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► **To cite this version:**

Gregory Dubourg, Mathieu Surénaud, Yves Lévy, Sophie Hüe, Didier Raoult. Microbiome of HIV-infected people. *Microbial Pathogenesis*, 2017, 106, pp.85-93. 10.1016/j.micpath.2016.05.015 . hal-01573803

HAL Id: hal-01573803

<https://hal.science/hal-01573803v1>

Submitted on 26 Jul 2021

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Microbiome of HIV-infected people

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Consistent interactions between the gut microbiome and adaptive immunity recently led several research groups to evaluate modifications of human gut microbiota composition during HIV infection. Herein we propose to review the shifts reported in infected individuals, as their correlation to disease progression. Though the gut microbiota is consistently altered in HIV individuals, the literature reveals several discrepancies, such as changes in microbial diversity associated with HIV status, taxa modified in infected subjects or influence of ART on gut flora restoration. Similarly, mechanisms involved in interactions between gut bacteria and immunity are to date poorly elucidated, emphasizing the importance of understanding how microbes can promote HIV replication. Further research is needed to propose adjuvant therapeutics dedicated to controlling disease progression through gut microbiome restoration.

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1. Introduction

Humans harbour nearly 100 trillion intestinal bacteria that are essential for health. These microorganisms establish symbiotic relationships with their hosts, making essential contributions to mammalian metabolism while occupying a protected, nutrient-rich environment. The relationship between the host and its microbiota is described as a superorganism that plays an essential role in maintaining health and immunity. Its interplay with the immune system has been demonstrated over the past decade, highlighting its involvement in several infectious diseases. Indeed, loss of bacterial diversity induced by antibiotics is well-known to be associated with *Clostridium difficile* infections [1,2]. Also recently, the same low diversity was shown to be a predictive factor of *Campylobacter* infections [3], independently of antibiotic treatment. Above all, the gut microbiome is a significant source of immunological interactions whose mechanisms are currently only partially known. As an example, the gut flora acts as an adjuvant that promotes influenza vaccination through co-stimulation by microbiota-released flagellin [4]. Complex interactions between microbes constitute a barrier against enteric pathogens as has been demonstrated for *Salmonella typhimurium* colonization [5] or through production of antimicrobial molecules [6]. Experiments performed in germ-free mice infected with *Klebsiella pneumoniae* showed that these animals are highly susceptible to bacterial infection in an IL-10-dependent manner. Thus, Lipopolysaccharide (LPS) pretreatment also rendered germ-free mice resistant to pulmonary *K. pneumoniae* infection. These results suggest that symbiotic gut colonization enables proper inflammatory response to harmful insults to the host [7]. In the same way, the presence of segmented filamentous bacteria within gut microbiota is associated with protection against *Staphylococcus aureus* pneumonia [8]. The role of Gram-negative bacteria from gut microbiota in immune activation and chronic inflammation in HIV individuals is known since circulating LPS had been used as an indicator of microbial translocation [9]. In this context, the possible role of the intestinal microbiome in disease progression had led several teams to assess changes in gut microbial communities during HIV infection. In this review, we have mainly reviewed articles focusing on gut flora compositional disruptions observed in infected subjects, as possible mechanisms involved in disease progression. We also provide perspectives as first conclusions from recent therapeutic strategies dedicated to restoring the gut flora in HIV-subjects.

2. Gut microbiota composition and susceptibility to infections of mucosal origin

Among opportunistic infections, HIV-related disease had long been associated with bacteremia, especially before the existence of antiretroviral therapy (ART). The spectrum of microorganisms seemed to be specific to HIV infection. Thus, *Streptococcus pneumoniae* and *Salmonella* spp. were the organisms the most frequently associated with seropositive status [10]. While immunological status, such as CD4 T-cell counts, had been shown to play a role in the occurrence of invasive pneumococcal diseases (IPD) [11], this is unclear for *Salmonella* bacteremia for which risk factors have not been elucidated [12]. As a matter of fact, even though its incidence is raised among men who have sex with men (MSM), HIV status had been shown in a case control study to increase host susceptibility to *Shigella* infections [13]. This raises the question of gut mucosal microbiota involvement. Indeed, recent studies had shown that low oxygen tension in gut tissue promotes *Salmonella* multiplication, and increases its virulence. Besides, its virulence effectors play a role in intra-species dynamics for colonization [14]. By extension, invasive pneumococcal disease had been associated

with modifications of the airway microbiome. These data support the claim that the mucosal microbiota and its interactions with the immune system could facilitate invasion of infectious agents from mucosal surfaces. This should be contrasted with a recent report, in which desertification of the gut microbiota induced by antibiotics from a patient suffering from XDR-tuberculosis had been associated with fatal sepsis due to IPD [15]. Considering recent advances in understanding the pathophysiological and immunological mechanisms associated with HIV disease progression, these findings led to a focus on gut microbiota composition disruptions in HIV-infected subjects.

Gut microbiota analysis

For many years, culture techniques were the only ones available to assess gut microbiota composition. Since the advent of molecular techniques, in particular high-throughput sequencing technologies, our knowledge of the bacterial repertoire has exploded, permitting rapid, reproducible and cost-effective comparison between large cohorts of different specimens. However, despite significant advantages, these methods exhibit several pitfalls from sample collection to data interpretation. As a matter of fact, microbial signatures differ depending on whether samples are mucosal biopsies, feces or rectal swabs [16,17] and these discrepancies were highlighted in studies dedicated to the HIV gut microbiota. These findings raise the question about the appropriate specimen to collect for studying gut flora composition and in particular its interactions with the immune system, for which adherent mucosal microorganisms are largely involved. Extraction bias has also been extensively reported, from sample lysis to DNA purification. Thus, a heating step at 95 °C, combined with repeated silica bead beating is currently considered as the reference method for releasing DNA [18,19]. Altogether, the use of commercial kits to extract nucleic acids is tempting to improve workflow, but induces multiple significant biases [20–23]. While the 16S approach is the most commonly used, several limitations are now well-established. Thus, the copy number of the 16S rDNA gene fluctuates among bacterial species, and among strains within the same species [24–26], leading to under- or overestimation of relative abundances [27]. In addition, the choice of the targeted hypervariable region is crucial, as choice of the V1–V3 region results in higher richness than when V3–V5 is selected, but is unable to detect Bifidobacteria [28]. Finally, the inability of the high throughput sequencing technologies to detect microorganisms at concentrations lower than 10⁵/mL reflects the depth bias [29].

3. Gut microbiota and immune homeostasis

Homeostasis in the gut mucosa is maintained by a system of checks and balances between potentially pro-inflammatory cells, including Th1 cells that produce interferon- γ , Th17 cells that produce IL-17A, IL-17F, and IL-22 and anti-inflammatory Foxp3⁺ regulatory T cells (Tregs). Th17 cells play a major role in both epithelial homeostasis and host defense against various extracellular pathogens such as *Candida albicans* and *Pseudomonas aeruginosa*. IL-17 and IL-22 stimulate the production of antimicrobial proteins (AMP) by the epithelium and thereby sustain its barrier function. They also induces the recruitment of neutrophils that eliminate bacteria having translocated across epithelium. Although Tregs and Th17 cells exert mostly opposing functions, these cells are two closely related CD4 T-cell subsets sharing reciprocal maturation pathways [30]. There is an active balance between the development of either Tregs or Th17 cells and even plasticity between the two subsets [31]. It has recently become evident that individual commensal species influence the balance between these T lymphocyte subsets. For example, polysaccharide-A (PSA) of *Bacteroides fragilis* has been shown to induce IL-10 expression in

intestinal T cells, which prevents the expansion of Th17 cells and potential damage to the mucosal barrier [32]. Mouse models have shown that species belonging to clusters IV and XIVa of the genus *Clostridium* promote the accumulation of CD4⁺ T regulatory cells [33]. Furthermore, recent work has also indicated that bacterial metabolites are involved in regulating the gut immune response. Butyrate, a colon microbial fermentation product, could induce the differentiation of Tregs in the colonic lamina propria and ameliorate experimental T cell-dependent colitis in mice [34]. Interestingly, normal commensal microbes in the gut appear to be essential for initiating Th17 cell differentiation. Indeed, segmented filamentous bacteria (SFB) induce Th17 cell differentiation. Recently, it has been shown that in response to attachment of SFB or other adherent bacteria, intestinal epithelial cells upregulate and release serum amyloid A (SAA) [35]. Colonization of mice with SFB results in rapid up-regulation of serum amyloid A proteins in intestinal epithelial cells, and SAA1 and/or SAA2 induce the activation of myeloid cells specialized in polarizing Th17 cells. Moreover, both obesity and dietary fat intake can alter the production of cytokines involved in Th17 differentiation and potentially promote the development of Th17.

It is also clear that the immune system affords some control over the composition of the resident microbial communities. For example, certain secreted antibacterial proteins produced by epithelial cells can shape the composition of intestinal microbial communities. In many cases, decreased functionality of the host immune system is associated with a narrowing of the range of microbial species with which mutualism can be achieved: microbes that are normal constituents of the microbiota and classified as 'pathobionts' or 'opportunistic pathogens' in a wildtype host become *bona fide* pathogens in immunodeficiency.

4. Gut homeostasis disturbances during HIV infection

HIV infection causes rapid and profound depletion of lamina propria CD4⁺ T cells, impacting gut homeostasis. Structural damages in the gut mucosa during primary HIV-1 infection lead to profound defects of immunological functions in the gut. This leads to the formation of "leaky gut" that allows microbial translocation from the gut to the bloodstream, which, together with HIV-1 replication, contributes to activation of the immune system. Several investigations have pointed to the resident gut microbiota as an integral driver of pathological inflammation that persists in patients even during the administration of antiretroviral therapy. However the mechanisms of human gut microbiota involvement in inflammatory processes that may influence disease progression in HIV, however, are still poorly elucidated.

An important feature of pathogenic HIV and SIV infections is the preferential loss of mucosal Th17 cells, which does not occur for nonpathogenic lentiviral infections [36,37]. Intestinal homeostasis is achieved in large part by a balance between Th17 and Tregs in the context of genetic and environmental factors, particularly the commensal microbiota [38]. The ratio of Th17 cells to Treg cells is decreased in both peripheral blood and rectosigmoid biopsy samples from individuals with HIV infection. The low Th17/Treg ratio in these individuals correlates with high plasma levels of 16S rDNA, which are indicative of microbial translocation, and with a higher frequency of activated CD8⁺ T cells, which is one of the strongest predictors of mortality and low CD4⁺ T cell reconstitution after starting ART [39]. This change in Th17/Treg ratios is associated with the induction of the immunomodulatory enzyme indoleamine 2,3-dioxygenase 1 (IDO1), which is an intracellular haem-containing enzyme that catalyzes the oxidative catabolism of tryptophan to kynurenine [40]. IDO is an enzyme produced by macrophages and dendritic cells upon stimulation by inflammatory signals such as

IFN- γ that metabolizes tryptophan through the kynurenine pathway. Indeed, tryptophan catabolites can inhibit the differentiation of IL-17 secreting CD4⁺ T cells as quinolinic acid, another metabolite, is involved in neurological signs in AIDS [41]. It had recently been shown that the Kyn/Trp ratio increases in early phases of infection and is positively correlated to CD8⁺ T-cell activation and Treg frequency [42]. The ratio is further increased in untreated subjects whereas it is normalized in ART individuals. Zelante et al. however, showed that highly adaptive lactobacilli in the gut, in particular *Lactobacillus reuteri*, by switching from sugar to tryptophan as an energy source, were expanded and produced an AhR ligand, 3-IALd, active in innate lymphoid cells (ILCs) where it would contribute to mucosal resistance against the opportunistic pathogen *Candida albicans* [43].

Microbial translocation begins during the late acute phase of infection (~day 21–28 post infection) [44]. The ability of the host to prevent microbial products from reaching the bloodstream in the short term is highlighted by the normal plasma levels of LPS in acutely HIV-infected individuals [9]. Indeed, during the acute phase of infection most microbial products found within the lamina propria of the GI tract are localized within tissue macrophages, thus limiting their circulation [45]. However, the preferential loss of mucosal Th17 cells contributes to the damage of the epithelial barrier [46]. As well, HIV-infected subjects have lower frequencies of intestinal CD13⁺ myelomonocytic cells, including dendritic cells, macrophages and granulocytes, compared with HIV-uninfected individuals, which would further impair the removal of microbial products from the intestine and allow them to enter the bloodstream. Using an HIV-infected humanized mice model, Hofer et al. demonstrated that LPS elevation depends on intestinal barrier dysfunction and defective LPS clearance by macrophages [47]. HIV-infected patients display defective phagocytosis of microbial products by monocytes/macrophages [48].

Chronic immune activation and inflammation have been implicated as better predictors of HIV disease outcome and are now considered to be the main hallmarks of HIV infection. In addition to LPS, other indicators of systemic microbial translocation such as sCD14, sCD163, intestinal fatty acid-binding protein, kynurenine/tryptophan ratio, soluble tumor necrosis factor receptor 1 level, high-sensitivity C-reactive protein level, and D-dimer have also been associated with disease progression in untreated patients and with mortality in treated HIV-infected patients [49,50].

4.1. Gut microbiota modifications in HIV individuals: bibliographical methods

The literature review was performed using the PubMed database. Searched terms used included the keywords "gut microbiota", "gut flora", "HIV". Concerning studies that were selected for comparison of compositional modifications or diversity changes (Table 1), only those including feces or mucosal specimens and using the 16S approach were included.

4.2. Significance of diversity changes associated with HIV-infection

Species diversity in a given community takes into account two components: richness, which corresponds to the number of different species within the community and the species relative abundance. Measurement of α -diversity is the most widely used in medical microbiology, representing diversity within a single ecosystem. Several indexes such as Simpson or Shannon-Wiener indices are commonly used to assess gut microbiota α -diversity, while the Chao1 index assesses species richness. Thus, many studies have linked changes in gut flora diversity to human diseases, and the recent use of high-throughput sequencing

Table 1
Characteristics of 7 studies using high-throughput sequencing techniques dedicated to the gut/mucosal microbiota in HIV-individuals. -: unchanged or unknown.

Reference of the study	[56]	[53]	[55]	[57]	[58]	[52]	[51]
Participants							
HIV (of which ART)	21 (19)	32 (18)	28 (14)	21 (21)	31 (19)	40 (20)	18 (0)
Controls	22	9	13	16	9	20	14
Sample site(s)	Multiple	rectal biopsies	feces	feces	feces	mucosal (sponge)	colon biopsies
Technology used	454 pyrosequencing	Phylochip analysis	454 pyrosequencing	454 pyrosequencing	Illumina sequencing	Illumina sequencing	Illumina sequencing
Diversity	↓	—	↑	—	↓	↓	—
Relative abundance							
Class							
<i>Proteobacteria</i>		↑		↑	↑		↑
<i>Clostridia</i>		↓					
Family							
<i>Enterobacteriaceae</i>	↑	↑	↑	↑	—	—	—
<i>Ruminococcaceae</i>		—	↑	—	—	—	↓
<i>Lachnospiraceae</i>	↓	—	—	—	↓ (<i>Lachnospira</i>)	↓	↓
<i>Rickenellaceae</i>	↓	—	↓	↓	—	↓	↓
Genus							
<i>Bacteroides</i>	↓	↓	↓			—	↓
<i>Clostridia</i>	↓	↓		—		↓	↓
<i>Faecalibacterium</i>	↓	—	—	—	↓	—	—
<i>Blautia</i>	↓	—	—	—	—	—	↓
<i>Prevotella</i>	↑	—	↑	—	↓ (after ART)	—	↑
<i>Coprococcus</i>	↓	—	—	—	—	↓	↓
<i>Dorea</i>	↓	—	—	—	—	—	—
<i>Ruminococcus</i>	↓	—	↑	—	—	↓	—
<i>Parabacteroides</i>	↓	—	↓	—	—	—	—
<i>Barnesiella</i>	—	—	—	↑	—	—	↓
<i>Alistipes</i>	—	↓	↓	↓		↓	↓

technology applied to several HIV-cohorts has led to the first statements on gut microbiota modifications in HIV-subjects. Three studies were dedicated to the shifts observed in the rectal mucosa microbiome from infected patients [51–53]. The sampling method was based on the postulate that microorganisms involved in immune homeostasis are mucosal adherent rather than present in feces. Thus, in a study comparing rectal biopsies microbiomes from 32 infected subjects when compared to 9 controls, it was concluded that richness and evenness were not different [53]. McHardy et al. on the other hand, assessed the compositional shift in rectal mucosal microbiota from 20 healthy HIV-negative subjects; 20 healthy HIV-positive subjects on chronic ART [cART] and 20 healthy untreated HIV-positive subjects. They found a decreased Chao1 index in untreated subjects compared to cART or controls, leading them to conclude a positive of impact of antiviral therapy on restoration of species richness [52]. Shannon index results, however, were not mentioned in the paper, although this index seems to have been measured. Besides these studies, Yu et al. reported reduced alpha diversity in anal microbiota from 25 HIV-positive men compared with 51 negative subjects [54], partially but not completely attributable to the use of antibiotics (trimetoprim-sulfamethoxazole). Among work focusing on fecal microbiota, a robust study by Lozupone et al. surprisingly concluded that there was a significantly higher diversity and richness in stool samples from infected patients [55]. The difference was significant when compared with both ART-treated and seronegative individuals. The most recent studies re-launched the debate, as shown by a decreased diversity reported in 21 HIV subjects of which 19 receiving antiviral therapy [56], whereas Dinh et al. found no difference in their cohort including 21 treated patients [57]. Loss of diversity starts to have its real significance in the paper published by Nowak et al. [58]. Shannon and reciprocal Simpson's diversity indexes were calculated from stool samples from 31 HIV-1 infected patients of whom 28 were viremic and 18 were receiving ART. These indices were significantly lower in infected patients compared to controls. Interestingly, patients with the lowest CD4⁺

T-cells counts had the lowest alpha diversity as the Shannon index was a predictor of CD4⁺ T-cell count. Moreover, several parameters of disease progression such as CD4/CD8 ratio or sCD14 were correlated with diversity indexes. Introduction of ART did not restore gut microbiota that became surprisingly less diverse after introduction of antiviral therapy. Finally, stomach fluid flora diversity has been described as reduced in HIV-subjects in the study published by von Rosenvinge et al. on a small sample size [59].

Most studies dealing with the changes observed in gut microbiota in HIV-subjects agree with a significant decrease in diversity, with the exception of the study conducted by Lozupone et al. Similar findings had been to date linked with several diseases, such as inflammatory bowel disease and chronic gut inflammation [60,61] or metabolic disorders including obesity or diabetes mellitus [62,63]. Additionally, loss of diversity is well-known to be associated both with infectious diarrhoea, and with *Clostridium difficile* [64] or *Salmonella* infections [65]. Similar findings had recently been found in travelers developing *Campylobacter* infections [3], leading to a decrease in gut diversity in HIV subjects as a possible door for opportunistic pathogens.

4.3. Structural disruptions are associated with immune reconstitution in HIV-subjects

4.3.1. Clostridia class depletion

Several studies have highlighted the depletion of the Clostridia class, of which many members are involved in immune homeostasis. Interestingly, McHardy et al. reported a significant decrease in Clostridia in cART subjects, mainly due to depletion of *Ruminococcaceae* and *Lachnospiraceae* families [52] (Fig. 1). To the best of our knowledge, however, a clear role for these families in health and disease has not been established yet. However, the role of *Ruminococcaceae* in intestinal gut barrier homeostasis had already been mentioned [66]. Interestingly, the activation and maturation status of colonic myeloid Dendritic Cells (mDC) in HIV patients has been negatively correlated to low prevalence of mucosal species of which

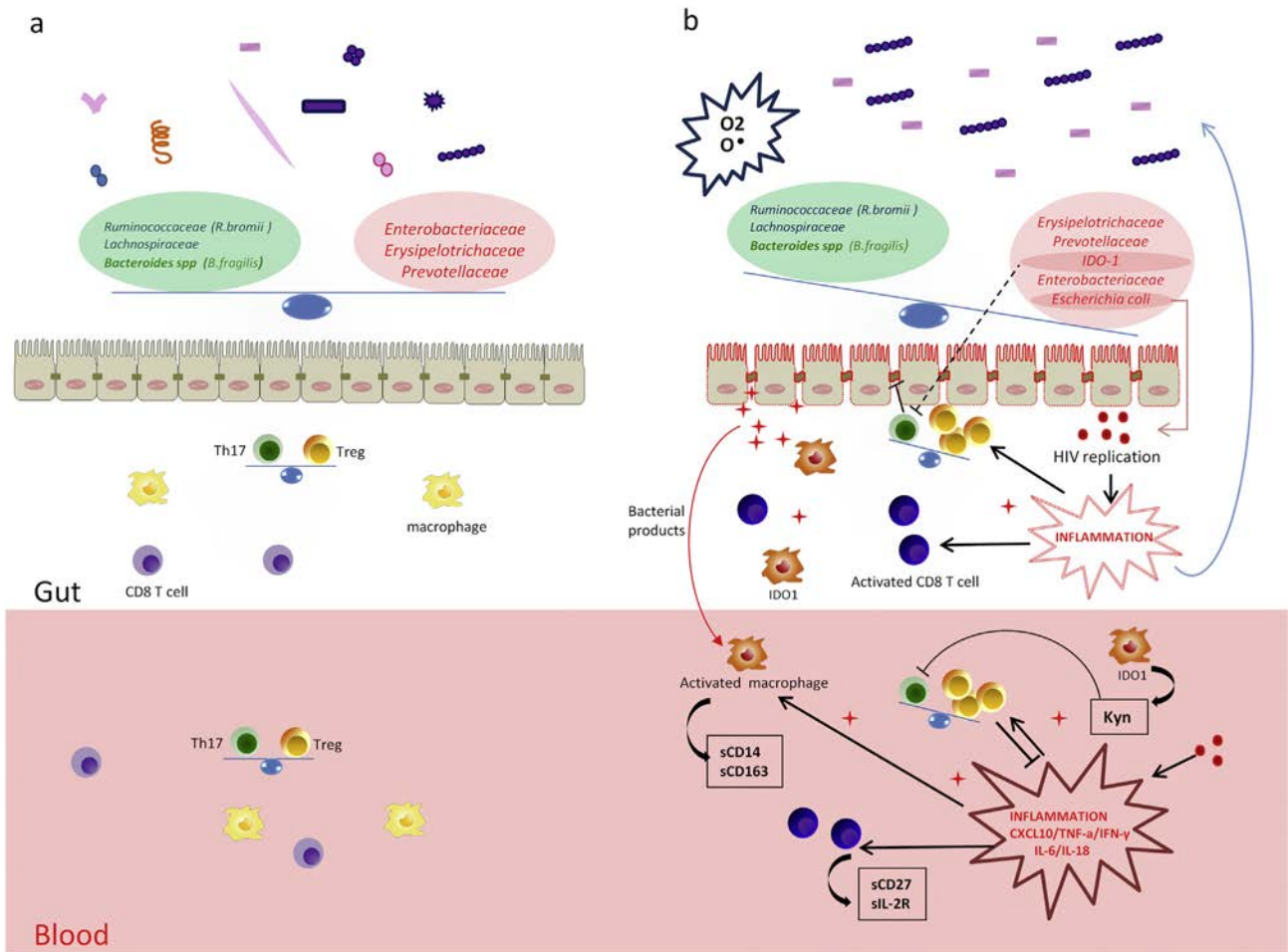


Fig. 1. Immunological disruptions due to compositional gut microbiome shifts in HIV individuals (1b) compared to immune homeostasis in healthy subjects (1a).

Ruminococcus bromii [67]. The *Lachnospiraceae* family, whose depletion is frequently associated with that of *Ruminococcaceae* in HIV subjects, is primarily known for one of its members. Thus, particular consideration had been given these last years to *Faecalibacterium prausnitzii*, an obligate anaerobe belonging to *Lachnospiraceae*. Depletion of this commensal bacterium had been frequently associated with inflammatory bowel disease (IBD) or Crohn's disease, suggesting its anti-inflammatory properties [60,68]. Indeed, depletion of the *Lachnospiraceae* family was reported during HIV infection in three studies [51,52,56] (Table 1). However, the study conducted by Mutlu et al. was the only one to reach the *Faecalibacterium* genus [56]. Nowak et al. showed similar findings but without statistical significance [58]. Further studies are needed to determine whether this decrease is an artifact due to Clostridia class depletion.

Also, *Coprococcus* sp., was significantly depleted in three studies among those included in Table 1 [51,52,56]. While no significance has yet been established due to the low number of publications, several studies have hypothesized role of *Coprococcus* species in inflammatory diseases [69] whereas the genus has been found to be depleted in irritable bowel syndrome [70] Finally, *Lachnospiraceae* and *Ruminococcaceae* decreases have been associated with susceptibility to *Clostridium difficile* infections along with rarefaction of anaerobic fermenters [71]. These disruptions were combined with reduction of diversity. However, none of the studies included in Table 1 observed a concomitant decrease in these three parameters, suggesting that the mechanism remains to be elucidated.

4.3.2. *Prevotella* and *Bacteroides*

Three studies concluded on an increase of the genus *Prevotella* in HIV gut microbiota [51,55,56], despite differences in sampling methods (e.g. mucosa, biopsies or feces), patient enrollment (ART or untreated), or techniques used to characterize flora. Nowak et al. also observed a slight but non-significant increase in the genus, but especially its significant reduction in patients who underwent antiretroviral therapy when compared to naive individuals [58]. The relative abundance of the genus *Prevotella* has been correlated with activation of CD4⁺ and CD8⁺ T cells in mucosal tissue [51]. Recently, the same team associated high prevalence of *Prevotella copri/Prevotella stercorae* with mDC stimulation through CD40 expression in mucosal tissue from untreated HIV individuals [67]. These findings were also associated with mucosal viral load and cytokine production, both in tissue and in blood. These recent studies help shed light on *Prevotella* involvement in gut microbiota homeostasis, which remains partially unknown. To date, the *Prevotella* enterotype has been associated with carbohydrate-based diets [72]. *Prevotella* increase has also been associated with Non-alcoholic fatty liver disease concomitantly with Gammaproteoacteria [73] or the genus *Alistipes* [74] and in ulcerative colitis [75]. Though the underlying mechanisms need to be elucidated, it seems important to mention that *Prevotella* is a mucin degrader [76].

Along with *Prevotella* increase, *Bacteroides* spp. have frequently been reported to be depleted in HIV subjects [51,53,55,56]. Among species belonging to the *Bacteroides* genus, *Bacteroides fragilis*, which had been found to be significantly reduced in HIV mucosal

samples [53] as well in colon biopsies [51] may play a key role in the persistence of the adaptive immune system, inducing conversion of CD4⁺ T cells into Foxp3⁺ Treg cells [77]. Other Treg inducers, such as *B. massiliensis*, *B. thetaiotaomicron*, *Parabacteroides distasonis*, or *B. uniformis* [78–81], have been reported to be depleted during chronic HIV. Recently, Neff et al. screened bacterial genomes for PSA-like gene elements and concluded that many species containing information to encode for PSA were considerably reduced in HIV [82]. These findings suggest a reduction in IL-10 production by Tregs, contributing to maintain a chronic inflammatory state in HIV subjects. Finally, the increased *Prevotella* rich/*Bacteroides* poor gradient in HIV microbiota had been clearly highlighted by the study conducted by Lozupone et al. [81]. Surprisingly, meta-analysis revealed that this community composition resembled that observed in Healthy individuals in agrarian cultures with diets comprised mainly of plant-derived oligosaccharides. These data suggest the need to assess the impact of HIV on gut microbiota in a broader context, including different cultures, diets and lifestyles, in order to avoid confounding factors. It should be mentioned that among the *Bacteroidales*, the *Rikenellaceae* family was reported decreased during HIV infection in five studies, making this statement the least controversial among the different studies. While the genus *Alistipes* seems the most affected [52,58] these data have not been interpreted yet. To date, *Alistipes* spp. has only been associated within IBD when depleted [60].

4.3.3. Overgrowth of *Enterobacteriaceae*

The role of Gram-negative bacteria in immune activation and chronic inflammation in HIV individuals is known, since circulating Lipopolysaccharide (LPS) has been used as an indicator of microbial translocation [9]. It had been shown to be bioactive in mice and a modulator of innate and adaptive immune activation. Moreover, circulating LPS as well as *Enterobacteriaceae* DNA has been associated with T-cell hyperactivation in immunologic non-responders [83]. Subsequently published discrepant findings however, such as the absence of association between sCD14 counts and LPS levels [84] highlights that causal link is not elucidated yet. An increase in Proteobacteria relative to HIV gut flora was observed in four studies [51,53,57,58] making this statement robust, even if, as mentioned previously, mucosal and fecal microbiota can display different composition patterns. Dinh et al. significantly associated an increase in *Enterobacteriaceae* in ART subjects with sCD14, IL-1 β and interferon levels. Moreover, enrichment of Proteobacteria has been also associated with markers of T cell activation, mucosal immune disruption, and chronic inflammation in untreated HIV viremic individuals [53] (Fig. 1). One possible explanation for this overgrowth is the presence of oxygen or nitrogen radicals in the HIV gastrointestinal tract due to chronic inflammation and gut impairment, fostering colonization by facultative anaerobes [85,86].

Dillon et al. studied the impact of exposure to commensal bacteria on human intestinal Lamina Propria CD4⁺ T cells [87]. Interestingly, *Escherichia coli* induced an increase in HIV-1 replication assessed by higher levels of HIV-1 p24 compared to exposure to virus alone. Moreover, T-cell activation and cytokines such as IL-17 and IFN- γ were increased under the same experimental conditions. While LPS purified from *Escherichia coli* did not induce higher viral replication than whole bacteria, antigens involved in the observed enhancement of activation and infection had to be determined. Finally, LPS does not only influence disease progression; but is also involved in morbidity and mortality in HIV patients. Thus, a correlation was established between LPS levels and hypertension in HIV subjects. This association was independent of T CD4 cell count [88].

4.3.4. Bacteria harboring IDO-1 enzyme activity

Tryptophan catabolism by IDO alters the balance of TH17 to regulatory T cells in HIV disease. Interestingly, Vujkovic-Cvijin et al. reported a correlation between dysbiosis, kynurenine production and inflammatory markers such as IL-6 [53]. Several enriched members among Proteobacteria were also found to bear tryptophan metabolism coding genes. Few, however, had been frequently reported to be increased in the HIV microbiota (e.g. *Xanthomonas*, *Burkholderia*, *Stenotrophomonas*, or *Shewanella*). Among these, the genus *Pseudomonas* had been successfully tested for kynurenine production in a culture assay. It can be nevertheless linked with the high proportion of 57 HIV asymptomatic subjects found positive for *P. aeruginosa* qPCR in feces (90%) when compared to the general population (20%) in a study conducted by Gori et al. [89]. In addition, the relative abundance of *P. aeruginosa* was 10-fold higher in infected subjects than in healthy controls in the same study. Finally, further studies are needed to determine if and how gut microbiota is involved in kynurenine increase during HIV infection and disease progression.

4.4. Influence of ART and virological parameters

The influence of ART on gut microbiota restoration has been mainly assessed. Further reduction in alpha diversity has been reported after ART therapy [58]. Unifrac PCOA analyses showed that, among 8 HIV-individuals with long term ART, 5 clustered with untreated subjects and 3 with HIV-negative subjects, suggesting an inconstant restoration of the gut microbiota by ART, for which duration was not a predictive factor [55]. Several relative abundances for long-term HIV individuals have been shown to return to levels comparable to HIV uninfected subjects such as for *Peptococcus*, *Catenibacterium*, or *Desulfovibrio* spp, whereas those of *Bacteroides* or *Prevotella* were more similar to those of untreated patients [55]. The genus *Prevotella* however, has been shown to be depleted after ART introduction in viremic patients [58]. When compared to mucosal samples, subjects receiving ART displayed intermediate levels of enrichment and depletion for several tested genera [52], and diversity that was previously decreased returned to a level similar to that of healthy controls. This community dissimilarity among aviremic individuals on effective ART [53] confirms that ART cannot consistently restore gut microbiota composition to a naïve state. Few conclusions have nevertheless been drawn from other virological parameters [53,55,56] as they were not systematically taken into account or that cohort included long-term ART subjects that were viral suppressed [57]. The most spectacular finding was that the loss of alpha-diversity, which had been reported to be a predictor of CD4 T-cell count in viremic patients [58], that had not been found elsewhere [56]. Concerning the viral load, only Dillon et al. reported a negative correlation between *Lachnospira* and plasma HIV-1 viral load [51].

5. Perspectives

The microbiota of elite controllers has been reported to be distinct from that of viremic subjects. Indeed, Nowak et al. included three of them, for which analysis of β -diversity showed less inter-individual variation between viremic patients compared to both elite controllers and healthy controls. Strikingly, the only long-term progressor included in the study by Vujkovic-Cvijin et al. displayed a gut microbiota similar to that of uninfected subjects. Even though no conclusion can be drawn due to the low number individuals, this should lead to further studies to specifically investigate the gut microbiota of Elite controllers. This would provide answers to several hypotheses such as i) Impairment of the gastrointestinal tract occurs at an early stage of infection and gut microbiota is

subsequently modified ii) Elite controllers are able to control through mucosal factors the initial viremia during primo-infection. This is also supported by the inability of ART to restore gut flora communities, leading to the discovery of adjuvant therapeutics for reducing chronic inflammation state during HIV infection. Using pyrosequencing, a study conducted by Pérez-Santiago et al. highlighted that increased abundance of *Lactobacillales* in the distal gut of recently HIV-infected individuals was associated with lower microbial translocation marker levels, higher CD4% and lower viral loads before initiation of ART [90]. Since then, the studies included in this review failed to report similar findings (Table 1). This did not prevent studies dedicated to the impact of the use of prebiotics during HIV infection on inflammation parameters to flourish. As an example, a randomized trial including 32 patients receiving ART exhibited a significant decrease in D-Dimer levels, along with a slight decrease in CRP and IL-6 [91]. Translocation markers however, were not markedly changed. The microbiota in the probiotic group was consistently enriched in *Lactobacilli* and *Bifidobacteria* whereas *Bacteroides* were decreased, linked to the pro-inflammatory state above. Discrepant results were observed in the “Probio-HIV” Clinical Trial, in which 20 infected subjects with cART displayed a significant decrease in inflammation and translocation markers such as CD38, HLA-DR, LPS Binding Protein –LBP), CRP and IL-6 [92]. Unhappily, the microbiota of participants was not characterized. Further studies should be conducted on larger cohorts to assess the impact of different probiotics on both gut flora and serum markers. It seems difficult however, to normalize all disruptions observed in chronic HIV infection with only one probiotic cocktail, as revealed by the *Bacteroides* decrease observed by the trial conducted by Stiksrud et al., which could be seen as an adverse effect. Considering the initial viremia as the possible cause of shifts induced by the infection, the question of performing fecal grafts on aviremic individuals warrants consideration.

6. Conclusions

HIV-infection is markedly associated with significant compositional gut microbiota modifications. *Enterobacteriaceae* or *Prevotellaceae* seem to be clearly promoted, whereas taxa maintaining mucosal immune homeostasis like *Rikenellaceae*, *Bacteroidaceae*, *Lachnospiraceae* or *Ruminococcaceae* are depleted, resulting in a loss of bacterial diversity. Indeed, while the complex interplay between gut microbiota and the immune system during HIV infection needs to be further investigated, gut flora modifications are clearly involved with disease progression. These data underline the need to design new therapeutic strategies whose objective would be to restore the microbiome as closely as possible to its naïve state, the effect of ART being marginal regarding this issue.

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