not detected), NS (no significant) indicates p-value > 0.05, *compared between IR and INR group.

Total 3,549,077 high-quality sequences were obtained (average sequence length 440 bp) from 94 participants. Average of 38,849 sequences per sample was obtained from control subjects, while 35,947 and 38,134 sequences per sample from IR and INR patients. Rarefaction was conducted on the OTU table to 30,174 reads per sample to avoid methodological artefacts. Specifically, 609 OTUs in the control group, while 486 OTUs and 567 OTUs in IR and INR group respectively were defined at a 97% similarity level. Significant difference of bacterial diversity (Shannon, Simpson, and Sobs), richness (ACE, Chao1) and

Good's coverage were observed among the three groups, while there was no significant difference was found between IR group and INR group. The summary information was shown in Table 2.

Compositional analysis of fecal microbiota

Principal coordinate (PCoA) analysis by weighted UniFrac matrices showed obvious differentiation of bacterial communities between IR and controls (PERMANOVA, pseudo-F: 8.99, R^2 = 0.13, P = 0.001, Fig. 1a), INR and controls (PERMANOVA, pseudo-F: 8.77, R^2 = 0.12, P = 0.001, Fig. 1b); while no significant difference were observed between IR and INR group (PERMANOVA, pseudo-F: 0.80, R^2 = 0.01, P = 0.71, Fig. 1c). The average abundance of each bacterial phylum and genus level in patients and controls were showed respectively (S1 and S2 Figs).

The Wilcoxon rank sum test was used to detect taxa that showed significant differences in abundance among groups (confidence interval method). At the phylum level, Bacteroidetes, Actinobacteria, Tenericutes and Lentisphaerae were more abundant in health control than IR group. Proteobacteria, Fusobacteria and Saccharibacteria were significantly enriched in IR group (Fig. 2a). The relative abundance of 11 families was significantly different between IR and control groups. The relative abundance of 93 genera, including 15 predominant (> 1% of the total sequences in either group) and 78 less-predominant genera, were significantly different between the control and IR groups. Among the different predominant genera, *Lachnoclostridium*, *Megasphaera*, *Escherichia-Shigella*, *Veillonella*, *Streptococcus*, *Fusobacterium*, and *Ruminococcus_gnavus* were found overrepresented in IR patients. *Faecalibacterium, Eubacterium_rectale_group, Alistipes, Subdoligranulum, Bifidobacterium, Roseburia, Ruminococcaceae* and *Parasutterella* were enriched in controls (Fig. 2b). A taxonomy-based bacterial comparison was conducted to define the differences between the control and INR groups. At the phylum level, Bacteroidetes, Actinobacteria, Lentisphaerae were more abundant in control group than INR group, while Proteobacteria, Fusobacteria, Tenericutes, Saccharibacteria and unclassified k norank were more abundant in INR group (Fig. 2c). At the genus level, the relative abundance of 83 genera (including 11 predominant genera) was different between the control and INR group. The relative proportions of *Faecalibacterium, Eubacterium*_rectale_group, *Alistipes, Bifidobacterium*, *Blautia, Roseburia and Ruminococcaceae* were more abundant in health control than INR group. *Parasutterella, Megasphaera, Fusobacterium, and Ruminococcus_gnavus* were found overrepresented in INR patients (Fig. 2d). Although there were no significant difference between IR and INR group at the phylum level (Fig. 2e), the abundances of 12 genera (including 2 predominant genera) differed between the IR and INR groups. The two predominant genera *Escherichia-Shigella and Blautia* enriched significantly in IR than INR group (Fig. 2f).

In order to identify the key phylotypes responsible for the difference in distinguishing fecal microbiota of different groups, linear discriminant analysis (LDA) effect size (LEfSe) was performed and an effect-size threshold of 3 was used. Taxonomic cladograms that represent the microbiota structure and predominant bacteria in the three groups were displayed; and the largest differences between the two communities were shown in S3 Fig.

Compare of T-cell activation in IR and INR group

As expected, nadir CD4 + T-cell, current CD4 + T-cell counts and CD4/CD8 ratio were lower in INR than IR group ($p < 0.0001$). Proportions of CD8 + CD57 + T-cell in INR group was significantly lower than IR group ($p < 0.001$). The proportion level of CD4 + and CD8 + T-cells immune activation (CD4/8 + T-cell by expression of CD25+, HLA-DR+, and HLA-DR+/CD38+) was similar in INR and IR group (Table 1).

Bacterial translocation markers and inflammation profiles comparison

Lipopolysaccharide (LPS) which translocate from the gut to blood stream was commonly used as the major antigens driving chronic immune activation. Levels of LPS were significantly increased in INR group when compared with others ($p < 0.0001$). However the soluble immune activation marker sCD14 was not different between groups. Of the 13 markers studied, the level of IL-13 was not different among groups and twelve markers (IL-2, IL-4, IL-5, IL-6, IL-9, IL10, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α) were shown to be significantly higher in INR and IR groups when compared with the control, but there was no significant difference between INR and IR groups (Table 1).

Association between fecal microbiota and immune activation

Spearman correlations among the relative abundance of bacteria genera and levels of T-cell activation, inflammation or translocation biomarkers were evaluated (Fig. 3). Interestingly, nadir CD4 + T-cell counts levels were positively correlated to the abundance of *Ruminococcaceae* and *Alistipes*, while current CD4 + T-cell counts were strongly positively to the abundance of *Ruminococcaceae* and *Subdoligranulum*. *Fusobacterium* were negatively correlated to nadir and current CD4 + T-cell. CD4/CD8 ratio were positively correlated to genus *Faecalibacterium* and *Ruminococcaceae*, but negatively correlated to *Escherichia-Shigella*. Moreover, CD8 + CD57 + T-cell was positively correlated to *Escherichia-Shigella*, but was negatively correlated to genus *Ruminococcaceae* and *Alistipes*. Inflammation markers and LPS were positively correlated to the abundance of genus *Ruminococcus* and *Fusobacterium*, but were negatively correlated to genus *Faecalibacterium* (Fig. 3).

Discussion

The goal of highly active antiretroviral therapy (HAART) is immune reconstitution following successful viral suppression in HIV-infected patients [2]. Despite with complete viral suppression, the CD4 + T cell is not reversed completely and microbial translocation continues after peripheral CD4 + T cell restoration [19–22]. The gut microbiota has been reported had critical impact on human biology and pathophysiology. The gut microbiota as a key factor for immune homeostasis is accepted widely now [22]. Compositional and functional changes of the gut microbiota have pointed a novel link between the gut bacterial community and immunity in treated HIV-infected individuals [7, 23]. However, the way the microbiome contributes to immune activation in HIV positive individuals is poorly understood. We

supposed that gut microbiome may play an important role in different immune response to long-term ART in HIV + individuals. So in this study, we investigated the fecal microbiome and immune activation in HIV + individuals with different immune response to long-term ART in China.

The microbiome dysbiosis in HIV-infected individuals has been described in several studies [7], however, there is only few study included a handful of IR and INR patients and without considering the immune response to HAART [24]. The current study, which included the so far higher number of IR and INR patients, expand the previous observations and investigate the relationship between gut microbiota and immune activation. It found that ecological indices of microbiota (including community diversity, richness and observed species numbers) were significantly lower in IR and INR group when compared to health control. Additionally, PCoA analysis showed remarkable differentiation of bacterial communities between IR, INR and health control. These suggested that the diversity and composition of gut microbiota could not be recovered completely as normal gut environment in HIV-infected individuals although with immunological response after long-term effective ART.

Furthermore, we found that IR and INR group had a unique bacterial signature at phylum level. Proteobacteria, Fusobacteria, and Saccharibacteria were more abundant, whereas Bacteroidetes, Actinobacteria and Lentisphaerae were depleted in IR and INR group. At the genus level, the predominant genera *Fusobacterium, Ruminococcus_gnavus and Megamonas* were more abundant, whereas *Faecalibacterium, Alistipes, Bifidobacterium, Eubacterium rectale*, and *Roseburia* were depleted in IR and INR group than health control. Taxa from bacterial phyla Proteobacteria, Fusobacteria and Bacteroidetes have been reported different between HIV positive and negative individuals in studies. Our result is consistent with most of published studies which reported Proteobacteria were more abundant in HIV-infected individuals although others reported no change [10, 11, 25–30]. The phylum Fusobacteria (most driven by its constituent genus *Fusobacterium*) which was reported associated with intestinal inflammation [31, 32] and enriched in HIV-infected individuals in some studies [26, 30, 33, 34]. Here, we found *Fusobacterium* enriched in IR and INR group. Furthermore, inflammation markers and translocation biomarkers (LPS) were positively correlated with *Fusobacterium*, while nadir and current CD4 + T-cell counts were negatively correlated to *Fusobacterium* in this study.

The phylum Bacteroidetes which include the families Bacteroidaceae, Prevotellaceae, Porphyromonadaceae, and Rikenellaceae, exhibited a more heterogeneous pattern of changes in HIV-infected individuals [10, 11, 22, 25–30, 33–37]. The family Bacteroidaceae (mostly driven by the abundance of the genus *Bacteroides*) is generally considered to play an anti-inflammatory role [38–41], were depleted in IR and INR group. The bile-tolerant family Rikenellaceae (mostly driven by the abundance of the genus *Alistipes*) which is protective properties against C. difficile infection [41, 42], were overall depleted in IR and INR group. Actinobacteria which was reported similar in the proximal gut of HIV-infected patients and negative controls [29], were depleted in IR and INR group in this study. The *Alistipes* was negatively correlated to CD8 + CD57 + T-cell and positively correlated to nadir CD4 + T-cell counts. Notably, the commonly used probiotics *Bifidobacterium* which belong to the phylum of Actinobacteria were depleted in IR and INR group than health control [43].

Although the abundance of phylum Firmicutes is similar in the three groups, the genus such as *Faecalibacterium* and *Ruminococcaceae* were depleted in IR and INR group than health control. *Ruminococcaceae* have been associated with both protective and disruptive roles within the gut microbial community, such as the production of anti-inflammatory SCFA[44] or the degradation of host mucus and potential proinflammatory role in IBD[45]. In this study, the *Faecalibacterium* and *Ruminococcaceae UCG-002*, which belong to the family of *Ruminococcaceae* were depleted in IR and INR group than health control, Interestingly, *Ruminococcaceae* were positively correlated to the current and nadir CD4 + T-cell counts while negatively correlated to CD8 + CD57 + T-cell. In additionally, inflammation markers and LPS were positively correlated to the abundance of genus *Ruminococcus*. The *Faecalibacterium* has been reported as anti-inflammatory commensal genus [26, 46]. In this study, the genus *Faecalibacterium* was positively correlated to CD4/CD8 ratio, while negatively correlated with inflammation markers and LPS. The family Enterobacteriaceae is associated with inflammation[47], *Escherichia-Shigella* which belonged to Enterobacteriaceae were negatively correlated to CD4/CD8 ratio, but positively correlated to CD8 + CD57 + T-cell which is hallmark of immunosenescence in HIV infection[48, 49]. Furthermore, *Escherichia-Shigella* was more abundant in IR group than INR group in this study. These suggested that *Fusobacterium, Alistipes, Ruminococcaceae, Faecalibacterium* and *Escherichia-Shigella* maybe the major genus contributed to different immune response in immunodiscordant and immunoconcordant patients on long-term suppressive ART.

Altogether, these results highlight that immune activation in HIV-infected patients is associated with the observed gut microbiota dysbiosis. Based upon these findings, we speculate that the gut microbiota may be one of the factors contributing to different immune response to HAART. We acknowledge that the extensive dietary data and living conditions of these subjects which are not controlled may lead to biases in our analysis. On the other hand, while correlation analysis is useful to have clues of biological links to the impact of dysbiosis in immune response in these patients, a direct manipulation of microbiome is needed to validate their cellular and biochemical action in vitro or in vivo. And the exact mechanisms how HIV infection induced dysbiosis in the gut need to be studied in future study.

Conclusions

In summary, we report the gut microbiota dysbiosis in HIV-infected immunological nonresponders and immunological responders. The differences are robust in number of observed species, richness and community composition. This report also provides the gut microbiota difference between immunological nonresponders and immunological responders. Furthermore, these compositional dysbiosis correlated with different immune response in HIV-infected patients.

Methods

Recruitment of subjects

The process of participants' recruitment and sample collection are depicted in Suppl. FigS4. A total of 58 HIV-infected individuals were recruited, including 28 immunological responders (IR), 30 immunological non-responders (INR), and 36 healthy subjects (control). IR and INR were defined as CD4 + T-cell counts/μl ≥ 500 or < 200 after 2 years of virus controlled, respectively. Subjects were recruited from the HIV clinic of the first affiliated hospital of Zhejiang University from November 2015 to October 2017. All HIV-positive participants were diagnosed by the Disease Control and Prevention Center of Zhejiang Province. In the HIV + cohort, all subjects were on two nucleoside reverse transcriptase inhibitors (NRTIs) + nonnucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitor based therapy: Zidovudine/Tenofovir Disoproxil Fumarate(AZT/TDF) + Lamivudine(3TC) + Efavirenz (EFV) or Lopinavir/ritonavir (LPV/r). Controls were healthy HIV negative volunteers and most of them were staff of the institution. Age, gender, and body mass index (BMI) of the controls matched those of HIV individuals (Table 1). The exclude criteria were following: age > 60 y; opportunistic infection; hepatitis B or C infection; antibiotics, immunosuppressive regimen, probiotics, prebiotics, or synbiotics usage in previous 6 months; use of rectally administered medications within 48 h; BMI > 30; inflammatory bowel disease (IBD) history; active inflammation affecting the gastro intestines.

Ethics Statement

All participants provided written informed consent before entering the study. This study conformed to the ethical norms of the 1975 Helsinki Declaration. The research protocol was approved by the Institutional Review Committee of the First Affiliated Hospital of Zhejiang University on October 7, 2015. All data analyzed were anonymized.

Fecal samples collection and DNA extraction

Fecal samples of participants were collected in sterile container before their clinic visit and stored at − 80 °C until DNA extraction. DNA was extracted by QIAamp DNA stool mini kit (QIAGEN, Hilden, Germany) and glass-bead beating on Mini-bead beater (FastPrep; Thermo Electron Corporation, Boston, MA, USA) according to the manufacturer's instructions. DNA quantification and purity were assessed by NanoDrop ND-1000 spectrophotometer (Thermo Electron Corporation). The integrity and size of DNA were checked by 1.0% agarose gel electrophoresis. DNA was stored at − 20 °C for further analysis.

Polymerase chain reaction (PCR) and 16S rRNA gene sequencing

PCR amplification of the bacterial 16S rRNA gene V3–V4 region was performed using universal primers (338F 5'- ACTCCTACGGGAGGCAGCAG-3', 806R<5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μl mixture containing 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST

(Promega, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database and was accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA533202.

Bioinformatics and statistics

The raw fastq files were demultiplexed and quality-filtered using Trimmomatic, and then merged by FLASH. The original reads were trimmed with a minimum length of 50 bp and an average quality score of 20. Two mismatches were allowed in primer sequences and reads containing ambiguous bases were removed. Ten hompolymers authorized in sequences was limited. Operational taxonomic units (OTUs) were picked with 97% similarity threshold by UPARSE(http://drive5.com/uparse/) and chimera identification sequence was performed using UCHIME. Taxonomy-based analyses were performed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) in combination with 16 s rRNA Silva128 database with 70% cut-off confidence.

Viral Load, Flow Cytometry and Immunophenotype

Quantifications of CD4 + and CD8 + T-cells and HIV-1 RNA were carried out in HIV- infected individuals by flow cytometry and Cobas Amplicor (Roche Molecular Systems Inc., Branchburg, New Jersey, USA) as clinical routine. The percentage of CD4 + and CD8 + T cells expressing markers of activation (CD25+, CD38+, HLADR+, or CD38+/HLA-DR+) and senescence(CD57+) were quantified by BD FACS Canto II flow cytometer (BD Biosciences, California, USA) using fresh anticoagulated whole blood. Antibody such as CD3-FITC, CD4- PerCP/Cy5.5, CD8-Brilliant Violet 510™, CD38-Brilliant Violet 421, CD25-PE, HLA-DR-APC/Fire™ 750, and CD57-allophycocyanin (APC) were purchased from Biolegend (San Diego, CA).

Bacterial translocation and immune activation markers

Sera samples from 27 IR, 30 INR, and seventeen heathy participants were collected to measure immune activation markers. These markers were quantified using LEGENDplex™ Human Th Cytokine Panel (Biolegend,San Diego, CA): IL-2, IL-4, IL-6, IL-9, IL10, IL-13, IL-17A, IL-17F, IL-21, IL-22, TNF-α and interferon (IFN)-γ in line with the manufacturer's instructions. Human Lipopolysaccharides (LPS) ELISA Kit (CUSABIO; Wuhan, China) and Human and soluble CD14 (sCD14) ELISA Kit (MultiSciences, Hangzhou, China) were used to test plasma LPS and sCD14 according to the standard protocols. Two replicate was performed for each assay.

Statistics analysis

OTUs that reached a 97% level of nucleotide similarity level were used for alpha diversity (Shannon, Simpson, and Sobs), richness (ACE and Chao1), Good's coverage, rarefaction curve and phylogenetic beta diversity measures analyses by mothur. PERMANOVA (permutational multivariate analysis of variance) was used to assess beta diversity based on UniFrac distances. The number of permutations was 999 for PERMANOVA. The results were imported into Phyloseq for subsampling normalization,

manipulation, and graph visualization by R (V.3.1.3, The R Project for Statistical Computing)[50]. Linear discriminant analysis (LDA) effect size (LEfSe) uses the Kruskal-Wallis rank sum test to detect features with significant different abundances between assigned taxa. LEfSe are available online in the Galaxy workflow framework[51]. Principal Coordinates analysis (PCoA) was generated for weighted Unifrac data to visualize the microbial communities. The one-way ANOVA, non-parametric test, Wilcoxon rank sum test and Mann-Whitney U test which used for comparisons between groups were conducted in R package and SPSS software (version 21, SPSS, Inc., Chicago, IL, USA). The average abundance values for each bacterium are depicted as mean ± SD. A significant alpha of 0.05 and LDA effect size threshold of 3 were used for all biomarkers. Correlations between variables were calculated using Spearman's rank-correlation analysis by R package; and associations with a Benjamini – Hochberg adjusted p-value lower than 0.01 were considered relevant.

Declarations

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Competing interests

The authors declare that they have no conflict of interest.

Informed consent:

Written consent was obtained from the patient.

Author Contributions

Yirui Xie and Jia Sun participated in the design of the study and performed the statistical analysis and wrote the paper. Li Wei, Haiyin Jiang, Caiqin Hu and Jiezuan Yang carried out the experiment. Caiqin Hu and Ying Huang collected biopsy samples. Bing Ruan helped to review the manuscript. Biao Zhu participated in the design and review the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database and was accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA533202.

Ethics approval and consent to participate

This study conformed to the ethical norms of the 1975 Helsinki Declaration. The research protocol was approved by the Institutional Review Committee of the First Affiliated Hospital of Zhejiang University on October 7, 2015. All data analyzed were anonymized. All participants provided written informed consent before entering the study.

Consent for publication

Not applicable.

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Figures

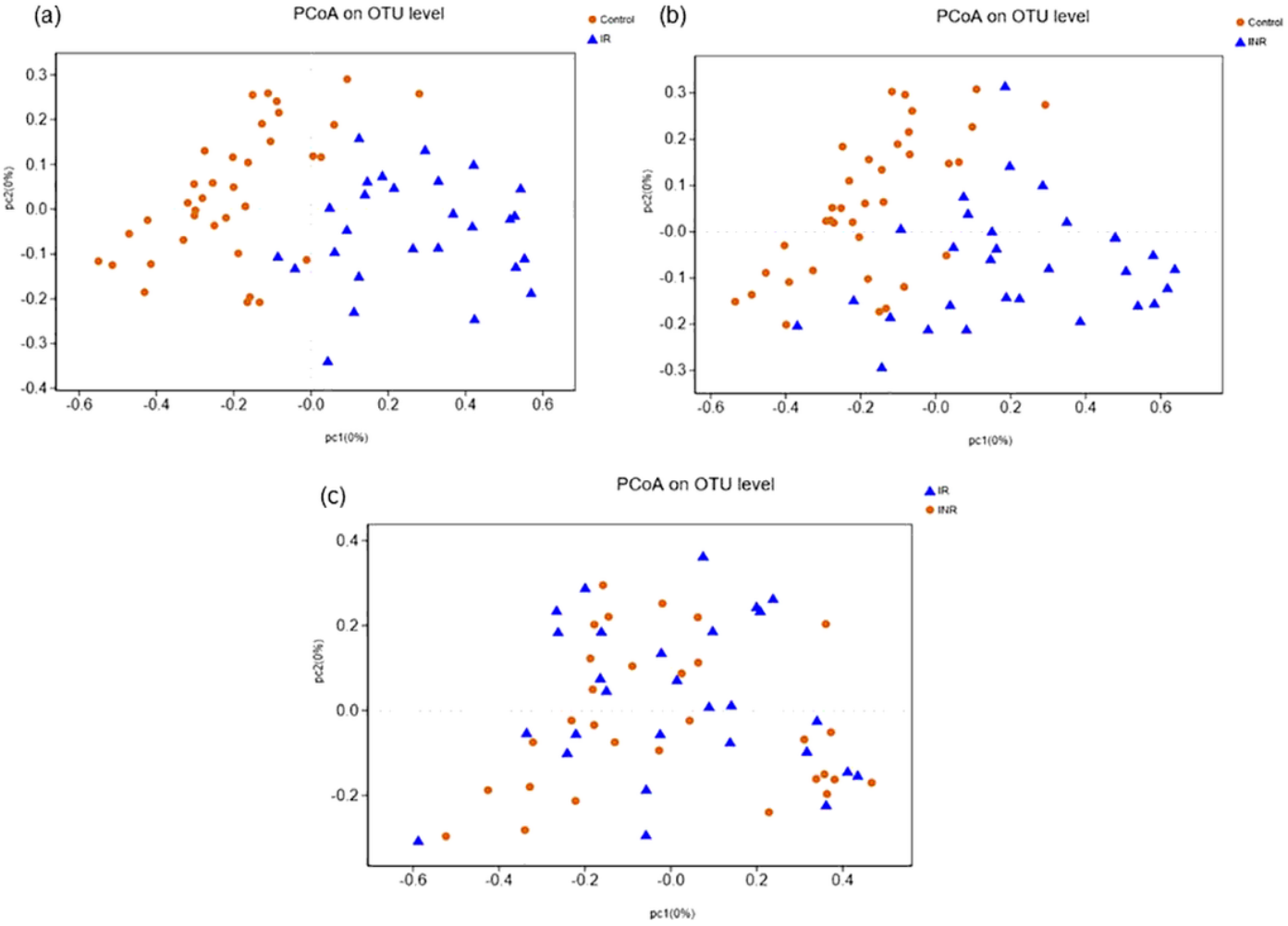

Figure 1

Principal coordinates analysis (PCoA) for microbiomes of the patients and health control groups. Remarkable bacterial communities difference between immunological responders (IR), immunological

non-responders (INR) and health control (Control) group (Fig 1a; Fig 1b); no significant difference between IR and INR group (Fig 1c).

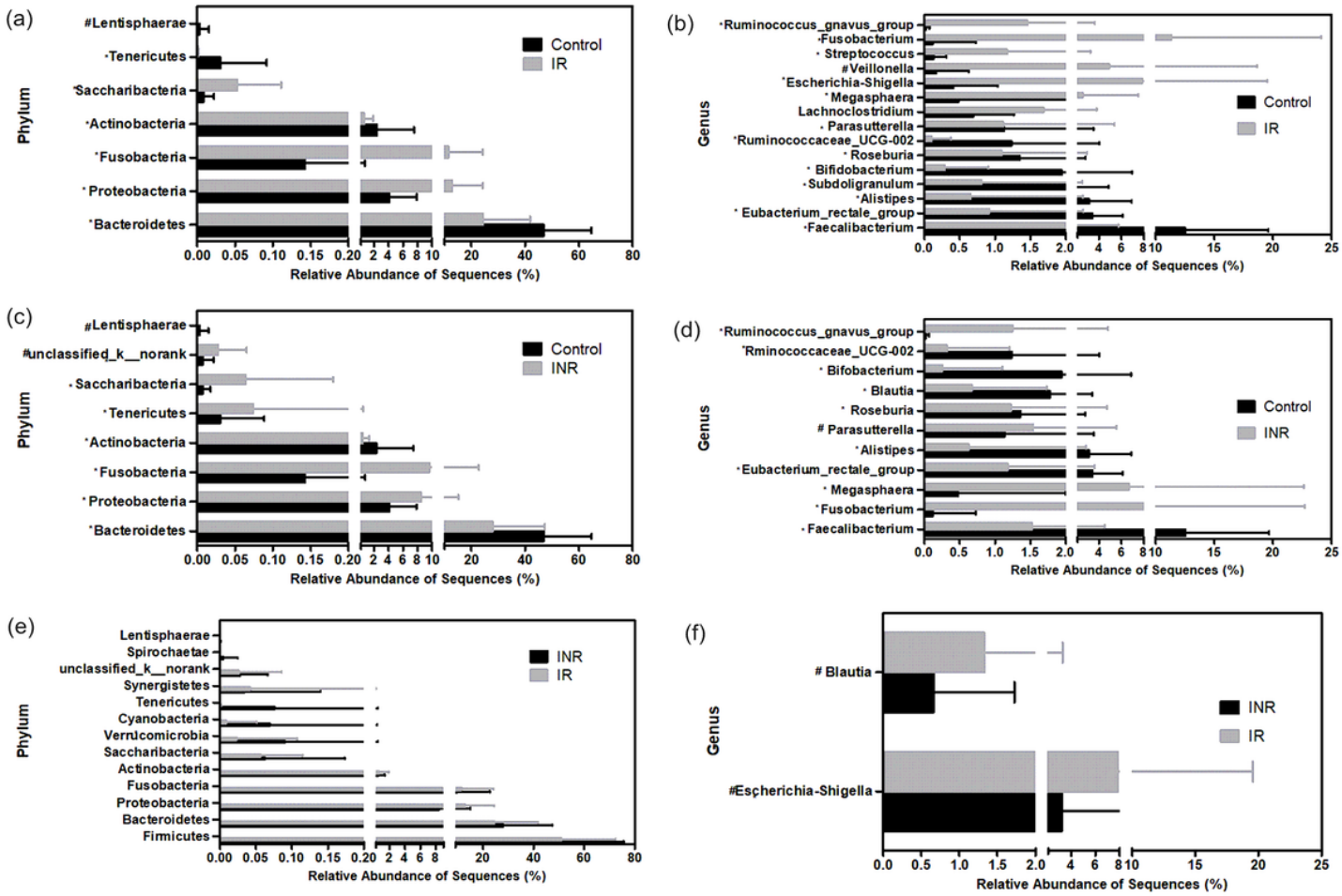

Figure 2

Taxonomic differences of fecal microbiota between the patients and health control groups. Comparison of relative abundance at the bacterial phylum (a,c,e), and genus (b,d,f) levels between immunological responders (IR), immunological non-responders (INR) and health control (Control) group. # indicates $P < 0.05$. * indicates $P < 0.01$. The average abundance values for each bacterium are depicted as mean ± SD.

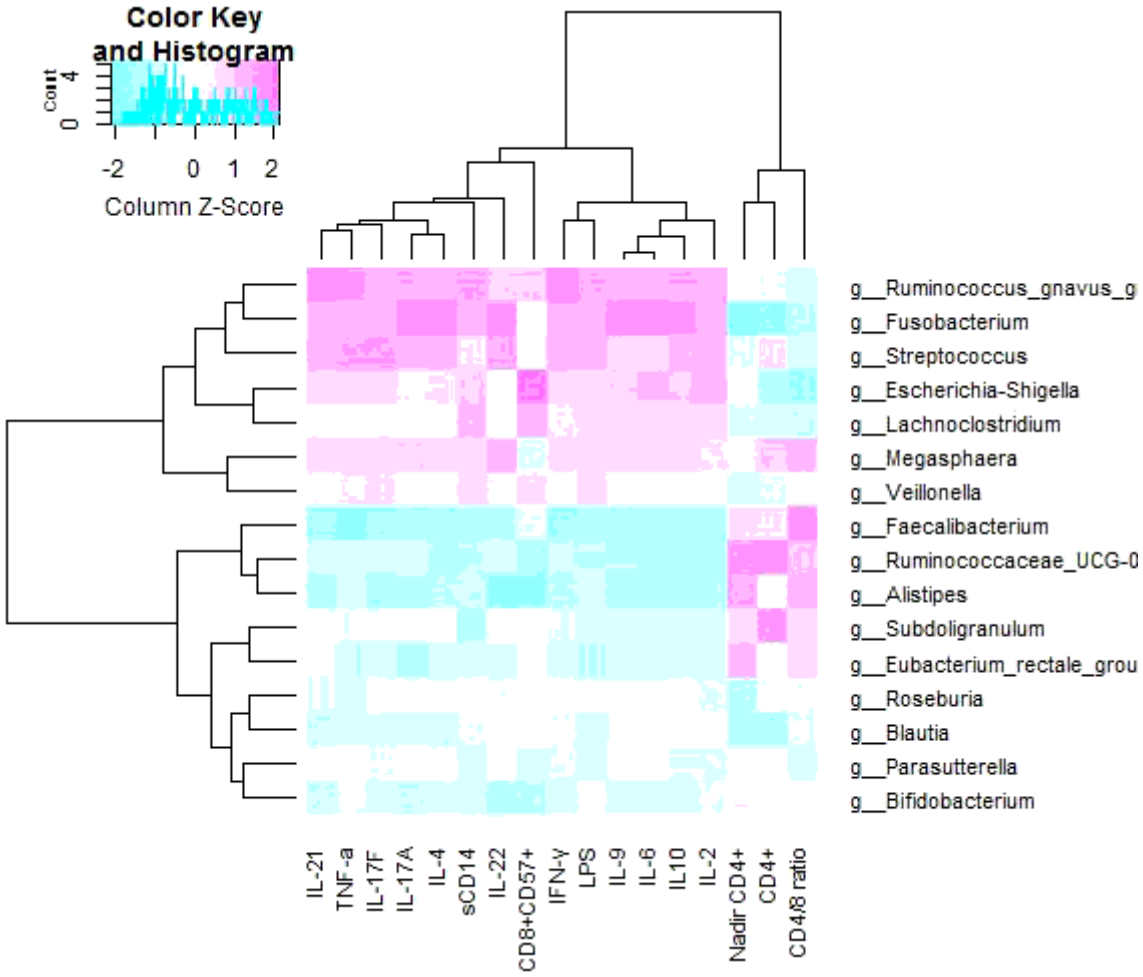

Figure 3

The composition of fecal microbiota correlates with markers of immune activation. Some cellular and soluble markers of immune activation correlated to specific genera of gut microbiota. Spearman's correlation was used. Associations with a Benjamini – Hochberg adjusted p-value lower than 0.01 were considered relevant.

Supplementary Files

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