MINIREVIEW

“The interaction between endogenous bacterial flora and the latent HIV”

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Abstract

Human commensal bacteria do not normally cause any diseases. However, in certain pathologic conditions, they exert a number of curious behaviors. In HIV infection, these bacteria exhibit bidirectional relationships: whereas they cause opportunistic infections based on the immunological deterioration, they also augment HIV replication, in particular viral replication from the latently-infected cells, which is attributable to the effect of butyric acid produced from certain anaerobic bacteria by modifying chromatin status. Here, we review recent evidence supporting the contributory role of such endogenous microbes in disrupting HIV latency, and its potential link to the clinical progression of AIDS.
Microbes and its products in relation to HIV latency

HIV attacks the immune system, the body's defense against infectious organisms or other illnesses, creating vulnerability to various infections. Thus, microbial coinfection contributes to the course of disease progression of HIV and the development of AIDS-related deaths (1-4).

A number of bacteria are normal residents in body cavities surfaced by mucous membranes including the oral cavity, gut and vagina. As they endogenously colonized such niches, they seldom cause illness except when the host immunity is impaired. Recent evidence indicates that the mucosal surfaces of both the gut and vaginal cavities are predominant sites for HIV replication (5-7). These mucosal sites are densely populated with CD4+ T cells, the primary target of the virus (1, 6). While mucosal sites contain the same or less number of CD4 T cells when compared to other sites (for example lymph nodes), they are enriched with activated CD4 T cells that express HIV co-receptors such as CCR5 and alpha4beta7 (8). Also, Th17, a subset of CD4 T cells producing IL-17, homes to the gut, and it has been shown to be preferentially infected with HIV (9, 10). The profound loss of these cells has been associated with disease progression in both SIV and HIV infections (11-14). Estes et al. demonstrated the presence of not only LPS, but also of Escherichia coli, in colonic lamina propria and in lymph nodes of chronically infected rhesus macaques (15). In addition, Dillon et al. (16) presented evidence suggesting the preferential infection of IL-17 producing intestinal CD T cells by HIV and the enhancement of HIV productive infection.
in the presence of *E. coli*. Meanwhile, Ahmed *et al.* reported that certain
commensal bacteria preferentially stimulating TLR4 suppressed HIV-1
expression, whereas some with enhancing effects stimulated TLR2 (17).
These findings indicate that HIV-associated impairment of epithelial barrier
integrity and CD4 T cell depletion are most likely involved in systemic
microbial translocation that may give rise to an immune activation, which
could either drive HIV further or out of latency.

Like the gut, commensal organisms thrive in the oral cavity. *Candida albicans*,
a polymorphic fungus which is a commensal microbe in the healthy individual,
could express its pathogenic potential as HIV infection progresses due to the
decay of fungal containment on the oral epithelium associated with the loss of
Th17 cells (18). We have also explored the effect of certain anaerobes that
are part of the oral or gut flora in reactivating latent HIV as most of them
produce butyric acid, the oldest known inhibitor for HDAC, under anaerobic
condition. Initially, we demonstrated that the culture supernatant of
*Porphyromonas gingivalis*, a periodontogenic bacteria, could induce the
expression of quiescent HIV from latently infected T and macrophage cell
lines accompanied by inducing a hyperacetylation of histones H3 and H4 (19).
We found that among the various virulence factors produced by this Gram-
negative anaerobe, a high concentration of butyric acid in the culture
supernatant and the augmenting effect of HIV replication was abolished upon
its removal. To confirm these observations, we attempted to comprehensively
examine the human resident butyric acid-producing bacteria from various
tissues (20). We found that bacterial culture supernatants of *P. gingivalis, F.*
nucleatum, C. cochlearium and A. tetradius increased histone acetylation and efficiently induced HIV gene expression from the latent status. Interestingly, these organisms (except P. gingivalis) produced the highest amount of butyric acid in culture supernatants collected from among representative anaerobic organisms found in oral, oral and gut, gut, and vaginal cavities, respectively (20). Furthermore, chromatin immunoprecipitation analysis revealed loss of HDAC1-AP-4 (transcriptional repressor complex of the HIV-1 provirus) occupancy at the LTR whereas RNA polymerase II recruitment was increased. These effects were correlated with the presence of elevated levels of butyric acid and were not observed in culture supernatants from non-butyrate-producing bacteria. In addition, Gonzalez et al. (21) reported that extracts of P. gingivalis and F. nucleatum enhanced HIV reactivation in monocytes/macrophages via toll-like receptors (TLR) 2 and 9 activation. This same group of investigators recently demonstrated the HIV reactivation in monocytes/macrophages by the oral commensal Streptococcus gordonii to be Tat-dependent and appears to involve NF-κB activation (22). Moreover, TLR5 stimulation could sufficiently induce reactivation of latent HIV in CD4+ T lymphoid cells. It was also reported earlier that P. gingivalis could upregulate CCR5 expression in oral keratinocytes, thus facilitating the transfer of infectious HIV-1 to permissive cells such as macrophages (23). Taken together, these findings support the hypothesis that periodontal and other commensal pathogens, such as butyrate-producing anaerobes, play contributory role in the clinical progression of AIDS.
The vaginal microflora constitutes different bacterial species and some anaerobic bacteria, such as *A. tetradius*, *A. vaginalis*, *P. asaccharolyticus*, and *A. lactolyticus*, have been reported to induce HIV replication. Prior studies have shown the correlation of HIV infection with an abnormal vaginal flora morphology and bacterial vaginosis, with the latter considered as a risk factor in further acquiring the virus (24-27). Interestingly, these bacteria produce significant amounts of butyric acid in anaerobic conditions. *In vivo* as well as *in vitro* studies demonstrated that BV-associated bacteria (e.g. *Candida vaginitis* and HSV) could also influence HIV replication and genital tract shedding (24, 28, 29). Interestingly, Spiegel *et al.* (30) noted the increased levels of butyrate, succinate, acetate and propionate, and decreased lactic acid levels in nonspecific vaginitis. Butyric acid production was caused by *Peptococcus (Anaerococcus)* bacterial species. It is noted that butyric acid but not other short chain fatty acids is responsible for these effects. Moreover, others have shown genital mycoplasmas stimulate TNF-α production from a murine macrophage cell line. Jiang *et al.* (31) recently reported the association of HIV infection with impaired regulation of innate defenses (e.g. human beta defensins) at mucosal sites and increased bacterial colonization of the female genital tract.

**HIV latency as a critical obstacle to the eradication of HIV infection**

Although antiretroviral therapy (ART) has been efficient in reducing the morbidity and mortality of people living with HIV/AIDS, the eradication of the virus has not yet been achieved. The major roadblock in the treatment of HIV
is the persistence of latent HIV-1 reservoirs that are constant sources of rebound virus upon cessation of ART therapy or treatment failure and emergence of drug-resistant viral clones.

Maintenance of HIV latency is a multifactorial event involving several factors including (1) chromatin environment at the site of integration, (2) transcriptional interference, (3) lack of host transcription factors needed for viral gene expression, (4) presence of host transcriptional repressors, and (5) epigenetic silencing of viral transcription (32-36). The elucidation of molecular mechanisms by which HIV-1 virus persists within the infected cells provides a basis for a new therapeutic approach aimed towards combining HIV gene expression therapy and ARV regimen. In general, it involves the use of agents that will bring the latent HIV out of hiding in the cells with the hope that ARV or the adaptive immune response will block new infection events (32, 37-39).

Disruption of chromatin organization at the HIV-1 LTR promoter sets a threshold for transcription factor activation and eventually reversal of the state of chromatin that is responsible for the repression of HIV proviral DNA within the host genome. Interestingly, proof-of-concept studies using various combinations of histone deacetylase (HDAC) or histone methyltransferase (HMT) inhibitors showed that purging of latent proviruses from latently-infected cells is practically attainable (40, 41).

The progression to AIDS is influenced by host inflammatory responses and co-infection with other pathogens such as viruses, fungi, parasites and bacteria (3, 20, 42-45). In particular, opportunistic infection frequently sets in...
when an HIV-infected individual is immunocompromised. AIDS progression is usually accompanied by the action of pro-inflammatory cytokines associated with inflammatory responses that are thought to be perpetuated by the cycle of immune activation brought by opportunistic infections. AIDS-defining events are most likely consequences of cyclical host-microbial interactions within HIV-1-infected individuals. In Figure 1, we depict one such condition caused by commensal bacteria of gastrointestinal and vaginal tissues. Interestingly, the gastrointestinal and vaginal mucosal tissues are major sites of HIV replication and amplification (6, 7). Impaired mucosal integrity and innate defense mechanisms against gut microbes result from HIV infection due to immune activation brought by, but is not limited to, the leakage of microbial products from the gut or skewering of homeostatic responses to inflammatory stimuli (11, 46-48). A recent study on SIV-infected rhesus macaques and African green monkeys revealed the importance of microbiome composition during inflammation and AIDS progression and indicated a significant contribution of the endogenous microbial flora on the course of HIV infection (49).

In the context of HIV latency, it is clearly shown that microbial interactions can regulate the epigenetic status of HIV-1 proviral DNA within the genome of infected cells and its transcriptional competence. We previously reported that human resident butyric acid-producing bacteria from various tissues could reactivate latent HIV-1 proviruses (19, 20). These observations indicate that microbial products or changes in microbiota composition could influence the progression of the disease. This review provides an overview of the
interaction between some endogenous bacterial flora occupying niches in the human body and HIV persistence that may have serious implication to the pathogenesis of HIV infection.

**Epigenetic regulation of HIV gene expression**

The chromatin organization and epigenetic regulation of HIV-1 promoter are critical in establishing and maintaining HIV latency. Immediately downstream of the transcription start site of the HIV-1 LTR, a repressive nucleosome (nuc-1) exists in its hypoacetylated state during latency. Transcriptional reactivation can be facilitated by enzyme complexes that covalently modify tails of the core histones in nucleosomes, promoting changes in chromatin structure and allowing the recruitment of positive transcription factors to the LTR for full transcription (Figure 1, box) (50-53).

Histone deacetylases (HDACs) and methylases (HMTs) are two classes of enzymes closely linked to transcriptional activation and repression of HIV.

A) Histone deacetylases.

HDACs act to repress transcription by catalyzing the hydrolytic removal of acetyl groups from histone lysine residues (54). Moreover, HDACs can interact with nonhistone proteins such as p53, NF-κB, and form multiprotein complex where other components help HDAC carry out its functions (55, 56).

Currently, there are 18 known mammalian HDACs which are phylogenetically...
divided into four groups: Class I (HDAC1, 2, 3 & 8) are related to Rpd3 yeast HDAC and often present in multiprotein complexes harboring Nurd and SIN3 corepressors; Class II are homologous to the yeast HDA1 HDAC and are subdivided into classes IIa (HDAC4, 5, 7 & 9) and IIb (HDAC6 & 10); Class III HDACs represent the nicotinamide adenine dinucleotide (NAD)-dependent sirtuins and are homologs of yeast Sir2; and Class IV includes HDAC11, a constitutively nuclear protein displaying properties of both class I and II (35).

Apart from acting on histones, HDACs can mediate HIV silencing through its physical recruitment to the HIV-1 LTR. In fact, we previously reported the negative regulation of HIV transcription by AP-4 through the recruitment of HDAC1 to the promoter as well as by masking the binding of TATA-binding protein to the TATA box (57). Other HIV-1 LTR-bound transcription factors identified to directly recruit HDAC1, which then accomplish gene silencing, include YY-1/LBP-1 complex, NF-κB (p50) homodimer, Sp1/Myc complex and C-promoter binding factor 1 (58-61). HDAC3 is also reported to be present at the HIV promoter and causes transcriptional repression (62-64). Previously, we showed that the HIV-1 reactivating potential of our novel HDAC inhibitor NCH-51 was abolished when the Sp1 sites at the LTR was mutated or when Sp1 expression was knocked-down by siRNA or by treatment with the Sp1 inhibitor mithramycin A (65). These data indicated that Sp1 is responsible for the recruitment of HDAC1 to the LTR. It is possible that post-transcriptional modification of Sp1 might be involved. As Sp1 is widely believed to be a transcriptional activator, it is to be further elucidated by what biochemical or biological context Sp1 recruits HDAC1 and is converted to a transcriptional
repressor. Further studies are needed to depict a molecular mechanism of this phenotypic conversion of transcription factor.

B) Histone methyltransferases (HMTs).

Generally, HMTs catalyze the lysine methylation of histones, which can be linked both to transcriptional activation or repression (66). Methylation of histone 3 at lysine 4 (H3K4), H3K36 and H3K79 has been associated to gene activation, whereas H3K9 and H3K27 methylation has been correlated to gene repression (67). So far, latent HIV-1 proviruses have been reported to carry histones that are either trimethylated or dimethylated at Lys 9/27 or Lys 9, respectively (68-72). These repressive marks do not affect DNA or histone interactions but serve to recruit effector proteins that influence the transcriptional state of the chromatin.

SUV39H1 trimethylates histone H3 at Lys 9 and mediates repression of the HIV-1 LTR in microglial cells by interacting with HP1gamma (68). The COUPTF-interacting protein 2 (CTIP2) forms a multi-enzymatic chromatin-modifying complex containing SUV39H1, HP1, HDACs 1 and 2 to establish a repressive heterochromatin environment that leads to HIV-1 silencing (69). Recently, it was shown that recruitment of the histone demethylase LSD1 at the HIV-1 promoter was associated with both H3K4me3 and H3K9me3 epigenetic marks and acted synergistically with CTIP2 to repress HIV transcription and viral replication (73). Meanwhile, we previously found the involvement of G9a in maintaining proviral latency by promoting repressive
dimethylation at H3K9 in cell lines where HIV-1 proviral DNA is latently present (71). Either knockdown of G9a with siRNA or G9a inhibition with BIX01294 compound could successfully induce activation of transcription from latent HIV provirus. Furthermore, Friedman et al. reported the contribution of EZH2, the enzyme that catalyzed the trimethylation of H3K27, in silencing HIV proviruses (74). EZH2 is part of the multimeric protein complex PRC2 that serves as a recruiting platform for DNA methyltransferase 1 (DNMT1), the SWI/SNF component bromo-domain containing protein Brd7 and HDACs (75, 76). These additional components are known to be associated with the maintenance of proviral latency. For example, DNMT1 mediates the methylation of the HIV-1 LTR and reinforces HIV-1 latency (75, 77, 78).

Although considered as weak stimulators of HIV-1 LTR, DNA methylation inhibitors, such as 5-aza-2'-deoxycytidine, could reactivate latent HIV-1 provirus and had synergistic reactivation effect with NF-κB activators prostratin and TNF-α (77, 78).

**Potential therapeutic interventions aimed to decrease HIV reservoirs or eradicate HIV**

The disruption of HIV latency has been proposed as part of a strategy to eradicate HIV infection. This is carried out by producing a permissive environment for transcription through altering the degree of acetylation and methylation of histones and non-histone molecules. Inhibitors against HDACs such as trichostatin A, trapoxin, valproic acid, sodium butyrate or vorinostat (VOR; also known as SAHA) have the ability to disrupt latency of HIV infection.
in both cell culture models and ex vivo assays using cells from HIV-1 infected patients or latently infected cell lines (36, 79-85). Archin et al. (83) demonstrated that a single dose of VOR increased biomarkers of cellular acetylation, and simultaneously increased HIV RNA expression in resting CD4+ cells from HIV-1 infected patients. Also, we have previously demonstrated that a novel HDACi compound NCH-51, which has better pharmacological properties than SAHA, could activate latent HIV-1 gene expression with minimal cytotoxicity, through Sp1 sites (65). Meanwhile, methylation inhibitors like adenosine periodate (AdOX) could be employed to globally inhibit protein methyltransferase activity and induce viral production (86). The EZH2-specific HKMT inhibitor, 3-deazaneplanocin A (DZNep) (74), and the SUV39H1 inhibitor, chaetocin, could reactivate latent proviruses and could act cooperatively with HDACis to activate HIV transcription, indicating that combination therapy reverses epigenetic silencing more efficiently (41, 74). Moreover, Bouchat et al. (40) for the first time, demonstrated the recovery of HIV from ex vivo cultures of resting CD4+ T cells isolated from HIV-1-infected HAART-treated individuals by chaetocin or the G9a inhibitor BIX01294. Likewise, they observed that the reactivation activity of one HMTi was intensified when combined either with SAHA or prostratin. Although these findings strongly indicate the feasibility of this therapeutic approach, it has not been clearly observed that the use of such compounds led to a substantial reduction in the frequency of replication-competent cells among the resting CD4+ T cells examined (83, 87, 88).

Concluding Remarks
The commensal microbiota, populating all mucosal surfaces of the body, exerts its beneficial effect by offering nutritional and physiological advantages in exchange for a nutrient-rich habitat within the host. There appears to exist an interesting interplay between the host and these microbes. Just as these microorganisms help shape up the mucosal immune responses, the host also shapes up the microbial community by modulating both the innate and adaptive immune responses (89).

HIV infection is accompanied by functional immune deficiency and loss of mucosal barrier integrity, allowing microbial translocation and driving disease progression (1, 90, 91). A number of HIV-related opportunistic infections have been documented while other potential microbial factors promoting AIDS progression have slowly been unraveled in the past years. It is evident that a delicate balance between commensal microbiota and immune homeostasis is critical in the persistence and progression of HIV infection. The treatment of infections associated with AIDS should conceptually slow down AIDS progression, suggesting that the prevention and treatment of such non-HIV infections might be subsidiary but significant targets for AIDS therapy (1, 92). Further identification of the components of commensal microbiota and elucidation of the underlying mechanisms through which these microbes/microbial products potentiate or interfere HIV-1 pathogenesis could be very important for designing interventions against HIV/AIDS.
REFERENCES


causes induction of integrated HIV-1 without producing a T cell response.

FEBS letters 585:3549-3554.


Tyagi M, Karn J. 2007. CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. The EMBO journal 26:4985-4995.


Barton K, Margolis D. 2012. Selective Targeting of the Repressive Transcription Factors YY1 and cMyc to Disrupt Quiescent Human Immunodeficiency Viruses. AIDS research and human retroviruses.


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Obtained her Master of Science in Public Health (Medical Microbiology) at the University of the Philippines Manila, where she got exposed to HIV studies while working as a research assistant. In 2008, she completed her PhD (Doctor of Medical Sciences) on a Monbukagakusho scholarship at Nagoya City University Graduate School of Medical Sciences (NCU-GSMS) in Japan under the supervision of Takashi Okamoto. In 2009, she got a three-year Japan Foundation for AIDS Prevention postdoctoral fellowship and continued her work in Okamoto’s laboratory investigating the epigenetic mechanisms that regulate HIV latency, screening of small molecule drugs directed at novel HIV targets, and transcriptional regulation of HIV expression. She currently holds a position as an Assistant Professor in the same institution.

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Dr. Imai received his Bachelor’s and PhD degrees in Dental Science from Asahi University School of Dentistry and Mekai University School of Dentistry, respectively. He then joined the group of Takashi Okamoto as a postdoctoral fellow to pursue his interest on the transcriptional regulation and silencing of HIV. He was appointed as an Assistant Professor of NCU-GSMS and Nihon University School of Dentistry (NUSD) in 2008 and 2010, respectively. Dr. Imai has been a recipient of a Young Investigator award from the Society for Microbial Ecology and Disease and the ECC Yamaguchi Memorial AIDS Award from the Japanese AIDS Society for his work on HIV. Currently, he holds a position as an Associate professor at NUSD and studies on host-microbial interactions and infectious diseases with a focus on periodontitis-associated/other oral bacteria, Epstein-Barr virus and HIV latency.

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Obtained his degree of Medical Doctor (MD) and Doctor of Medical Sciences (equivalent to Ph.D.) in School of Medicine Keio University, Tokyo, Japan. He then became a clinical associate at the Department of Internal Medicine at Keio University and post-doctoral (Fogarty) fellow at National Cancer Institute, NIH, Bethesda, USA from 1983 to 1986 under the supervision of Dr. Flossie Wong-Staal and Dr. Robert C. Gallo. From 1986 to 1993, he joined the Virology Division of the National Cancer Center Research Institute in Tokyo, Japan. From 1993 to present, he has been a professor and chairman of the Department of Molecular and Cellular Biology at Nagoya City University Graduate School of Medical Sciences. He received Tamiya Memorial Award for Cancer Research and Award from Japanese Rheumatology Association. His current scientific interests include transcriptional control of HIV replication with specific focuses on Tat and NF-κB. He is also interested in other pathologies where NF-κB plays major roles such as cancer, leukemia, rheumatoid arthritis and autoimmunity.
Figure 1. Causal association of microbial interaction to HIV-1 latency and AIDS progression. HIV-1 infection weakens the immune system and making the body susceptible to opportunistic pathogens. CD4+ T cell depletion leads to impaired mucosal epithelial barrier integrity allowing microbial translocation. The influx of circulating microbial products are associated with systemic hyperimmune activation (e.g. through the TLR-NFκB pathway) that may aggravate and may enhance HIV-1 disease progression. Recently, the bacterial metabolite butyric acid that is produced at anaerobic conditions has been shown to reactivate latent HIV-1 by promoting the dissociation of HDAC1-AP4 repressor complex and hyperacetylation of histones, indicating its potential involvement in the progression of AIDS (see text for the details).
HAT Chromatin environment
Me
Ac
Ac
HATs
Active HIV-1
Positive transcription factors
HIV-infection
Loss of CD4+ T cells
Impaired mucosal epithelial barrier
Impaired immune defenses
Other microbes
Butyric acid
Cytokines
LPS
Other microbial products?
Opportunistic Infections
(viruses, bacteria, fungi, parasites)
TLR-NF-κB pathway
Latent HIV-1
(viruses, bacteria, fungi, parasites)
Figure 1, Victoriano et al.