Review article

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CHANGES OF THE INTESTINAL MICROBIOME–HOST HOMEOSTASIS IN HIV-INFECTED INDIVIDUALS – A FOCUS ON THE BACTERIAL GUT MICROBIOME

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Human immunodeficiency virus (HIV) infections cause severe CD4+ T cell depletion leading to chronic inflammation and immune activation, impaired barrier function, and microbial translocation. Even under effective antiretroviral therapy, these processes persist, leading to gut microbiome dysbiosis and disturbance of microbiome–host homeostasis. This systematic review aims at analyzing how gut microbiome and host immune system influence each other during HIV pathogenesis. An online search applying the PubMed database was conducted. The number of total results (n = 35) was narrowed down to 5 relevant studies focusing on the interaction between the host and gut microbiome, whereas strict exclusion criteria were applied, thereby assuring that no other comorbidities impacted study results. Our analyses revealed that gut microbiome diversity correlated positively with CD4+ T cell counts and negatively with microbial translocation markers. However, quantitative changes in bacterial richness did not consistently correlate with the numbers of metabolically active bacterial populations. Despite the reported increase in potentially pathogenic bacteria and, conversely, decrease in protective populations, the gut microbiota exhibited immune-modulating qualities given that mucosal inflammatory sequelae were dampened by decreasing pro-inflammatory and accelerating anti-inflammatory cytokine responses. Future research is needed to further elucidate these findings, to gain a deeper insight into host–microbiota interactions and to develop novel therapeutic strategies.

Keywords: HIV, intestinal microbiota, gut microbiome–host homeostasis, dysbiosis, bacterial richness, bacterial translocation, chronic immune activation, inflammation, immune recovery, probiotics

Abbreviations: AA, arachidonic acid; ART, antiretroviral therapy; BR, bilirubin; BV, biliverdin; Dol-b-G, dolichol-b-D-glucosyl phosphate; Dol-P, dolichol phosphate; ESR, erythrocyte sedimentation rate; GALT, gut associated lymphoid tissue; GIT, gastrointestinal tract; HIV, human immunodeficiency virus; Hs-CRP, high sensitivity C-reactive protein; INR, immune non-responder; IR, immune responder; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; LTB4, leukotriene B4; MeSH, medical subject headings; MSM, men having sex with men; Neu5Ac, *N*-acetylneuraminic acid; SIV, simian immunodeficiency virus; Th, T helper type; UGT, uridine glucuronyl transferases; URO, urobilinogen; VU, viremic untreated patients

Introduction

HIV infection and clinical manifestations within the gastrointestinal tract

Human immunodeficiency virus (HIV)-infected patients are known to suffer from gastrointestinal symptoms, even under effective antiretroviral therapy (ART). Clinical gastrointestinal manifestations such as diarrhea, weight loss, and malnutrition are symptoms most patients get confronted with [1]. In the late stages of HIV infection, even up to 90% of patients with acquired immune deficiency syndrome (AIDS) tend to develop infectious diarrhea [2]. This is both driven by the infection with common enteropathogens such as *Escherichia coli, Salmonella,* and *Shigella* as well as with opportunistic microorganisms including

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Cryptosporidium, Cystoisospora belli, and *Microsporidium* [3, 4]. One of the underlying causes, besides gastrointestinal side effects associated with the ART itself, lies in the rapid decrease in CD4+ T cells, especially T helper type (Th) -17 and -22 (i.e., T cells that are involved in normal mucosal defense and epithelial barrier maintenance), which quickly sets off several immunological domino effects leading to chronic inflammation, mucosal barrier dysfunction, immune dysfunction, profound changes in the gut microbiome composition, and subsequently to disturbances of host–microbiome homeostasis [5–9].

CD4+ T cell reservoir in gut associated lymphoid tissues (GALT)

Approximately 60% of all CD4+ T cells are estimated to reside in the lymphatic tissues of the gastrointestinal tract [10]. Due to higher expression levels of the chemokine receptor CCR5 in the intestinal mucosa, the initial decrease in CD4+ T cells is less pronounced in the peripheral blood during the acute phase of infection as compared to the gastrointestinal tract (GIT) [11]. Here, the entry and replication of the HIV in CD4+ T cells lead not only to a rapid and severe depletion of these cells but also to immediate changes in both the mucosal epithelia with subsequent structural and functional changes in the gut microbiome ecosystem [12, 13]. Studies with simian immunodeficiency virus (SIV)-infected macaques revealed that this CD4+ T cell destruction already takes place within the first week following HIV infection [14].

The role of mucosal epithelia in HIV infection

One of the gut epithelia's main functions is the digestion and absorption of nutrients. The gut mucosal epithelia also play an important role in protecting the host from pathogenic microorganisms residing in the gut lumen as well as in preventing the host from microbial translocation through its gut–blood barrier [15]. Furthermore, besides its protective immunological properties, the gut mucosa is also responsible for the regulation of its own local immune responses towards tolerance of the commensal microbiota, thereby preventing a potentially harmful overshooting immune reaction [16]. The integrity of the epithelial cell barrier of the gut mucosa therefore plays an important role in host–microbiome homeostasis, given that disturbances herein may lead to severe (i.e., fatal) consequences [15].

HIV infection results in the disruption of this balance and mucosal integrity. Upon initial infection of CD4+ T cells residing in the gut associated lymphoid tissues (GALT), these cells go into apoptosis and concentrations of pro-inflammatory cytokine such as TNF-α, IL-6, and IL-8 rise. This further leads to continuous recruitment and hyperactivation of new CD4+ T cell clones, which once again end up in apoptosis, finally resulting in impaired barrier function and dysregulation of the gastrointestinal immune-epithelial network [16, 17]. As a consequence of hyperactivation of the immune system, the constantly elevated levels of pro-inflammatory cytokines lead to a disruption of tight junctions, thus increasing the permeability of the gut–blood barrier with subsequent microbial translocation from the intestinal tract to extra-intestinal including systemic compartments [18].

Overall, both the abovementioned processes result in a vicious cycle where continuous CD4+ T cell infection leads to progressive cell apoptosis, increasing damage to the intestinal mucosa thus facilitating microbial translocation. Long-term consequences of this HIV infection-induced scenario are chronic inflammation with chronic immune activation finally leading to the exhaustion of the immune system, thereby raising morbidity and mortality in HIV-infected patients [7, 8, 19–21].

The gut microbiome in HIV infection

The human gut microbiota is composed of the following four main phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [22]. The relative abundances of respective phyla vary depending on a plethora of factors such as socioeconomic factors, age, geography, diet, and exercise, besides others [23–25].

Previous studies revealed that HIV infection also has an impact on the gut microbiome composition, given that HIV infection was associated with increased bacterial populations in the intestinal tract that are either pro-inflammatory and hence potentially pathogenic, and whose abundance correlated with immune status and immune recovery [7, 8, 19].

The here presented systematic literature review aims at portraying the most recent studies addressing the impact of HIV infection on changes in the balanced gut microbiome composition. Furthermore, the influence of commensal bacteria on host immune recovery and immune balance will be further unraveled by comparing HIV-infected patients undergoing ART with ART naive subjects.

Materials and methods

Search strategy

From the 15th to the 25th of June 2017, an online search was conducted using the PubMed database. Here, the advanced search builder was applied in order to find relevant literature. Using the search fields "MeSH Terms" and "Title", the keyword combination "HIV gut microbiome" was inserted. The search terms were therefore united as follows:

– (HIV gut microbiome [MeSH Terms]) OR HIV gut microbiome [Title]

To cover all relevant articles and to assure that no study was left out, synonyms of the keywords "HIV gut micro-

biome" were introduced in all possible combinations in the MeSH Term field. The synonyms were the following:

– HIV/AIDS gut/intestinal microbiome/microbiota/flora

However, search results did not differ from when the combination "HIV gut microbiome" was used.

In total, 35 publications were found. After screening all results, reviews (n = 8), commentaries (n = 2), addendums (n = 1), and studies involving SIV-infected macaques (n = 12) were excluded, leaving 11 studies to be considered. These 11 studies were then analyzed for eligibility. After a thorough assessment, 6 studies were excluded as for the following reasons:

Firstly, studies that did not mention or took into consideration the exclusion criteria of patients with a) medical history of inflammatory bowel disease or other intestinal inflammatory disorders; b) chronic or acute medical conditions such as cancer, diabetes, or hepatitis; c) antibiotic treatment 2 months prior to study conduction; and d) recent immunosuppressive therapy, immune modulators, or probiotics were excluded (n = 2) due to the potential impact of respective factors on the gut microbiome composition.

Secondly, studies that only analyzed blood samples were also excluded since the focus was rather on systemic effects of microbial translocation and on assessment of defined biochemical markers than on the link to the gastrointestinal tract and the potential changes of the gut microbiome itself (n = 3).

Thirdly, one study, which analyzed colon biopsies, was excluded, due to its detailed focus on the pathophysiologic processes involving CD1c+ and CD1c− myeloid dendritic cells and not on the host–microbiome interaction and the impact of the gut microbiome in HIV-infected patients (n = 1).

Finally, the numbers of studies screened, assessed for eligibility, and included in the review were narrowed down to 5.

Data extraction

All relevant information derived from the articles was extracted and sorted in columns using a table in Microsoft Word with the following criteria: study details, study type, study population, type of sample analyzed, microbiome analysis, applied technique, and main findings. The main findings of all the articles included in this review were summarized and inserted in *Table 1* to provide a comprehensive overview.

Results

α and β diversities

When analyzing gut bacterial composition, three studies first assessed numbers of different bacterial species, the α diversity, in HIV-negative and HIV-positive subjects. Nowak et al. described the α diversity to be decreased in untreated HIV-infected patients [26]. At baseline, i.e., before ART introduction, the numbers of bacterial species were lower in HIV-infected patients as compared to seronegative individuals *(Table 1)*. The CD4+ T cell counts positively correlated with bacterial richness. Hence, subjects with the lowest CD4+ T cell counts also displayed the lowest number of intestinal bacterial species [26].

When comparing bacterial species variations between the different cohorts, i.e., the β diversity, higher β diversities could be observed in HIV-positive patients as compared to healthy subjects [26]. These results are further supported by a study conducted by Vazquez-Castellanos and colleagues who documented a decreased α diversity but increased β diversity in HIV-positive individuals who had already undergone ART *(Table 1)* [27].

Nowak et al. further analyzed β diversity changes in elite controllers, defined as patients with undetectable HIV RNA since the diagnosis of HIV infection. These patients displayed lower inter-individual variation compared to viremic patients. Interestingly, the gut microbiota of elite controllers was characterized by a decreased β diversity when compared to HIV-positive patients. However, taking into account that there were only three patients with undetectable HIV-1 RNA, no definite conclusions can be drawn from these results [26].

A study performed by Noguera-Julian et al. provides a more detailed depiction of α diversity in HIV-negative patients with ART [28]. Comparing the gut microbiome of HIV-positive men having sex with men (MSM) with non-MSM, the authors concluded that the fecal microbiota was significantly more enriched (i.e., diverse) in MSM as compared to non-MSM HIV-positive individuals. However, the gut microbiome in this former cohort was characterized by a still lower α diversity as compared to healthy controls [28]. This study is further supported by a previous report [27], when comparing immune responders (IR) with immune non-responders (INR) defined as subjects who do not recover CD4+ count >300 cells/mm^3 in spite of at least 2 years of effective ART. Interestingly, the gut microbiota of the INR cohort exhibited a lower α diversity, whereas IR displayed a higher bacterial richness in the intestinal microbiome [28]. ART treatment, however, did not reverse decreased α diversity, further underlining that bacterial richness is linked to immune dysfunction despite the fact that IR still showed reduced intestinal bacterial richness [28]. This conclusion is further supported by the continuous decrease of α diversity observed in microbiome following ART introduction in the study by Nowak et al., irrespective of the applied ART regimen *(Table 1)* [26]. Hence, this correlation between reduced CD4+ T cell counts and bacterial richness suggests a pivotal role of microbiota diversity in host immune balance and recovery.

Serrano-Villar et al., however, could not observe a significant change in α diversity when comparing viremic untreated patients (VU) with healthy control subjects [29]. In contrast to the previous conclusions, ART-treated patients, irrespective whether IR or INR, however, exhibited significantly higher richness of metabolically active gut bacterial species as compared to VU and healthy controls [29].

Table 1. Summary of the main findings

Study details and study type	Study population	Type of sample analyzed	Microbiome analysis technique	Main findings (HIV+ compared to controls)
Nowak et al. 2015 (26) Observational study with longitudinal components	31 HIV+ ART naive individuals (28 viremic patients, 3 elite controllers) 9 HIV− individuals	Stool and blood samples	16S rRNA sequencing	Before ART: • ↓ α diversity in untreated HIV+ patients • ↑ β diversity • Elite controllers' gut microbiome composition resembles healthy controls' gut microbiome more than that of viremic patients • ↑ Bacteroidetes in elite controllers compared to viremic patients ($p = 0.02$) • ↑ Actinobacteria and Proteobacteria in viremic patients compared to elite controllers ($p = 0.02$) Viremic patients compared to HIV− individuals: Firmicutes: • ↑ *Lactobacillus* • ↓ *Lachnobacterium* ($p = 0.018$) • ↓ *Faecalibacterium* ($p = 0.008$) Proteobacteria: • ↓ *Haemophilus* ($p = 0.04$) Viremic patients after ART introduction: • Further decrease of α diversity ($p = 0.001$) Phyla: • ↓ Firmicutes *(Lachnospiraceae)* • ↓ Bacteroidetes *(Prevotella)* ($p = 0.0007$)
Vázquez-Castellanos et al. 2015 (27) Case-control study	15 HIV+ individuals on ART, 15 HIV− individuals	Stool samples	16S rRNA gene, meta-genome sequencing	• Healthy subjects cluster separately from positive subjects based on 16S rRNA sequencing. • HIV positive ↑ Bacteroidetes *(Prevotella)* ↑ Proteobacteria *(Succinivibrio, Desulfovibrio)* ↑ Firmicutes *(Catenibacterium)* ↑ LPS biosynthesis • HIV negative ↑ Bacteroidetes *(Bacteroides)* ↑ Firmicutes *(Faecalibacterium)* ↓ Starch and sucrose metabolism ↓ Glycolysis/gluconeogenesis

Table 1. *(cont'd)*

Study details and study type	Study population	Type of sample analyzed	Microbiome analysis technique	Main findings (HIV+ compared to controls)
Noguera-Julian et al. 2016 (28) Cross-sectional study	Barcelona: 129 HIV+ (15 untreated) 27 HIV– Stockholm: 77 HIV+ (all untreated) 7 HIV–	Stool and plasma samples	16S rRNA sequencing	• α diversity ↑ in MSM richer versus non-MSM • ↓ α diversity both in MSM and non-MSM compared to controls • Lowest α diversity was found in INR • MSM: ↑ *Prevotella,* Non-MSM: ↑ *Bacteroides* Independent of HIV status • HIV+ status: ↑ *Bacteroides*, ↓ *Prevotella*

At this point, it is important to emphasize major differences in the applied analysis methodologies in the studies above that might explain the divergent results and hence drawn conclusions. Whereas most studies derived bacterial 16S rRNA sequencing based data sets, Serrano-Villar and colleagues analyzed the bacterial proteomes (i.e., proteomics). When comparing both completely different methodological approaches, Serrano-Villar et al. rather portrayed the bacterial gut microbiome from a functional point of view by measuring which bacteria taxa were metabolically more active [29], whereas the other studies presented a merely qualitative/relative or quantitative depiction of the bacterial microbiome [26–28].

Changes of bacterial gut microbiome composition

Consistent findings among the 16S rRNA sequencing based studies were the higher relative abundances of Bacteroidetes *(Bacteroides),* Firmicutes (*Lachnobacterium* spp., *Faecalibacterium* spp., and *Ruminococcus* spp.) in HIV-negative subjects as compared to HIV-positive patients *(Table 1)* [26, 27, 29, 30]. Notably, *Faecalibacterium* spp. are considered to exert anti-inflammatory properties, and their depletion has been previously reported in Crohn's disease [31, 32]. HIV-infected patient, however, showed increased abundances of distinct Bacteroidetes, Actinobacteria, Proteobacteria (i.e., *Succinivibrio* and *Desulfovibrio* spp.), and Firmicutes (i.e., Clostridia, Lactobacilli) in their intestinal microbiomes *(Table 1)* [26, 27, 29, 30].

These findings are well in line with results assessed by Noguera-Julian et al. showing that the HIV status of patients positively correlated with the abundances of intestinal bacterial genera clustering with *Bacteroides,* but negatively with genera associated with *Prevotella (Table 1)* [28]. When comparing bacterial populations of the gut microbiome before and after ART introduction, Nowak et al. documented decreased abundances of defined Firmicutes (Lachnospiraceae) and Bacteroidetes (*Prevotella* spp.) in HIV-infected patients undergoing ART, when compared to ART-naive patients *(Table 1)* [26]. Comparing the intestinal microbiome of viremic patients before ART introduction to elite controllers revealed that the *Bacteroidetes* and *Proteobacteria* were enriched in elite controllers. Viremic patients, on the other hand, harbored a microbiome that was enriched with Actionobacteria when compared to patients with undetectable HIV RNA. Overall, the gut microbiome of elite controllers was more similar to that of healthy controls than to the one in viremic patients. This similarity to healthy controls suggests that the change of the gut microbiome composition is profoundly dependent on immune status and recovery.

Villar-Garcias et al. investigated potential beneficial (i.e., health promoting) effects of the probiotic yeast *Saccharomyces boulardii*. After *S. boulardii* treatment, distinct Firmicutes (Clostridiales, *Catenibacterium* spp.) decreased in the gut microbiome, whereas Proteobacteria (such as Desulfovibrionales) increased *(Table 1)* [30]. Remarkably, both ART introduction and probiotic treatment helped to partially restore or at least direct the gut microbiome towards its original, "healthy" state [30].

Nevertheless, analysis of bacterial proteomes by Serrano-Villars et al. revealed different results [29]. From a metabolic point of view, Clostridiaceae and Ruminococcaceae were significantly decreased in the gut microbiome of HIV-infected patients. However, when focusing on active biomarkers by analysis of the most active bacterial populations in each patient group, results were more consistent with those derived from 16S rRNA based sequencing analyses [26–28]: Ruminococcaceae, Clostridiaceae, and Bifidobacteriaceae families were significantly enriched in healthy controls, whereas VU patients exhibited increased Prevotellaceae and Brachyspiraceae levels in their intestinal microbiome [29]. Biomarkers found for IR patients, and therefore as-

sociated with immune recovery, were Succinivibronaceae and Erysipelotrichaceae, whereas, however, no significant biomarkers could be identified for the INR group [29]. Of note, Succinivibronaceae, which were increased from both a quantitative and metabolic point of view in the gut microbiome of IR patients, have previously been associated with anti-inflammatory properties in the porcine colon during nematode infection [33]. Furthermore, increased concentrations of pathogenic clostridial species, which are considered pro-inflammatory mucotropic bacteria, were shown in the intestines of HIV-infected patients [29]. The question, why the metabolically active intestinal Clostridiaceae were shown to be decreased in the study conducted by Serrano-Villar and colleagues, remains unanswered [29].

Nevertheless, given that changes in the taxonomic bacterial composition of the gut microbiome have been shown to correlate with low CD4+ T cell counts during HIV infection, this further underlines the impact of a distinct intestinal microbiota (with a defined balance between bacterial species exerting pro-inflammatory effects and others with anti-inflammatory, immune protective properties) in immune status and recovery.

Correlations between microbial translocation markers, systemic inflammation and gut microbiota

Markers of mucosal damage, microbial translocation, and systemic inflammation were generally higher in INR and patients who presented late in the course of disease and were therefore subjected to a rather late ART introduction [28, 30, 34]. When measuring markers of microbial translocation and systemic inflammation, INR patients tended to exhibit higher fibrinogen and soluble CD14 levels as well as increased lipopolysaccharide-binding protein (LBP) concentrations in their sera. LBP was further positively correlated with high sensitivity C-reactive protein (Hs-CRP), erythrocyte sedimentation rate (ESR), and soluble CD14 [30]. In line with these results, lipopolysaccharide (LPS) was negatively correlated with CD4+ T cell counts [26]. Showing further increased gut microbiome involvement, the number of total observed bacterial species correlated both positively with CD4+ T cells and CD4+/CD8+ ratios, and negatively with LPS and LBP [26].

Serrano-Villar et al. further analyzed the interactions of intestinal gut bacteria and their metabolism with immune function in HIV patients in more detail [29]. The authors concluded that the overall impact of HIV infection and ART treatment *per se* on the microbes' metabolic activity was rather (moderately) low. The patients' immune status, however, seemed to have greater influence on the gut microbiome composition [29]. To support this hypothesis, the authors proved a positive correlation between ceramide-related metabolites (C16 ceramide) and the frequencies of %CD8+, HLA-DR+, and CD38+ T cells in all patient groups. Lower levels of these variables were associated with healthy controls and IR, whereas a worse immune status was associated with higher metabolic activity [29]. Additional metabolite markers of epithelial barrier integrity, hepatic function, and inflammation, distinguishing respective cohorts, were analyzed and are summarized in *Table 2*.

The accumulation of Neu5Ac in bacteria of VU patients only suggests that this cohort may have harbored increased pathogen numbers, which scavenge sialic acid from the host membrane [29]. Moreover, the biomarker LTB4 which exclusively accumulated in IR patients, is one of the first soluble pro-inflammatory metabolite produced from arachidonic acid (AA) metabolism [35]. It is tempting to speculate that gut bacteria may support host immune recovery by lowering mucosal inflammation, since LTB4 only accumulated in immune responders and not in immune discordant and viremic untreated patients. The secretion of LTB4 and other pro-inflammatory molecules derived from AA metabolism might also be modulated by the reported increased AA accumulation in the gut bacteria of all HIV-positive patients [29]. *N*-acyl amide oleamide was also significantly elevated in gut bacteria of IR patients. Of note, *N*-acyl amide oleamide is known to regulate gastrointestinal inflammation [29, 36].

Lastly, each of the three metabolites derived from haem catabolism was associated with one of the HIV-

Table 2. Metabolite markers for HIV infection associated dysfunctions (29)

Metabolite marker	Compared to controls, accumulated in the gut bacteria of:	Associated dysfunction
N-Acetylneuraminic acid (Neu5Ac)	VU patients	Markers of structural changes of the epithelial barrier
Dolichol phosphate (Dol-P) Dolichol-β-D-glucosyl phosphate (Dol-β-G)	All HIV+ patients (VU, IR, INR)	
Arachidonic acid (AA)	All HIV+ (VU, IR, INR); not in HIV–	Markers of inflammation and immune recovery
Leukotriene B4 (LTB4)	IR patients	
N-Acyl amide oleamide	IR patients	
Biliverdin (BV)	IR patients	Markers of the hepatic function, HIV viral infectivity and inflammation
Bilirubin (BR)	INR patients	
Urobilinogen (URO)	VU patients	

positive cohorts [29]. Biliverdin (BV), the first product of haem breakdown, was increased in gut bacteria abundant in IR, whereas bilirubin (BR) was found to accumulate in immune discordant patients [29]. Furthermore, urobilinogen (URO), the final product of bilirubin breakdown, was exclusively detectable in intestinal bacteria of VU patients [29].

ART has been reported to interfere with haem catabolism by inhibiting uridine glucuronyl transferases (UGT), hepatic enzymes, which are essential for the disposal of bilirubin and have been found in some bacteria [29, 37]. This could thus explain the lack of bilirubin in untreated patients, given that bilirubin is not retained through ART inhibition of UGT, and gut bacteria simultaneously contribute to its breakdown to URO, which subsequently accumulates. This might further explain why patients undergoing ART exhibit accumulated bilirubin and biliverdin levels within their gut bacteria and why ART is associated with hyperbilirubinemia [29, 38]. However, this does not explain why gut bacteria in IR patients display accumulated biliverdin, whereas INR subjects exhibit accumulated bilirubin. Biliverdin, but not bilirubin, has been reported to reduce HIV viral infectivity and constitutes an important anti-inflammatory molecule [29, 38, 39]. Thus, immune recovery is suggested to be linked to bacterial biliverdin accumulation in the GIT of HIV-infected patients [29].

Overall, the abovementioned results suggest that gut bacteria play a major role in modulating and counteracting ("buffering") pro-inflammatory processes [29].

Vazquez-Castellanos et al. also found relevant changes in metabolic processes of gut bacteria in HIV-infected patients. Results revealed a general enrichment of the genes involved in various pathogenic processes. As such, HIV-infected patients harbored bacterial microbiomes with increased ribosomal and LPS biosynthesis [27]. This further underlines the previously described increase in LPS levels associated with CD4+ T cell counts [26].

Discussion

Main findings

Our literature survey revealed that HIV infection was associated with reduced bacterial richness, independently of the sexual orientation of infected subjects. This decrease in α diversity positively correlated with CD4+ T cell counts. ART introduction in HIV-naive patients did not result in significant changes of the gut microbiota towards increased and hence beneficial gut microbiome diversity. After simultaneous therapy with the probiotic yeast *S. boulardii*, however, a shift towards a beneficial (i.e., more health-promoting) bacterial microbiota composition could be observed, with a decrease of some *Clostridiales*, such as *Clostridiaceae* and *Catenibacterium,* as well as of microbial translocation and systemic pro-inflammatory parameters [26–28, 30].

The main changes in the bacterial gut microbiome as assessed by 16S rRNA based sequencing technology were the increase of Bacteroidetes (*Prevotella*), Proteobacteria (Succinivibrio, Desulfovibrio), and Firmicutes (*Clostridia, Lactobacillus*) in HIV-infected patients. However, when analyzing the bacterial proteomes, divergent results were obtained, indicating that the relative abundances of bacteria and their total amounts do not necessarily correlate with their levels of metabolic activity. However, results were consistent with the increases in Prevotellaceae and Succinivibronaceae, which were elevated from a functional and quantitative point of view in HIV-infected individuals [26, 27, 29]. Correlation analyses of the gut microbiota with markers for microbial translocation and systemic inflammation suggest that gut bacteria do in fact significantly impact immune recovery. Thus, despite the reported increases in potentially harmful (i.e., pathogenic) bacteria and, conversely, decreases in presumably beneficial (i.e., health promoting) groups, the gut microbiota exhibited immune-modulating qualities, given that mucosal inflammatory sequelae were dampened by decreasing pro-inflammatory and preserving or even accelerating anti-inflammatory cytokine responses [27, 29].

Limitations

One should be careful to draw coherent conclusions from the surveyed publications for the following reasons. Since the importance of the gut microbiome in HIV pathogenesis has only become a research focus in the last decade, there are only very limited numbers of studies that address the complexity of host–microbiome interactions in HIV-infected patients. Moreover, many studies did not include sufficient and representative case numbers. Furthermore, there are no sufficient longitudinal studies that are comprehensively assessing the impact of ART on the gut microbiome composition. Moreover, the complete lack of longitudinal studies comparing HIV gut microbiome before and after HIV infection conceals a broad spectrum of important information that needs to be uncovered. Hence, in order to draw coherent conclusions, follow-up studies with long-term observation periods of more than 12 months would be required. Lastly, the controversial results of studies applying substantially different methodological approaches, such as 16S rRNA sequencing technology and bacterial proteomics, make it virtually impossible to draw significant and clear conclusions.

Summary, conclusion, and future perspectives

In summary, HIV infection leads to CD4+ T cell depletion, chronic inflammation, impaired barrier dysfunction, and significant changes of the gut microbiome composition. The reported decreases in gut microbiome diversity and

increases in microbial translocation and pro-inflammatory markers predict immune status and recovery of HIV-infected patients.

One of the reported changes in the bacterial gut microbiome composition was characterized by the increased abundances of certain Clostridia and Proteobacteria in HIV-infected individuals. Notably, in this context, many AIDS patients develop infectious diarrhea caused by gram-negative pathogens including *Salmonella, Shigella,* and *E. coli* [40]. However, this increase could have relevant clinical manifestations that go beyond the GIT. It is tempting to speculate that elevated Enterobacteriaceae such as *E. coli* and *Klebsiella* spp. in the GIT might lead to a higher risk of developing extra-intestinal infections such pneumonia, urinary tract and wound infections, for instance. This would also increase the risk of pathogenic transmission to and nosocomial infection of patients in case of hospitalization. Lastly, one should be aware of potential bacterial translocation from the leaky GIT to extra-intestinal including systemic compartments leading to increased morbidity and mortality of HIV-infected patients due to bacteremia/septicemia.

All in all, further research is needed to gain a deeper understanding of host–microbiota interactions and the importance of the gut microbiome for immune recovery in HIV-infected patients. To this end, the size of all cohorts should be increased, follow-up periods should be prolonged, and the study of bacterial microbiome should be extended to other microorganisms such as fungal, parasitic, and viral (entero)pathogens. Additionally, more studies applying proteomic approaches should be conducted, as they provide a better and clearer functional portrayal of bacterial influence and impact of HIV infection, ART, and immune recovery on the gut microbiota composition. Deeper insights gained from these studies would presumably open the door to novel therapeutic approaches including probiotics such as *S. boulardii,* which has been shown to reduce bacterial translocation and systemic inflammation [30]. Fecal transplantation should also be taken into account as a possible adjunct therapy in the future. This approach has already been successfully applied in treating recurrent and refractory *Clostridium difficile* infections, for instance, and could therefore be used to modify gut microbiome composition towards a beneficial (i.e., health-promoting) direction and to treat infectious diarrhea in the late stages of HIV infection [41, 42].

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Conflicts of interest

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