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

Yirui Xie (1312019@zju.edu.cn)
Zhejiang University School of Medicine First Affiliated Hospital https://orcid.org/0000-0002-0591-4122
Jia Sun
Zhejiang University School of Medicine First Affiliated Hospital
Li Wei
Zhejiang University School of Medicine First Affiliated Hospital
Haiyin Jiang
Zhejiang University School of Medicine First Affiliated Hospital
Caiqin Hu
Zhejiang University School of Medicine First Affiliated Hospital
Jiezuan Yang
Zhejiang University School of Medicine First Affiliated Hospital
Ying Huang
Zhejiang University School of Medicine First Affiliated Hospital
Bing Ruan
Zhejiang University School of Medicine First Affiliated Hospital
Biao Zhu
Zhejiang University School of Medicine First Affiliated Hospital

Research article

Keywords: HIV-1, Gut microbiota, immune activation, immunological responders, immunological nonresponders, HAART

Posted Date: September 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-44062/v2

Version of Record: A version of this preprint was published on January 6th, 2021. See the published version at https://doi.org/10.1186/s12866-020-02074-1.

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 a 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 gut microbiota dysbiosis could not be recovered completely in HIV-infected immunological responders and immunological non-responders although with long-term suppressive antiretroviral treatment. At the 97% similarity level, *Fusobacterium, Ruminococcus_gnavus* and *Megamonas* were more abundant, whereas *Faecalibacterium, Alistipes, Bifidobacterium, Eubacteriumrectale* and *Roseburia* were more depleted in the IR and INR groups than that in the health control. *Ruminococcaceae* and *Alistipes* were positively correlated with nadir and current CD4+ T-cell counts, but negatively correlated with CD8+CD57+ T-cell counts. Inflammation markers and translocation biomarkers (LPS) levels were positively correlated with the abundance of genus *Ruminococcus* and *Fusobacterium,* but were negatively correlated with the genus *Faecalibacterium*. The relative abundances of *Escherichia-Shigella* and *Blautia* were significantly higher in the IR than that in the INR group. *Escherichia-Shigella* were negatively correlated with the CD4/CD8 ratio but positively correlated with the amount of *C*D8+CD57+ T-cells. *Roseburia* and *Blautia* were negatively associated with nadir CD4+ T-cell and positively associated with CD8+CD57+ T-cell counts.

Conclusions: Gut microbiota dysbiosis may be one of the factors contributing to different immune response to HAART. *Fusobacterium, Alistipes, Ruminococcaceae, Faecalibacterium*, *Escherichia-Shigella, Roseburia* and *Blautia* maybe the major genus contributed to different immune response in immunodiscordant and immunoconcordant patients on long-term suppressive ART. *Blautia* and *Roseburia* might be associated with treatment outcome.

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 compared with nadir CD4 + T-cell count (the patient's lowest CD4 count). However, the extent of immunologic recovery varied greatly with each individual. 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% to 30% in the ART cohorts [3-5]. The INR were defined when

absolute CD4+ T cells failed to reach 200 cells/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 after receiving ART for years.

Emerging evidence suggested 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 implement few in China [7]. Recently, several studies reported that gut microbiota was associated with CD4 recovery in HIV-infected patients [8-10]. The microbiome of chronic HIV infected individuals were studied in China, however, these patients had heterogeneous HIV progression and immune response to ART [11, 12]. Studies showed that HIV-mediated destruction of gut mucosa lead to local and systemic inflammation [13]. Moreover, chronic inflammation was reported to be associated with gut microbiome in non-AIDS population [14]. These suggest the gut microbiome may take part in immune activation in HIV-infected individuals with ART [13, 15-17]. Despite 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 [18, 19]. 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. There was no significant difference between the rate of the transmission route in the IR and INR groups (p=0.779). The rate of MSM transmission route was 57.1% vs 51.7% in the INR and IR group, while the rate of the heterosexual (HTS) transmission route was 20.7% vs 21.4%, and others were missing from their records. The viral load of all HIV-infected individuals on ART was not detected. Nadir and current CD4+ T cell counts were significantly higher in the IR group than that of INR group (Table 2). No differences in duration of ART and ongoing ART regimen were observed between the IR and INR groups. Other characteristics such as gender, age and body mass index (BMI) were generally matched among the IR, INR and control 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 from the control subjects, while 35,947 and 38,134 sequences per sample were obtained from the 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 found between the IR and INR groups. 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 the IR and control groups (PERMANOVA, pseudo-F: 8.99, R^2=0.13, P =0.001, Fig.1a), the INR and control groups (PERMANOVA, pseudo-F: 8.77, R^2=0.12, P =0.001, Fig.1b); while no significant difference were observed between the IR and INR groups (PERMANOVA, pseudo-F: 0.80, R^2=0.01, P =0.71, Fig. 1c).

The average relative abundance of each bacterial phylum and genus in patients and controls were showed respectively (Fig. 2; Fig. 3; S1, S2 and S3 Fig; S1 and S2 Table). The Wilcoxon rank sum test was used to detect taxa with significant differences in relative abundance among groups (confidence interval method). At the phylum level, Bacteroidetes, Actinobacteria, Tenericutes and Lentisphaerae were more abundant in the control group than the IR group. The relative abundances of Proteobacteria, Fusobacteria and Saccharibacteria were significantly higher in the IR group (Fig. 2 and S1 Fig. 2a) than that in the control group. The relative abundance of 11 families was significantly different between the IR and control groups. The relative abundances 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 the IR group. The relative abundances of *Faecalibacterium, Eubacterium_rectale_group, Alistipes, Subdoligranulum, Bifidobacterium, Roseburia, Ruminococcaceae* and *Parasutterella* were higher in the control group (Fig. 3 and S1 Fig. 2b) than in the IR group. 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 the control group than that in INR group, while Proteobacteria, Fusobacteria, Tenericutes, Saccharibacteria and unclassified k norank were more abundant in the INR group than that in the control group (Fig. 3 and S1 Fig. 2c). At the genus level, the relative abundances of 83 genera (including 11 predominant genera) were different between the control and INR groups. The relative proportions of *Faecalibacterium, Eubacterium*_rectale_group, *Alistipes, Bifidobacterium*, *Blautia, Roseburia* and *Ruminococcaceae* were more abundant in the control group than that in the INR group. *Parasutterella, Megasphaera, Fusobacterium, and Ruminococcus_gnavus* were found overrepresented in the INR group (Fig. 3 and S1 Fig. 2d). Although there was no significant difference between the IR and INR group at the phylum level (Fig. 2 and S1 Fig. 2e), the abundance of 12 genera (including 2 predominant genera) differed between the IR and INR groups. The abundances of two predominant genera *Escherichia-Shigella* and *Blautia* were significantly higher in the IR than that in the INR group (Fig. 3 and S1 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 S4 Fig.

Compare of T-cell activation in the IR and INR groups

As expected, nadir CD4+ T-cell, current CD4+ T-cell counts and CD4/CD8 ratio were lower in the INR group than in the IR group ($p<0.0001$). The proportion of CD8+CD57+ T-cell in the INR group was significantly lower than in the 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 the INR and IR groups (Table 1).

Bacterial translocation markers and inflammation profiles comparison

Lipopolysaccharide (LPS) which translocated from the gut to blood stream was commonly used as the major antigens driving chronic immune activation. The level of LPS was significantly increased in the INR group when compared with others ($p<0.0001$). However, the soluble immune activation marker sCD14 was not different between the 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 the INR and IR groups when compared with the control, but there was no significant difference between the 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. 4). Interestingly, nadir CD4+ T-cell counts were positively correlated with the abundance of *Ruminococcaceae* and *Alistipes*, while current CD4+ T-cell counts were strongly positively correlated with the abundance of *Ruminococcaceae* and *Subdoligranulum*. The genus *Fusobacterium* was negatively correlated with nadir and current CD4+ T-cell. The CD4/CD8 ratio was positively correlated with the genus *Faecalibacterium* and *Ruminococcaceae,* but negatively correlated with *Escherichia-Shigella*. Moreover, the CD8+CD57+ T-cell counts was positively correlated with *Escherichia-Shigella*, but was negatively correlated with the genus *Ruminococcaceae* and *Alistipes*. The genus *Roseburia* and *Blautia* were negatively associated with nadir CD4+ T-cell and positively associated with CD8+CD57+ T-cell counts. Inflammation markers and LPS were positively correlated with the *Ruminococcus* and *Fusobacterium*, but were negatively correlated with the genus *Faecalibacterium* (Fig. 4).

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 [20-

23]. 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 [23]. 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, 24-26]. However, the way the microbiome contributes to immune response in HIV positive individuals is poorly understood. 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, 25, 26], however, there is only few study including a handful of IR and INR patients[8, 10] and without considering the immune response to HAART [27]. The previous studies reported that the relative abundance of *Fusobacterium* was independently associated with poorer CD4 T-cell recovery[10], and the relative abundance of unclassified *Subdoligranulum sp*. and *C. comes* were positively correlated with CD8+HLA-DR+ T-cell counts and CD8+HLADR+/CD8+ percentage in HIV-infected subjects[8]. The current study, including the so far higher number of IR and INR patients, expanded the previous observations and investigate the relationship between gut microbiota and immune activation. Here we found that ecological indices of microbiota (including community diversity, richness and observed species numbers) were significantly lower in IR and INR group when compared with the control group. Additionally, PCoA analysis showed remarkable differentiation of bacterial communities between the IR, INR and control groups. These suggested that the gut microbiota dysbiosis could not be recovered completely in immunodiscordant and immunoconcordant HIV-infected individuals although with long-term suppressive antiretroviral treatment.

Furthermore, we found that the 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 the IR and INR group. At the 97% similarity level, the predominant genera *Fusobacterium, Ruminococcus_gnavus* and *Megamonas* were more abundant, whereas *Faecalibacterium, Alistipes, Bifidobacterium, Eubacterium rectale*, and *Roseburia* were depleted in the IR and INR group than that of control group. Taxa from bacterial p*hyla Proteobacteria, Fusobacteria* and *Bacteroidetes* have been reported different between HIV positive and negative individuals in studies. Our result was consistent with most of published studies which reported that Proteobacteria were more abundant in HIV-infected individuals although others reported no change [8, 10-12, 28-33]. The family Enterobacteriaceae has been reported positively associated with markers of monocyte activation (sCD14), inflammation and colonic T cell activation and inversely correlated with blood CD4+ T cell counts [30, 32, 34]. *Escherichia-Shigella* which belonged to Enterobacteriaceae were negatively correlated with the CD4/CD8 ratio, but positively correlated with the CD8+CD57+ T-cell which was hallmark of immunosenescence in HIV infection[35, 36]. Furthermore, *Escherichia-Shigella* was more abundant in the IR group than that in INR group in this study.

The phylum Fusobacteria (most driven by its constituent genus *Fusobacterium*) was reported associated with intestinal inflammation [37, 38] and abundant in HIV-infected individuals in some studies [29, 33, 39,

40]. Here, we found increased abundance of *Fusobacterium* in the IR and INR groups. Furthermore, inflammation markers and translocation biomarkers (LPS) were positively correlated with *Fusobacterium*, while the nadir and current CD4+ T-cell counts were negatively correlated with *Fusobacterium* in this study. This was consistent with the previous published studies which reported that the relative abundance of *Fusobacterium* was independently associated with poorer CD4 T-cell recovery and enrichment of *Fusobacterium* was associated with reduced immune recovery and persistent immune dysfunction following ART[10].

The phylum Bacteroidetes which include the families Bacteroidaceae, Prevotellaceae, Porphyromonadaceae, and Rikenellaceae, exhibited a more heterogeneous pattern of changes in HIV-infected individuals [11, 12, 23, 28-34, 39-42]. The family Bacteroidaceae (mostly driven by the abundance of the genus *Bacteroides*) which was generally considered to play an anti-inflammatory role [43-46], were depleted in the IR and INR groups. The bile-tolerant family Rikenellaceae (mostly driven by the abundance of the genus *Alistipes*) which was protective properties against *C. difficile* infection [46, 47], were overall depleted in the IR and INR groups. Actinobacteria which was reported similar in the proximal gut of HIV-infected patients and negative controls [32], were depleted in the IR and INR groups in this study. The *Alistipes* was negatively correlated with the CD8+CD57+ T-cell and positively correlated with the nadir CD4+ T-cell counts. Notably, the commonly used probiotics *Bifidobacterium* which belong to the phylum of Actinobacteria was depleted in the IR and INR group compared with the control group [48].

Although the abundance of phylum Firmicutes was similar in the three groups, the genus such as *Faecalibacterium* and *Ruminococcaceae* were depleted in the IR and INR groups compared with the control group. *Ruminococcaceae* have been associated with both protective and disruptive roles within the gut microbial community, such as the production of anti-inflammatory short-chain fatty acids (SCFA) [49] or the degradation of host mucus and potential proinflammatory role in IBD[50]. In this study, the *Faecalibacterium* and *Ruminococcaceae UCG-002*, which belong to the family of *Ruminococcaceae* were depleted in the IR and INR groups compared with the control group. Interestingly, *Rudminococcaceae* was positively correlated with the current nadir CD4+ T-cell counts while negatively correlated with the CD8+CD57+ T-cell counts. In additionally, inflammation markers and LPS were positively correlated with the abundance of genus *Ruminococcus*. The *Faecalibacterium* has been reported as anti-inflammatory commensal genus [29, 51]. In this study, the genus *Faecalibacterium* was positively correlated with CD4/CD8 ratio, while negatively correlated with the inflammation markers and LPS.

Normal large important groups which commonly present in high amounts in healthy controls interested us more than various pathogens. Both *Roseburia* and *Blautia* (belonging to the phylum Firmicutes, class Clostridia, family Lachnospiraceae) were normal large and important groups of commensals and have been described as SCFA producers with beneficial effect on the intestinal barrier and an important energy source for epithelial cells [52]. In this study, *Blautia, and Roseburia* were more abundant in the control group and the abundance of Blautia increased significantly in the IR group than the INR group. *Roseburia* and *Blautia* were negatively associated with the nadir CD4+ T-cell and positively associated with the

CD8+CD57+ T-cell counts. These suggested that *Blautia* and *Roseburia* might be associated with treatment outcome.

Altogether, these results highlight that immune activation in HIV-infected patients was 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. *Fusobacterium, Alistipes, Ruminococcaceae, Faecalibacterium*, *Escherichia-Shigella, Roseburia* and *Blautia* maybe the major genus contributed to different immune response in immunodiscordant and immunoconcordant patients on long-term suppressive ART. *Blautia* and *Roseburia* might be associated with treatment outcome. However, we didn't collect samples before ART treatment, so whether the gut microbiome composition influences treatment success need future research and this study couldn't directly address whether changes in the microbiota were causative or a rather a result of systemic HIV-1-associated immune activation. We acknowledge that the extensive dietary data and living conditions of these subjects which were not controlled may lead to biases in our analysis. On the other hand, while correlation analysis was useful to have clues of biological links to the impact of dysbiosis in immune response in these patients, a direct manipulation of microbiome was needed to validate their cellular and biochemical action in vitro or in vivo. And the exact mechanisms how HIV infection affected 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 and gut microbiota dysbiosis may be one of the factors contributing to different immune response to HAART. *Fusobacterium, Alistipes, Ruminococcaceae, Faecalibacterium*, *Escherichia-Shigella, Roseburia and Blautia* maybe the major genus contributed to different immune response in immunodiscordant and immunoconcordant patients on long-term suppressive ART. *Blautia* and *Roseburia* might be associated with treatment outcome.

Methods

Recruitment of subjects

The process of participants' recruitment and sample collection are depicted in Suppl. S5 Fig. A total of 58 HIV-infected individuals, including 28 immunological responders (IR), 30 immunological non-responders (INR), and 36 healthy subjects (control) were recruited. IR and INR were defined as CD4+ T-cell counts/μl ≥500 or <200 after 2 years of complete viral suppression 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 48h; 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, 30s for annealing at 55 °C, and 45s 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 50bp and an average quality score of

20. Two mismatches were allowed in primer sequences and reads containing ambiguous bases were removed. Ten homopolymers 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 16s 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 replicates were 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)[53]. Linear discriminant analysis (LDA) effect size (LEfSe) uses the Kruskal-Wallis rank sum test to detect features with significant different abundance between assigned taxa. LEfSe are available online in the Galaxy workflow framework[54]. 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

Acknowledgments

We gratefully acknowledge the patients who participated in this study.

Funding

This work was funded by the Natural Science Foundation of China (Young Scientist Fund, 81500491) and National Science Foundation of China (Major Research Plan, 2018ZX10715-014).

Competing interests

The authors declare that they have no conflict of interest.

Informed consent: Written consent was obtained from the patients.

Author Contributions

Yirui Xie participated in the design of the study and performed the statistical analysis and wrote the paper. Jia Sun performed the statistical analysis and revised 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.

References

1. Kelley CF, Kitchen CM, Hunt PW, Rodriguez B, Hecht FM, Kitahata M, Crane HM, Willig J, Mugavero M, Saag M *et al*: **Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment**. *Clin Infect Dis* 2009, **48**(6):787-794.
2. Cenderello G, De Maria A: **Discordant responses to cART in HIV-1 patients in the era of high potency antiretroviral drugs: clinical evaluation, classification, management prospects**. *Expert Rev Anti Infect Ther* 2016, **14**(1):29-40.
3. Tsukamoto H, Clise-Dwyer K, Huston GE, Duso DK, Buck AL, Johnson LL, Haynes L, Swain SL: **Age-associated increase in lifespan of naive CD4 T cells contributes to T-cell homeostasis but facilitates development of functional defects**. *Proc Natl Acad Sci U S A* 2009, **106**(43):18333-18338.
4. Florence E, Lundgren J, Dreezen C, Fisher M, Kirk O, Blaxhult A, Panos G, Katlama C, Vella S, Phillips A *et al*: **Factors associated with a reduced CD4 lymphocyte count response to HAART despite full viral suppression in the EuroSIDA study**. *HIV Med* 2003, **4**(3):255-262.
5. Kaufmann GR, Perrin L, Pantaleo G, Opravil M, Furrer H, Telenti A, Hirschel B, Ledergerber B, Vernazza P, Bernasconi E *et al*: **CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study**. *Arch Intern Med* 2003, **163**(18):2187-2195.
6. Gazzola L, Tincati C, Bellistri GM, Monforte A, Marchetti G: **The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options**. *Clin Infect Dis* 2009, **48**(3):328-337.
7. Gootenberg DB, Paer JM, Luevano JM, Kwon DS: **HIV-associated changes in the enteric microbial community: potential role in loss of homeostasis and development of systemic inflammation**. *Curr Opin Infect Dis* 2017, **30**(1):31-43.
8. Lu W, Feng Y, Jing F, Han Y, Lyu N, Liu F, Li J, Song X, Xie J, Qiu Z *et al*: **Association Between Gut Microbiota and CD4 Recovery in HIV-1 Infected Patients**. *Front Microbiol* 2018, **9**:1451.
9. Ji Y, Zhang F, Zhang R, Shen Y, Liu L, Wang J, Yang J, Tang Q, Xun J, Qi T *et al*: **Changes in intestinal microbiota in HIV-1-infected subjects following cART initiation: influence of CD4+ T cell count**. *Emerg Microbes Infect* 2018, **7**(1):113.
10. Lee SC, Chua LL, Yap SH, Khang TF, Leng CY, Raja Azwa RI, Lewin SR, Kamarulzaman A, Woo YL, Lim YAL *et al*: **Enrichment of gut-derived Fusobacterium is associated with suboptimal immune recovery in HIV-infected individuals**. *Sci Rep* 2018, **8**(1):14277.
11. Sun Y, Ma Y, Lin P, Tang YW, Yang L, Shen Y, Zhang R, Liu L, Cheng J, Shao J *et al*: **Fecal bacterial microbiome diversity in chronic HIV-infected patients in China**. *Emerg Microbes Infect* 2016, **5**:e31.
12. Ling Z, Jin C, Xie T, Cheng Y, Li L, Wu N: **Alterations in the Fecal Microbiota of Patients with HIV-1 Infection: An Observational Study in A Chinese Population**. *Sci Rep* 2016, **6**:30673.
13. Deeks SG, Tracy R, Douek DC: **Systemic effects of inflammation on health during chronic HIV infection**. *Immunity* 2013, **39**(4):633-645.
14. West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, Prescott SL, in FMIG: **The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut**

microbiota therapies. *J Allergy Clin Immunol* 2015, **135**(1):3-13; quiz 14.

15. Klatt NR, Chomont N, Douek DC, Deeks SG: **Immune activation and HIV persistence: implications for curative approaches to HIV infection**. *Immunol Rev* 2013, **254**(1):326-342.
16. Klatt NR, Funderburg NT, Brenchley JM: **Microbial translocation, immune activation, and HIV disease**. *Trends Microbiol* 2013, **21**(1):6-13.
17. Lederman MM, Funderburg NT, Sekaly RP, Klatt NR, Hunt PW: **Residual immune dysregulation syndrome in treated HIV infection**. *Adv Immunol* 2013, **119**:51-83.
18. Siedner MJ: **START or SMART? Timing of Antiretroviral Therapy Initiation and Cardiovascular Risk for People With Human Immunodeficiency Virus Infection**. *Open Forum Infect Dis* 2016, **3**(1):ofw032.
19. Havlir DV, Currier JS: **CROI 2015: Complications of HIV Infection and Antiretroviral Therapy**. *Top Antivir Med* 2015, **23**(1):56-65.
20. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, Asher AI *et al*: **Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection**. *J Infect Dis* 2009, **199**(8):1177-1185.
21. Mavigner M, Cazabat M, Dubois M, L'Faqihi FE, Requena M, Pasquier C, Klopp P, Amar J, Alric L, Barange K *et al*: **Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals**. *J Clin Invest* 2012, **122**(1):62-69.
22. Neuhaus J, Jacobs DR, Jr., Baker JV, Calmy A, Duprez D, La Rosa A, Kuller LH, Pett SL, Ristola M, Ross MJ *et al*: **Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection**. *J Infect Dis* 2010, **201**(12):1788-1795.
23. Vazquez-Castellanos JF, Serrano-Villar S, Latorre A, Artacho A, Ferrus ML, Madrid N, Vallejo A, Sainz T, Martinez-Botas J, Ferrando-Martinez S *et al*: **Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals**. *Mucosal Immunol* 2015, **8**(4):760-772.
24. Cho I, Blaser MJ: **The human microbiome: at the interface of health and disease**. *Nat Rev Genet* 2012, **13**(4):260-270.
25. Lujan JA, Rugeles MT, Taborda NA: **Contribution of the Microbiota to Intestinal Homeostasis and its Role in the Pathogenesis of HIV-1 Infection**. *Curr HIV Res* 2019, **17**(1):13-25.
26. Vujkovic-Cvijin I, Somsouk M: **HIV and the Gut Microbiota: Composition, Consequences, and Avenues for Amelioration**. *Curr HIV/AIDS Rep* 2019, **16**(3):204-213.
27. Tincati C, Merlini E, Braidotti P, Ancona G, Savi F, Tosi D, Borghi E, Callegari ML, Mangiavillano B, Barassi A *et al*: **Impaired gut junctional complexes feature late-treated individuals with suboptimal CD4+ T-cell recovery upon virologically suppressive combination antiretroviral therapy**. *AIDS* 2016, **30**(7):991-1003.
28. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE: **HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy**. *Gut Microbes* 2014, **5**(4):562-570.
29. Mutlu EA, Keshavarzian A, Losurdo J, Swanson G, Siewe B, Forsyth C, French A, Demarais P, Sun Y, Koenig L *et al*: **A compositional look at the human gastrointestinal microbiome and immune**

activation parameters in HIV infected subjects. *PLoS Pathog* 2014, **10**(2):e1003829.

30. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, Hernandez RD, Lederman MM, Huang Y, Somsouk M *et al*: **Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism**. *Sci Transl Med* 2013, **5**(193):193ra191.
31. Dillon SM, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, Gianella S, Siewe B, Smith DM, Landay AL *et al*: **An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia**. *Mucosal Immunol* 2014, **7**(4):983-994.
32. Yang L, Poles MA, Fisch GS, Ma Y, Nossa C, Phelan JA, Pei Z: **HIV-induced immunosuppression is associated with colonization of the proximal gut by environmental bacteria**. *AIDS* 2016, **30**(1):19-29.
33. Dubourg G, Lagier JC, Hue S, Surenaud M, Bachar D, Robert C, Michelle C, Ravaux I, Mokhtari S, Million M *et al*: **Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen**. *BMJ Open Gastroenterol* 2016, **3**(1):e000080.
34. Dinh DM, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV, Wanke CA, Ward HD: **Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection**. *J Infect Dis* 2015, **211**(1):19-27.
35. Appay V, Sauce D: **Immune activation and inflammation in HIV-1 infection: causes and consequences**. *J Pathol* 2008, **214**(2):231-241.
36. Ivanov, II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR: **Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine**. *Cell Host Microbe* 2008, **4**(4):337-349.
37. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S: **Host-gut microbiota metabolic interactions**. *Science* 2012, **336**(6086):1262-1267.
38. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL *et al*: **Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment**. *Cell Host Microbe* 2013, **14**(2):207-215.
39. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, Anton P, Braun J: **HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota**. *Microbiome* 2013, **1**(1):26.
40. Yu G, Fadrosh D, Ma B, Ravel J, Goedert JJ: **Anal microbiota profiles in HIV-positive and HIV-negative MSM**. *AIDS* 2014, **28**(5):753-760.
41. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, Hov JR, Noyan K, Vesterbacka J, Svard J *et al*: **Gut microbiota diversity predicts immune status in HIV-1 infection**. *AIDS* 2015, **29**(18):2409-2418.
42. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, Lankowski A, Baldridge MT, Wilen CB, Flagg M *et al*: **Altered Virome and Bacterial Microbiome in Human Immunodeficiency Virus-Associated Acquired Immunodeficiency Syndrome**. *Cell Host Microbe* 2016, **19**(3):311-322.
43. Troy EB, Kasper DL: **Beneficial effects of Bacteroides fragilis polysaccharides on the immune system**. *Front Biosci (Landmark Ed)* 2010, **15**:25-34.

44. Round JL, Mazmanian SK: **Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota**. *Proc Natl Acad Sci U S A* 2010, **107**(27):12204-12209.
45. Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hamalainen AM *et al*: **Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans**. *Cell* 2016, **165**(6):1551.
46. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA *et al*: **Diet rapidly and reproducibly alters the human gut microbiome**. *Nature* 2014, **505**(7484):559-563.
47. Khanna S, Montassier E, Schmidt B, Patel R, Knights D, Pardi DS, Kashyap P: **Gut microbiome predictors of treatment response and recurrence in primary Clostridium difficile infection**. *Aliment Pharmacol Ther* 2016, **44**(7):715-727.
48. Veiga P, Gallini CA, Beal C, Michaud M, Delaney ML, DuBois A, Khlebnikov A, van Hylckama Vlieg JE, Punit S, Glickman JN *et al*: **Bifidobacterium animalis subsp. lactis fermented milk product reduces inflammation by altering a niche for colitogenic microbes**. *Proc Natl Acad Sci U S A* 2010, **107**(42):18132-18137.
49. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA: **Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis**. *Nat Rev Microbiol* 2008, **6**(2):121-131.
50. Png CW, Linden SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, McGuckin MA, Florin TH: **Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria**. *Am J Gastroenterol* 2010, **105**(11):2420-2428.
51. Shaw KA, Bertha M, Hofmekler T, Chopra P, Vatanen T, Srivatsa A, Prince J, Kumar A, Sauer C, Zwick ME *et al*: **Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease**. *Genome Med* 2016, **8**(1):75.
52. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, Turroni S, Biagi E, Peano C, Severgnini M *et al*: **Gut microbiome of the Hadza hunter-gatherers**. *Nat Commun* 2014, **5**:3654.
53. McMurdie PJ, Holmes S: **phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data**. *PLoS One* 2013, **8**(4):e61217.
54. Goecks J, Nekrutenko A, Taylor J, Galaxy T: **Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences**. *Genome Biol* 2010, **11**(8):R86.

Tables

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
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.

Figures

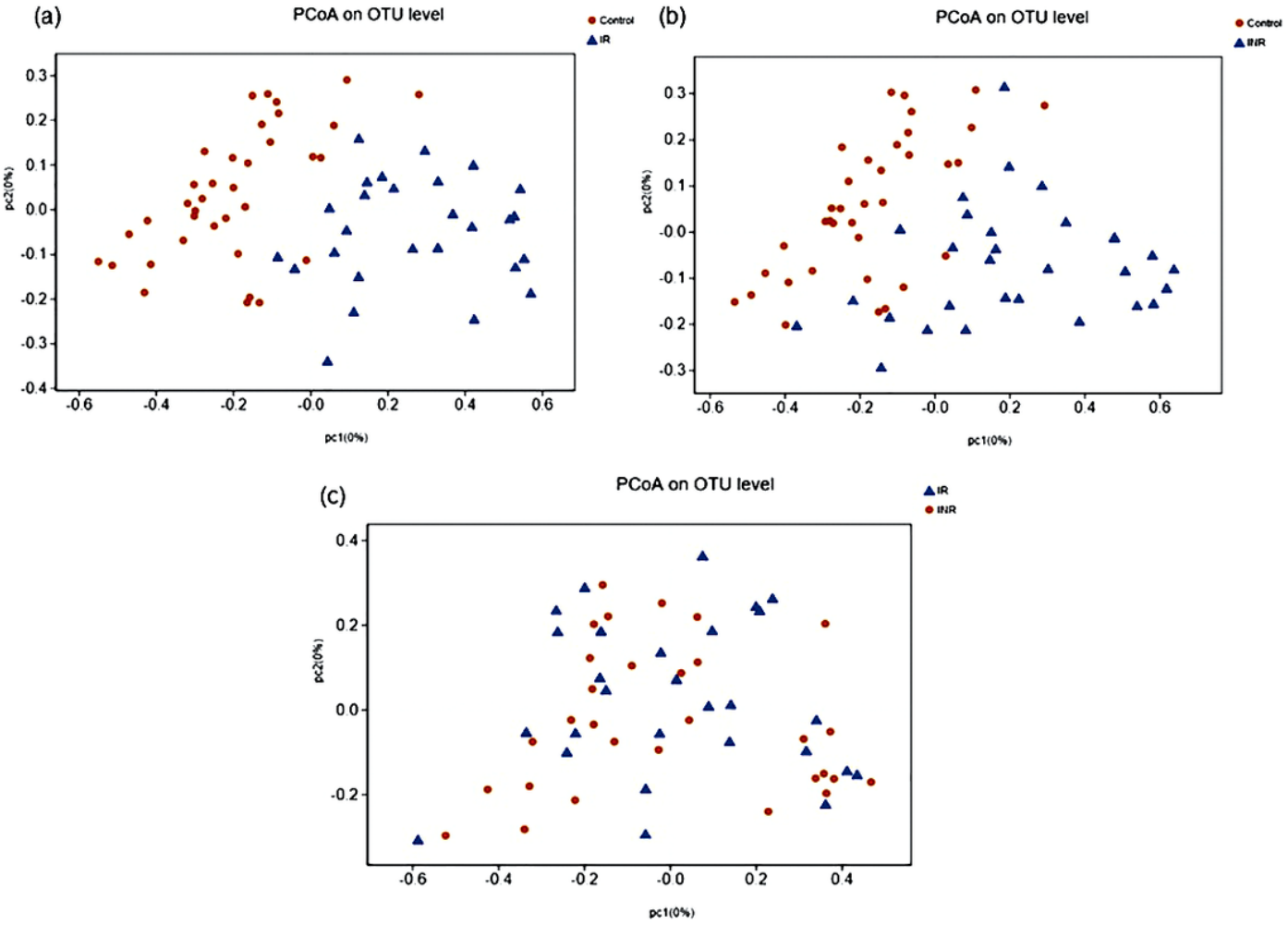

Figure 1

Principal coordinates analysis (PCoA) of microbiomes in the patients and the control group. Remarkable bacterial communities' difference among the immunological responders (IR), immunological non-responders (INR) and the Control groups (Fig. 1a; Fig. 1b); no significant difference between the IR and INR groups (Fig. 1c).

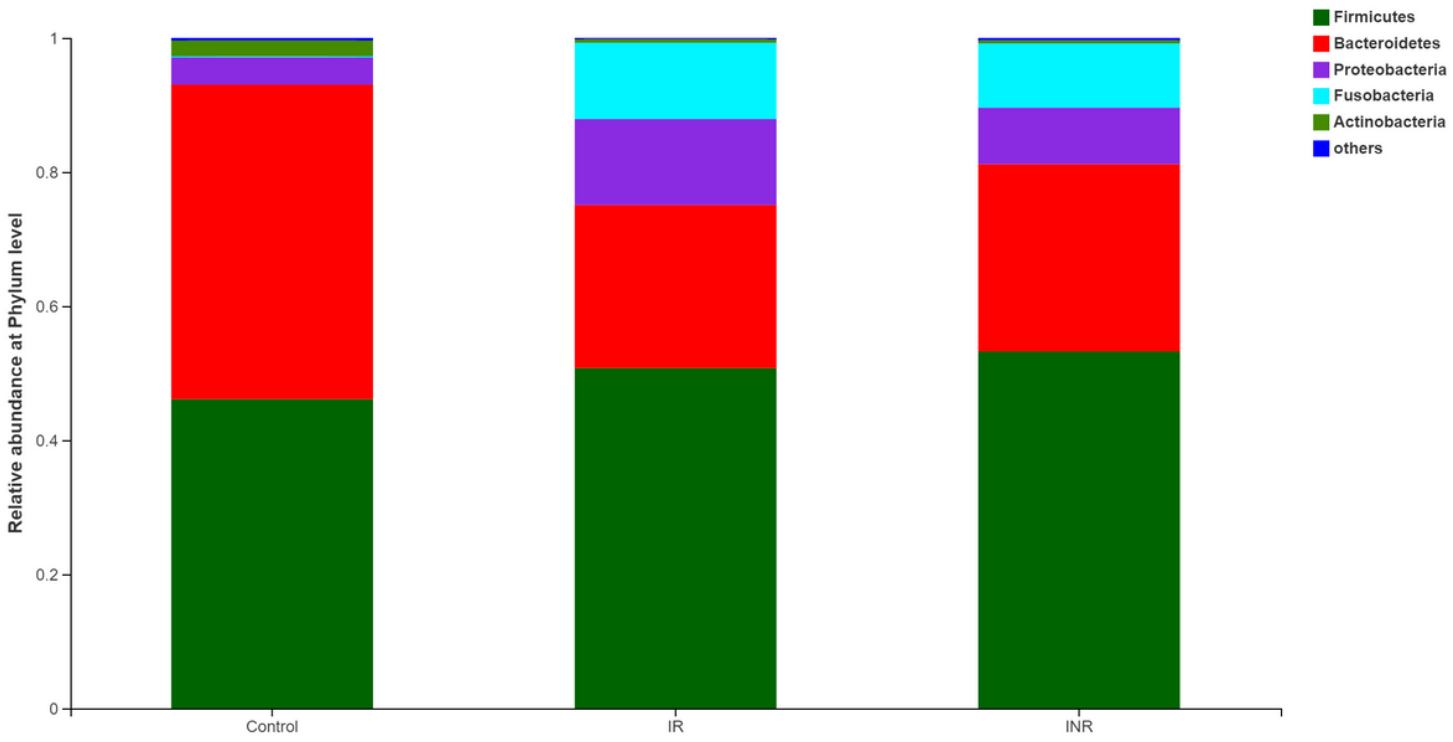

Figure 2

The relative abundance of fecal microbiota at phylum level in the patients and the control group. IR: immunological responders; INR: immunological non-responders; Control: health control.

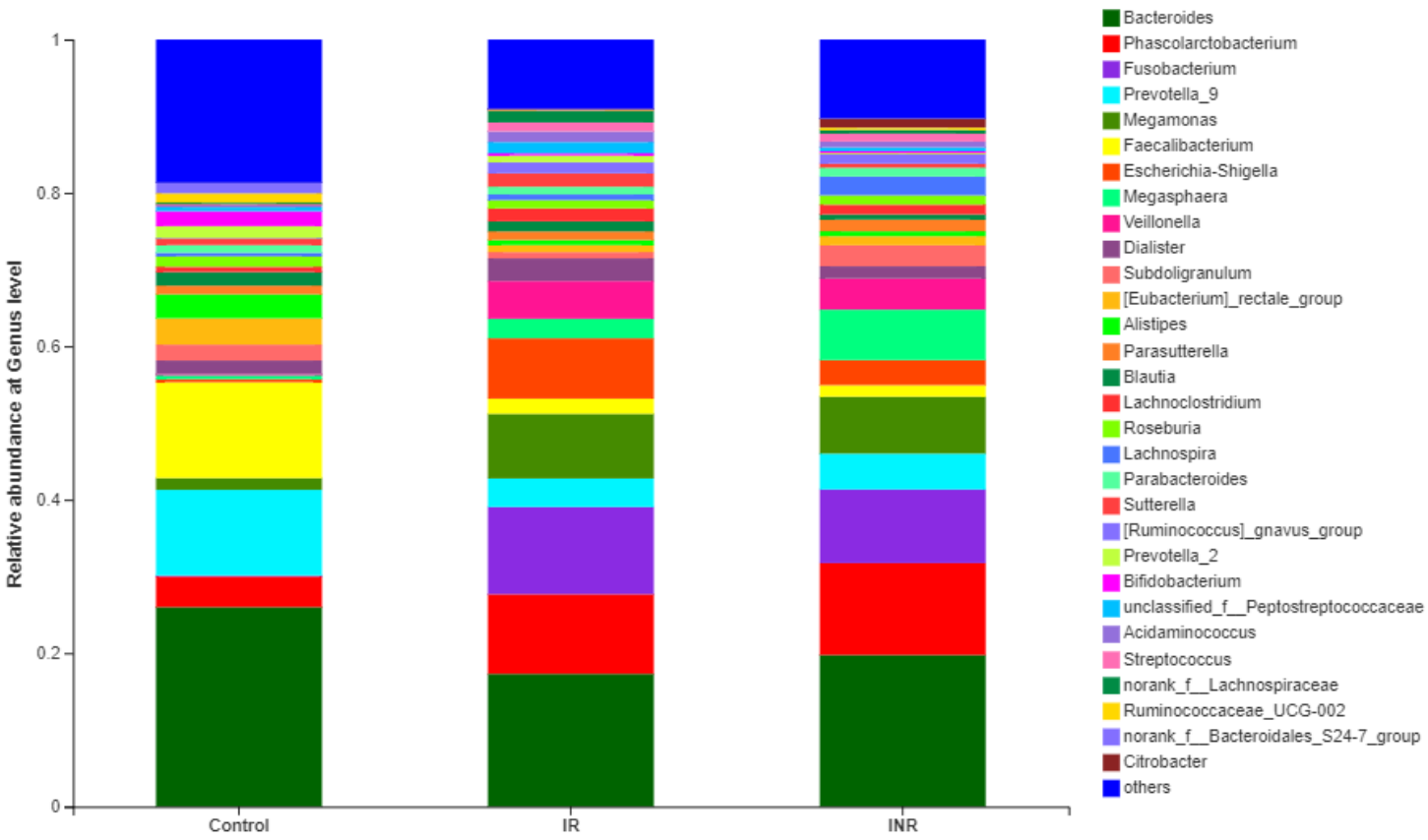

Figure 3

The relative abundance of fecal microbiota at genus level in the patients and the control group. IR: immunological responders; INR: immunological non-responders; Control: health control.

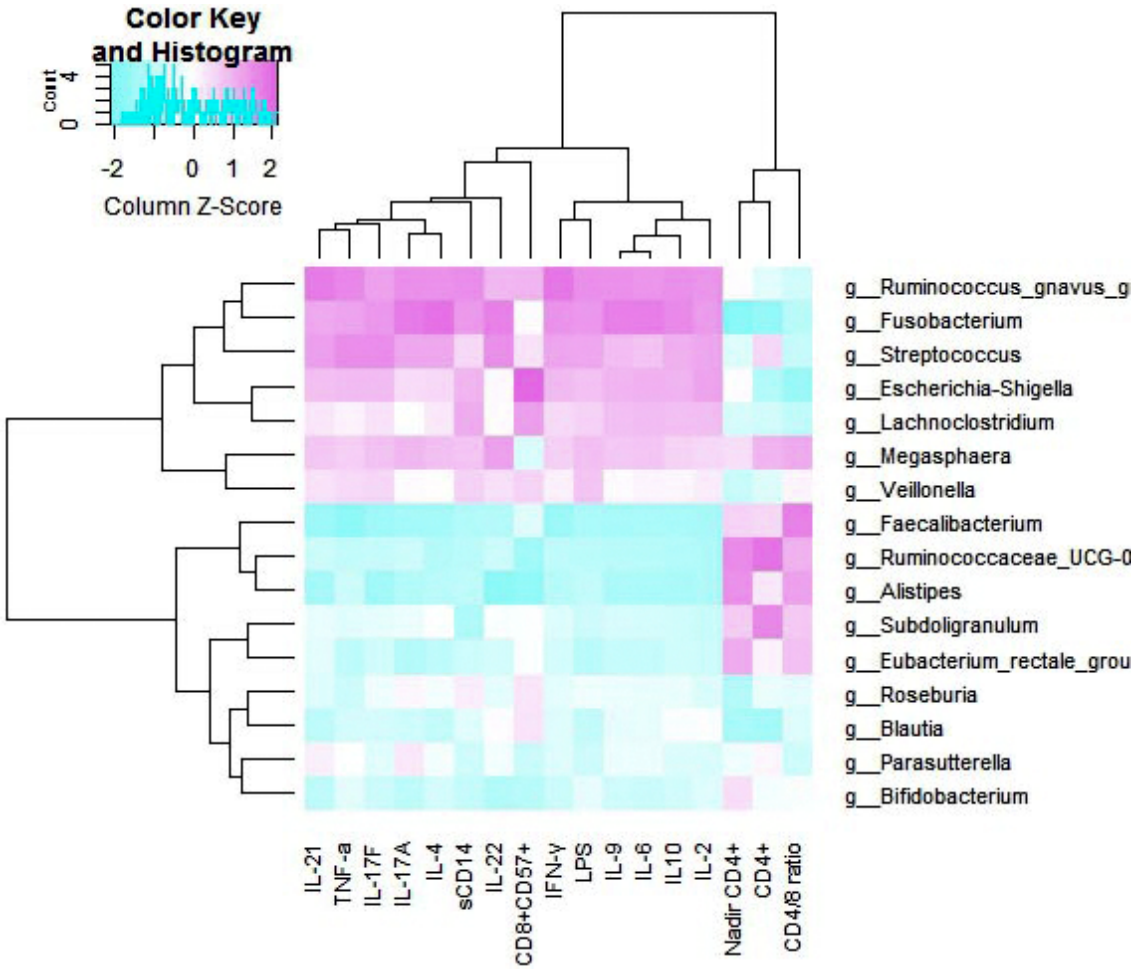

Figure 4

The composition of fecal microbiota correlates with markers of immune activation. Some cellular and soluble markers of immune activation correlated with 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

This is a list of supplementary files associated with this preprint. Click to download.

- S1Table.xlsx
- S2Table.xlsx
- S1Fig.png.png
- S2Fig.png.png
- S3Fig.png.png
- S4Fig.png.png
- S5Fig.png.png