Microbiome Studies in Non-human Primates

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Abstract

Purpose of Review Observations of differing bacterial, intestinal microbiomes in people living with HIV have propelled interest in contributions of the microbiome to HIV disease. Non-human primate (NHP) models of HIV infection provide a controlled setting for assessing contributions of the microbiome by standardizing environmental confounders. We provide an overview of the findings of microbiome contributions to aspects of HIV disease derived from these animal models.
Recent Findings Observations of differing bacterial, intestinal microbiomes are inconsistently observed in the NHP model following SIV infection. Differences in lentiviral susceptibility and vaccine efficacy have been attributed to variations in the intestinal microbiome; however, by-and-large, these differences have not been experimentally assessed.
Summary Although compelling associations exist, clearly defined contributions of the microbiome to HIV and SIV disease are lacking. The empirical use of comprehensive multi-omics assessments and longitudinal and interventional study designs in NHP models is necessary to define this contribution more clearly.

Keywords HIV · SIV · Non-human primate · Microbiome · Dysbiosis

Introduction

The commensal microbiome plays an essential role in the development and maintenance of systemic health through antagonism of pathobionts and the production of metabolites which tune immunity and epithelial integrity [1, 2]. In turn, perturbations of the commensal microbiome are associated with systemic shifts in immune homeostasis and contribute to disease susceptibility and outcome. Observations of differing bacterial, intestinal microbiomes in people living with HIV (PLWH) compared to population controls have led to extensive interest regarding the contribution of the microbiome to HIV disease, susceptibility, and vaccine responsiveness [3–14]. In PLWH, dysbiosis — an imbalance of the microbiome favoring an expansion of pathobionts relative to commensal species — correlates with viral load and markers of inflammation [3, 5, 6]. These observations are

particularly intriguing given the documented contribution of microbial translocation to persistent inflammation in disease progression [15–17]. Indeed, dysbiotic taxa have been identified to preferentially translocate [18–20]. It remains unclear whether observations of intestinal dysbiosis are a cause or a consequence of the immunopathologies associated with HIV disease, or how confounding variables contribute.

Non-human primates (NHPs) share ~ 90% sequence homology with humans and SIV-infection of Asian macaques with SIV_{mac} isolates recapitulates key aspects of HIV-1 infection [21–23]. Macaques and humans too share several dominant, gastrointestinal bacterial taxa, though differing at the species level [24, 25]. Herein we review recent published data describing potential contributions of the microbiome to various aspects of SIV disease. These studies have provided new insights and highlight experimental considerations that can be taken moving forward.

What's Dysbiosis Got to Do with It?

The contribution of intestinal microbial dysbiosis to HIV disease progression has emerged as a highly contentious specialty in HIV research. Several publications have reported the presence of a definable dysbiosis in

PLWH — an enrichment of Proteobacteria at the expense of Firmicutes (Gammaproteobacteria and Clostridia, in particular) and among Bacteroidetes, an increase in the *Prevotella:Bacteroides* ratio [3–6]. This dysbiosis has been shown to correlate with biomarkers of disease progression — including CD4 + T-cell depletion, microbial translocation, and inflammation — and to persist after initiation of antiretroviral therapy (ART) [3–6, 26–29]. These observations are not universal, however. Several publications have demonstrated that these microbial signatures are prevalent among HIV-uninfected men who have sex with men, and that failure to balance cohorts by sexual practice biases study outcomes [30–33]. Efforts to assess the presence of dysbiosis in the SIV NHP model have led to similarly conflicting results [20, 25, 34–41, 42••]. As shown in Table 1, variations in anatomic sites sampled, study design, infection stage, NHP species, and viral clone may contribute to differing outcomes. Where the microbiome is concerned, cross-sectional sampling can lead to drastically differing outcomes [43, 44••, 45]. Importantly, whereas studies in PLWH where dysbiosis has been observed have identified sweeping and largely consistent changes in the microbiome, reported SIV-associated changes to the microbiome are disparate and limited and result in widely varying interpretations. For example, whereas Klase et al. observed temporal fluctuations in Proteobacteria and *Lactobacillus* and did not interpret this to reflect dysbiosis, Glavan et al. observed changes to 4 genera and interpreted this as dysbiosis [20, 35]. Whereas dysbiosis itself may be ambiguous, it is clear that where taxonomic changes are observed, SIV infection does not recapitulate the changes in the intestinal microbiome reported in HIV studies. Data are still emerging regarding the potential of dysbiosis at non-intestinal sites [46–50].

Following the initiation of ART, studies agree that an altered intestinal microbiome in PLWH and SIV-infected macaques exists [4–6, 20, 26–29, 37, 39]. First described by Klase et al., ART initiation led to perturbations in bacterial phyla distribution, including a loss of Firmicutes — despite a specific increase of *Lactobacillus* — and an increase in Proteobacteria [20]. The ART-associated increase of Proteobacteria was further observed by Siddiqui et al. and the *Lactobacillus* increase by Blum et al. [37, 39]. It remains unclear whether these ART-induced changes represent a microbial disruption or a recalibration towards the pre-infection microbiome. Whereas neither longitudinal study comprehensively compared the pre-infection versus post-therapy microbiome, Blum et al. reported a significant cross-sectional difference in measures of beta-diversity [20, 37, 39]. Supporting a novel ART-initiated disruption to the intestinal microbiome, we recently examined the influence of individual antiretrovirals on the healthy macaque intestinal microbiome. In Ortiz et al., we observed that short-term administration (1–6 weeks) of antiretrovirals led to

instability of the fecal microbiome — no prolonged changes were observed in individual taxa [51]. These findings are in contrast to pre-exposure prophylaxis microbiome studies in humans, where changes in individual taxa were reported [52–54]. Further research is needed to understand whether these observed post-therapeutic differences are an effect of treatment longevity, route or formulation, longitudinal versus cross-sectional sampling, or host species specificity.

Irrespective of whether lentiviral infections result in a taxa-specific dysbiosis or microbial instability, there remains a question of whether dysbiosis contributes to disease. Lentiviral infections induce massive immunological and physical upheaval, and it is conceivable that any effect dysbiosis may normally have on intestinal immunity is overshadowed by infection. To this end, we treated rhesus macaques with vancomycin both prior to and throughout SIV infection in order to assess whether experimental dysbiosis contributes to SIV disease progression [42••]. Vancomycin induces a specific dysbiosis, inhibiting the ability of Gram + taxa to expand, thereby permitting Gram- taxa to flourish [55]. In our macaques, vancomycin treatment led to an enrichment of intestinal Gammaproteobacteria at the expense of Clostridia, an increase in *Prevotella:Bacteroides*, a shift in the intestinal bacterial metagenome, and a decline in the integrity of the intestinal epithelium. Despite these, vancomycin-treated animals did not exhibit a more rapid progression to AIDS. While humans and macaques are separate species, each harboring their own unique microbiome, the nearly parallel course of lentiviral disease progression in humans and macaques suggests that the microbiome in each species exerts a similar effect on disease outcome [24, 25]. While secondary bacterial infections — dysbioses per se — do complicate disease progression, antibiotic regimens to treat these infections are clinically indicated, with long-term use counter-indicated out of concern for the development of antibiotic resistance [56–58].

Clarity regarding the presence of intestinal bacterial dysbiosis and its contribution to progressive lentiviral disease progression has largely been mired by contextual ambiguity. Progressive HIV and SIV infections can be roughly divided into multiple stages: acquisition, acute and chronic infection, and AIDS. Perturbations in intestinal bacterial communities may exert an outsized influence on any one of these stages without uniformly contributing to disease progression. Sui et al. recently observed that cohorts of rhesus macaques obtained from different sources had significantly differing susceptibilities to rectal SHIV acquisition [44••]. Differences in susceptibility were attributed to significantly higher levels of target CD4 + T-cells and rectal inflammation which in turn, correlated with a an elevated intestinal *Prevotella:Bacteroides* ratio. Concerning established infection, Handley et al. observed that significant shifts in the bacterial microbiome were evident only in end-stage

Table 1 Publications that have assessed the presence of intestinal bacterial dysbiosis in SIV-infected macaques

Publication	Species	Source	Study design	Virus	Infection phase	α-diversity	β-diversity	Taxa observations as compared to uninfected
Allers et al. [34]	*Macaca mulatta*	Colonic mucosa	Longitudinal	SIVmac251	Acute	Significant increase	N/A	Bacteroidetes (Bacteroidia and *Alistipes*) increased. Firmicutes (*Ruminococcaceae* and *Eubacteriaceae*) increased
Glavan et al. [35]	*Macaca mulatta*	Jejunal mucosa	Cross-sectional	SIVmac251	Acute	N/A	N/A	Proteobacteria (*Actinobacillus*) increased
Klase et al. [20]	*Macaca nemistrina*	Feces	Longitudinal	SIVmac239	Acute	N/A	N/A	*Lactobacillus* decreased, Proteobacteria increased
Handley et al. [36]	*Macaca mulatta*	Feces	Longitudinal	SIVmac251	Acute	Not significant	Not significant	No significant differences observed
McKenna et al. [25]	*Macaca mulatta*	Feces	Longitudinal	SIVmac251	Acute	N/A	Not significant	Not reported
Siddiqui et al. [37]	*Macaca mulatta*	Feces	Cross-sectional	SIVmac239	Acute	Significant decrease	Significant difference	9 (unidentified) OTUs significantly differed
Vujkovic-Cvijin et al. [38]	*Macaca mulatta*	Feces	Longitudinal	SIVmac251	Acute	Significant decrease	Significant difference	*Lactobacillus* and *Streptococcus* genera and spp. decreased
Allers et al. [34]	*Macaca mulatta*	Colonic mucosa	Longitudinal	SIVmac251	Chronic	Not significant	N/A	Bacteroidetes (Bacteroidia) increased
Klase et al. [20]	*Macaca mulatta*	Colonic mucosa	Longitudinal	SIVmac239	Chronic	N/A	N/A	No significant differences observed
Glavan et al. [35]	*Macaca mulatta*	Jejunal mucosa	Cross-sectional	SIVmac251	Chronic	N/A	N/A	3 genera differed including an increase in Firmicutes *Streptococcus* and *Staphylococcus*
Blum et al. [39]	*Macaca mulatta*	Feces	Cross-sectional	SIVmac251	Chronic	Not significant	Significant difference	8 OTUs differed including a decline of 3 *Prevotella copri*
Klase et al. [20]	*Macaca nemistrina*	Feces	Longitudinal	SIVmac239	Chronic	N/A	N/A	*Lactobacillus* decreased, Proteobacteria decreased
Handley et al. [36]	*Macaca mulatta*	Feces	Longitudinal	SIVmac251	Chronic	Not significant	Not significant	No significant differences observed
Handley et al. [40]	*Macaca mulatta*	Feces	Cross-sectional	SIVmac251	Chronic	N/A	Not significant	No significant differences observed
Klatt et al. [41]	*Macaca nemistrina*	Feces	Longitudinal	SIVmac239	Chronic	N/A	N/A	No significant differences observed
Ortiz et al. [42••]	*Macaca mulatta*	Feces	Longitudinal	SIVmac239	Chronic	Not significant	Not significant	No significant differences observed
Ortiz et al. [42••]	*Macaca mulatta*	Feces	Longitudinal	SIVmac239	AIDS	Not significant	Not significant	No significant differences observed
Handley et al. [36]	*Macaca mulatta*	Feces	Longitudinal	SIVmac251	AIDS	Not significant	Significant difference	Firmicutes (*Ruminococcaceae* and Clostridiales) and Proteobacteria (*Moraxellaceae* and *Enterobacteriaceae*) decreased

N/A, not assessed

animals, absent prior to AIDS progression [36]. Macaques that had progressed to AIDS displayed an enrichment of *Enterobacteriaceae*, *Moraxellaceae*, *Ruminococcaceae*, and *Clostridiales*. A dysbiosis index in PLWH was similarly associated with nadir CD4 + T-cell count, suggesting dysbiosis is more prominent in individuals with advanced

disease [33, 59]. Importantly, studies in natural, non-progressing hosts of SIV infection have demonstrated microbiome instability in SIV-infected AGMs and chimpanzees as compared to uninfected counterparts, with significant perturbations evident only in animals displaying visible illness [60–62]. Collectively, these findings indicate that the presence of bacterial dysbiosis does not necessitate disease progression throughout acute or chronic infection though a causal relationship may emerge in late-stage disease.

Finally, a lack of cohesion regarding the identification of dysbiotic taxa may be associated with the use of phylogenetic determinations of dysbiosis. In humans, proteomic and metagenomic approaches have been used to concurrently identify bacterial taxa and bacterial functional capacity [29, 63]. The use of longitudinal, multi-omics approaches to bacterial identification may identify core dysbiotic signatures in SIV-infected macaques and in turn, networks of functionally related bacteria or low-abundance keystone taxa that are associated with different aspects of progressive disease [64, 65]. The use of empiric experimental methods will be necessary to determine the relevance of any identified changes.

Vaccination and the Bugs Within

Understanding the interaction between the microbiome and vaccine modalities may better inform the development and utilization of vaccines. Certain microbiome profiles may negatively impact immune responses to certain vaccine modalities, and the microbiome may influence optimal prime boost time windows. Moreover, modalities to alter the microbiome may enhance vaccine immune responses [66].

The microbiome has been shown to calibrate anti-HIV immunity in response to candidate HIV vaccine administration. In humans, acute HIV-1 antibody responses are characterized by non-neutralizing, anti-gp41 immunodominance, which was mirrored by vaccination with an HIV-1 envelope DNA/recombinant adenovirus virus type 5 (rAd5) strategy [67, 68]. This gp41-skewed response has been attributed to the presence of intestinal B-cells — generated in response to bacterial RNA polymerase and pyruvate-flavodoxin oxidoreductase — that produce antibodies which cross-react with gp41 [68, 69]. In an effort to understand the nature and development of this gp41-immunodominance, Han et al. vaccinated rhesus macaques with the HVTN 505 DNA/rAd5 vaccine and observed that for both adults and neonates, a tier 2 (moderate neutralization activity) gp41 immunodominant response was generated post-vaccination [70]. Both human and macaque gp41-reactive antibodies (mAbs) were cross-reactive against macaque fecal proteins, with a candidate gp41-reactive bacterial polymerase epitope present both

before and after vaccination [68, 70]. Intriguingly, 40% each of tested gp41-reactive and gp120-reactive macaque mAbs bound macaque fecal proteins. As the gp120 response lags behind the gp41 response, it is unlikely that microbial proteins prime the gp120 response [67]. This study underscores the utility of the NHP model in identifying bacterial components responsible for the development of poly-reactive mAbs.

Alterations to immunity by disease, diet, or pharmaceuticals can reciprocally calibrate the microbiome, and vaccine administration is no exception. Several groups demonstrated that irrespective of vaccine modality or route of administration, SHIV vaccination induces significant shifts in the rectal microbiome. In Sui et al., the assessment of macaques receiving adjuvanted MVA-SIV revealed that low-dose rectal SHIV$_{SF162P4}$ acquisition was not associated with anti-Env immunity [71]. Indeed, no to very low levels of SHIV-specific antibodies were found and neither anti-Env nor anti-Gag CD4 + and CD8 + T-cell responses correlated with a delay of viral acquisition. Instead, bacterial richness and beta-diversity were found to differ significantly between the vaccinated and naive group after vaccination which negatively correlated with viral acquisition. Musich et al. similarly observed significant differences in the rectal microbiome following vaccination and, intriguingly, observed that empty vector control animals displayed shifts in the rectal microbiome indistinguishable from vaccinated animals [72]. Here, both vaccinated and control animals exhibited significant shifts in measures of beta diversity, a loss of Bacteroidetes, and an increase in Proteobacteria. With both vaccinated and control animals exhibiting comparable infection risk, these shifts in the microbiome were not associated with protection from low-dose, rectal, SIV$_{mac251}$ challenge. However, frequencies of specific taxa correlated with significantly reduced peak viral load in vaccinated animals and Env-specific rectal IgA, which in turn correlated with acquisition risk.

A comparison of the female versus male rectal microbiome in Musich et al. revealed that females showed a greater magnitude of responsiveness following immunization and revealed sex-specific relationships between individual taxa and peak viral load and with biomarkers of protective efficacy [72]. In females alone, Elizaldi et al. observed that shifts in the rectal microbiome were not accompanied by significant or prolonged shifts in the vaginal microbiome following vaccination with plasmid-encoded SHIV Env/gp140 protein [73]. Here too, vaccination led to a loss of rectal Bacteroidetes, including *Prevotella*, and an increase in the Fimicute:Bacteroidetes ratio. Although vaccine efficacy was not assessed in this particular study, the pre-vaccination frequency of *Prevotella* predicted post-boost rectal HIV-1 Env IgG concentrations.

Although these particular studies highlight the importance of understanding the interplay between the microbiome and vaccine development, several outstanding questions remain. Vaccine components are unlikely to directly interact with the intestinal microbiome in cases of non-mucosal administration; however, they may influence the microbiome in mucosal vaccine delivery. Untangling cause and effect may be possible with very detailed time course experiments. Also, particular vaccine components may influence the composition of the microbiome. The studies detailed above used differing vectors, modalities, and adjuvants and all induced shifts in the microbiome, with Musich et al. reporting that alum-adjuvanted control macaques too had demonstrable shifts in the rectal microbiome [72]. A thorough assessment of the effects of individual vaccine components on the microbiome is warranted. Moreover, timing of prime/boost vaccine strategies may influence the microbiome. The studies detailed here spanned 0–38 weeks but it is unclear if observed microbiome shifts are permanent and whether longevity of perturbation might correlate with vaccine efficacy. Furthermore, as the composition of the microbiome impacts systemic health, it will be of interest to determine whether — weighed against the risk of contracting HIV — vaccine-induced shifts in the microbiome influence health and responsiveness to heterologous infections. Lastly, can one reconfigure the microbiome to improve vaccine efficacy? As discussed in more detail later in this article, a more robust understanding of whether and what influence probiotics have on lentiviral susceptibility is necessary for informed vaccine design.

Sex and Age Matter

The push to reduce sexual disparity in health research has led to a wealth of interest in determining how the microbiome influences aspects of HIV infection in women. Although it is established that frequencies of specific taxa within the intestinal microbiome of male and female primates differ and may contribute to differential vaccine efficacy, it remains unclear whether and how the steady-state intestinal microbiome contributes to lentiviral disease in infected females [25, 30, 72, 74–76]. However, several groups have begun to characterize the role of the vaginal microbiome in HIV acquisition [77, 78]. Initial studies which have aimed to develop the macaque model for studying acquisition aspects of HIV infection have revealed that there are striking dissimilarities between the human and macaque vaginal microbiomes. Whereas 50–80% of adult women harbor a *Lactobacillus*-dominant vaginal microbiome, adult rhesus, pigtailed, and cynomolgous female macaques exhibit polymicrobial

microbiomes [77, 79, 80•, 81–84]. Although it has been suggested that these differences make macaques an unsuitable model for female microbiome-associated studies, these differences may make the macaque an ideal model for assessing interventional regimens for women with polymicrobial microbiomes and as such, may be of great value to the HIV research field.

In human females, a *Lactobacillus* non-dominant vaginal microbiome is associated with a pro-inflammatory cervicovaginal milieu and a significantly increased risk of HIV acquisition [77]. It remains unclear what biological factors contribute to *Lactobacillus* dominance and whether the cervicovaginal microbiome can be durably modulated by therapeutic intervention [85]. Hallmaier-Wacker et al. found that menstruation is associated with a significant increase in alpha diversity and shift in beta-diversity among the rhesus vaginal microbiome [82]. Although vaginal community composition varies widely between individual macaques, Nugeyre et al. identified taxa that cycled with progesterone levels within individual cynomolgus macaques and similarly, Rhoades et al. observed that clinical markers of bacterial vaginosis differed significantly by menstrual status in rhesus macaques, as in women [80•, 81, 84, 86]. Menstrual-associated changes to the vaginal microbiome were not observed within the rhesus rectal microbiome, suggesting that menstrual hormones do not directly influence microbiomes at distal sites [80•]. Importantly, for most of the animals in these studies, a high degree of individuality was accompanied by a marked regularity to the vaginal microbiome over time suggesting an adaptation of the microbiome to its host. Efforts to colonize the female macaque microbiome with *Lactobacillus* by Lagenaur et al. have revealed that though robust through a single menstrual cycle, experimental colonization is not universally nor durably sustained [87–89]. Therapeutic colonization may require adjunct therapeutics such as the co-administration of keystone taxa, prebiotics or synbiotics [90, 91].

Mother-to-child transmission rates remain at 11%, with transmission most likely to occur through consumption of biological material during birth or breastmilk in the absence of maternal ART [92, 93]. There have been no studies thus far that have evaluated the relationship between the neonate microbiome and susceptibility to lentiviral infection. Like in humans, the microbiome of neonatal macaques is dramatically different from that of adults [94–97]. A detailed assessment of the rectal, oral, penile, and vaginal microbiomes of female and male infants, juveniles, and young, mid-aged, and older macaques by Janiak et al. demonstrated that infants have significantly differing measures of beta-diversity across all tissues as compared to non-infants as well as higher measures of alpha diversity in the non-oral

tissues [98•]. In non-infants, the microbiome of non-penile tissues did not significantly differ with age whereas the penile microbiome showed a great deal of plasticity.

These observations have two important implications in terms of HIV susceptibility. First — as juvenile macaques have the same microbiomes as adults — the juvenile and young adult microbiome composition is unlikely to uniquely contribute to HIV infection in young adult and adolescent humans. Indeed, Berard et al. observed that low-dose intra-vaginal SIV challenges did not reveal differential outcomes between juvenile and adult pigtailed macaques [99]. There were no observed differences by age in susceptibility to infection, vaginal immune cell subsets or inflammatory markers, or the vaginal microbiome. A second implication of neonatal microbiome plasticity is that there may exist a small window in neonates and infants in which to robustly elicit neutralizing, gp120-reactive antibodies, prior to B-cell imprinting by the microbiome [69, 70, 100]. To this end, Han et al. observed that vaccination of neonates with HIV-1 gp140 trimer induced more rapid gp120 neutralizing antibodies responses as compared to adults and overall higher levels of both gp41 and gp120 plasma antibodies [101]. Given the close relationship between diet, the maturing microbiome, and immune system development in human and macaque infants, it will be important to determine whether diet contributes to the development of natural and vaccine-elicited anti-SIV and anti-HIV humoral immunity [95, 102–104].

Microbiome Therapies: Too Little or Too Much?

Irrespective of the presence of a definable dysbiosis, extensive efforts are underway to alter the intestinal microbiome in progressive HIV and SIV infection. The administration of commensal taxa in the form of over-the-counter oral probiotics has gained favor as they are easy to administer, cost-effective, and generally well-tolerated [105]. In NHPs, these studies have begun to lay the groundwork for human studies. In SIV-infected macaques, probiotic therapy has largely been administered in the context of ART as probiotic therapy alone does not prevent SIV infection nor CD4 + T-cell losses [41, 47]. First demonstrated by Klatt et al., co-administration of probiotics and ART in chronically SIV-infected macaques led to improved CD4 + T-cell recovery and functionality, reduced colonic fibrosis, and increased expression of myeloid cell-related genes [41]. Looking to improve upon these results, Ortiz et al. further supplemented with IL-21 and observed a significant improvement in T_H17 cell recovery [106]. It remains unclear whether these therapeutics broadly altered the intestinal microbiome.

Though promising, the oral ingestion of limited communities of bacteria in commercial probiotics is unlikely to have durable, pervasive effects on intestinal community structure and does not appear to be uniformly beneficial [107, 108]. Fecal microbial transplantation (FMT) has risen as a more comprehensive, personalized approach to altering the intestinal microbiome that, while having caveats of its own, has been shown to durably improve microbial colonization and to preserve intestinal community structure [108–111]. Hensley-McBain et al. assessed the effect of a heterologous FMT on ART-treated, SIV-infected rhesus macaques and observed that although FMT was well-tolerated, treatment was not associated with significant immunological improvements [112]. While the colonic bacterial community composition quickly reverted to the pre-FMT community as assessed by beta-diversity, significant differences in minor taxa (uncategorized) remained evident.

With an observed role for the microbiome in influencing susceptibility to HIV and SIV infection, it follows that purposeful alteration of the intestinal microbiome may tune susceptibility [44••, 77]. Manuzak et al. recently assessed the influence of probiotic VSL#3 on intestinal immune parameters in healthy male rhesus and pigtailed macaques [113]. After probiotic administration, the authors observed significant increases in colonic and lymph node myeloid cells, IgA + B-cells, increased LN TFH cells and intestinal innate lymphocyte type 3 s, and decreased activation of colonic CD4 + T-cells. Similarly, Klatt et al. observed that co-administration of probiotic Visbiome with adjuvanted SIV Gag and HIV Env DNA/HIV gp140 trimer protein vaccination strategy, decreased target cell frequency, increased SIV gag-specific CD4 + and CD8 + T-cell responses, and increased IgA + LN B-cells [114].

Though widely reported to have beneficial effects on gastrointestinal immunity, oral probiotic therapy in macaques has not been shown to extensively remodel the intestinal microbiome [47, 114] perhaps suggesting the potential for a finite number of taxa to influence susceptibility to SIV or HIV. In an effort to create a gastrointestinal pathogen-free (*Campylobacter* spp. and *Shigella*) macaque model, Bochart et al. treated rhesus macaques with enrofloxacin, paromomycin, and fenbendazole for 10 days and observed that absent extensive changes to the microbiome, this therapy led to improved systemic immunity, including reduced microbial translocation, reduced colonic granulocytes, and systemically reduced frequencies of activated CD4 + T-cells [115•]. Furthermore, this particular therapeutic was associated with a significantly decreased susceptibility to low-dose, intrarectal SIV challenge.

Where comprehensively analyzed, the probiotic and antibiotic therapies described above do not reveal an obvious reciprocal commensal:pathogen dichotomy suggesting that immunological benefits of these therapies may be associated

with a recalibration of the metabolic networks shaped by the intestinal microbial ecosystem, more so than the presence or absence of specific taxa [41, 47, 114, 115•]. Indeed, observational studies that have described an association between the macaque or human rectal microbiome and lentiviral acquisition did not identify the taxa utilized in the pre-therapeutic studies described here [30, 31, 44••]. It will be of interest to see which metabolic pathways are associated with protection from lentiviral acquisition and whether and how these pathways may be targeted by next-generation probiotics [116, 117].

Odds and Ends

Research into the contribution of the microbiome to lentiviral disease progression has largely been limited to the bacterial intestinal microbiome. Although necessary efforts to characterize the contribution of the microbiome to SIV disease progression at other anatomical sites have begun to emerge, the bacterial microbiome is not uniform throughout the gastrointestinal system [46, 47]. As first described by McKenna et al., there exist significant differences in bacterial beta-diversity of rhesus macaques between the upper and lower tract, colonic contents, and stool [25]. Described in further depth by Yasuda et al., whereas rhesus macaque stool and luminal contents are predominantly comprised of Firmicute families and *Prevotellaceae*, both the small and large intestinal mucosa are highly colonized by Proteobacteria [118]. Proteobacterial families differed by site, with the small intestine exhibiting an overrepresentation of *Pasteurellaceae* and the large intestine, *Helicobacteriaceae*. Despite these differences, the stool is largely reflective of both the colonic mucosa and luminal contents (both small and large intestine), displaying 97% congruence at the operational taxonomic unit level. Lee et al. additionally considered a comparison between the stomach and colon of Japanese macaques (*Macaca fuscata yakui*) and observed significantly differing beta-diversity [119]. Compared to the colon, the stomach was enriched for Verrucomicrobia, with reduced abundance of Firmicutes which translated largely into metabolic differences.

The microbiome is not limited to bacteria but rather, consists of a complex ecosystem including bacteria, viruses, and eukaryotic symbionts. In both humans and macaques, a lentiviral-associated perturbation of the virome has been described [6, 36, 40, 120]. Whereas AIDS progression in humans is associated with expansions of *Anelloviridae* or *Adenoviridae*, progressive SIV infection is associated with expanded *Adenoviridae* alone [6, 36, 40, 120]. In SIV-infected macaques, *Picornaviridae* showed disease-state fluctuations — though overall expanded during chronic infection, *Sapelovirus* frequencies correlated with protection from infection and a loss of both *Sapelovirus*, and *Enterovirus* sequences accompanied AIDS progression. Although *Adenoviridae*, *Adeno-associated virus*, and *Enterovirus* sequences significantly correlated with the presence of gastrointestinal disease, causative effects of an altered virome remain unclear. In natural hosts of SIV infection, there are contradicting reports regarding the presence of an altered virome in chronic infection — whereas no expansion of the enteric microbiome was noted among captive, non-progressively SIV-infected African green monkeys, a significant expansion in disease-associated viruses was noted in wild gorillas [40, 121]. Efforts to understand whether and how the eukaryome contribute to HIV and SIV disease progression are even further unstudied. Although there are reports that fungal respiratory communities are disturbed in PLWH, it is unknown whether this may be a systemic phenomenon and mycobiome studies in the macaques have not advanced beyond surveys [122–126]. There are no reports thus far examining whether individual eukaryotes or eukaryotic networks associate with aspects of HIV and SIV disease progression. These areas of research remain underserved and may provide novel insight into mechanisms of chronic disease and AIDS progression.

Conclusions

Studies evaluating a contribution of the intestinal microbiome to SIV disease progression have not yet revealed commonalities in bacterial taxa that contribute to SIV acquisition, disease progression, or ART responsiveness. A lack of consensus may stem from experimental variation including cross-sectional analyses or reliance upon a phylogenetic assessment of the microbiome. Further studies are required to determine whether lentiviral-associated variations in the microbiome contribute to rather than reflect disease progression, and to evaluate the ability of microbiome-associated therapeutics to improve aspects of HIV acquisition or immunodeficiency.

Declarations

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