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The Fight is Not Over: Examining the Challenges in Modern HIV Treatment in Society and Science

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The Fight is Not Over: Examining the Challenges in Modern HIV Treatment in Society and Science

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INTRODUCTION

"*Pneumocystis* Pneumonia – Los Angeles". On June 5th, 1981, the Centers for Disease Control and Prevention released an unusual and baffling account of a rare type of lung infection, in what would be a landmark publication of their *Morbidity and Mortality Weekly Report*. "5 young men, all active homosexuals, were treated for biopsy-confirmed *Pneumocystis carinii* at 3 different hospitals in Los Angeles, California[1]." To an audience of non-medical professionals, reports of pneumonia among young men may seem peculiar, but hardly alarming. However, the fine print of the editorial note at the bottom of the report clarified that *Pneumocystis* in the United States is not only rare, but has also only been seen in either severely malnourished children or adults undergoing chemotherapy or organ transplantation[2,3]. These young men, all previously healthy and in their early 30's, had no clinically apparent underlying immunodeficiency that would predispose them to such an infection. Yet by the time the report had reached across the nation, 2 of the original 5 men had died. Soon, reports from New York, New Jersey, San Francisco, and other cities arrived at the CDC, detailing additional unexplained cases[4]. One month later, a second report came through detailing a cluster of cases surrounding another unusual diagnosis among previously healthy gay males. This time, it was a rare form of cancer in the US, Kaposi's sarcoma[5].

These reports of *Pneumocystis carinii* pneumonia and Kaposi's sarcoma in isolated groups of young, gay men in the United States, soon became known as two of the original defining illnesses of a new disease at the start of the 1980s. Despite treatment with powerful antibiotics and strong courses of chemotherapy, these patients failed to improve and nearly all

died within months of their hospitalization. Early physicians postulated that a "cellular-immune dysfunction related to a common exposure" was to blame for the emergence of these unusual illnesses[1]. Hence, the CDC named the new disorder "acquired-immunodeficiency syndrome" or AIDS. By September of 1982, the CDC reported that an average of one to two cases of AIDS were being diagnosed in the US every day[6]. As stated by G'dali Braverman, an AIDS activist living in San Francisco, "by mid-1982...people were starting to shake in their pants. It was clear that it was more than isolated incidents." Soon, the notion that AIDS resulted from behavior specific to homosexual men was dismissed as reports of AIDS in heterosexual people with hemophilia, intravenous drug users, and migrants from Haiti were released by the CDC[4,7,8,9]. By the end of 1982, risk groups for AIDS were identified as the "4 H's": homosexuals, hemophiliacs, heroin addicts, and Haitians[10].

THE CURRENT FACE OF HIV/AIDS

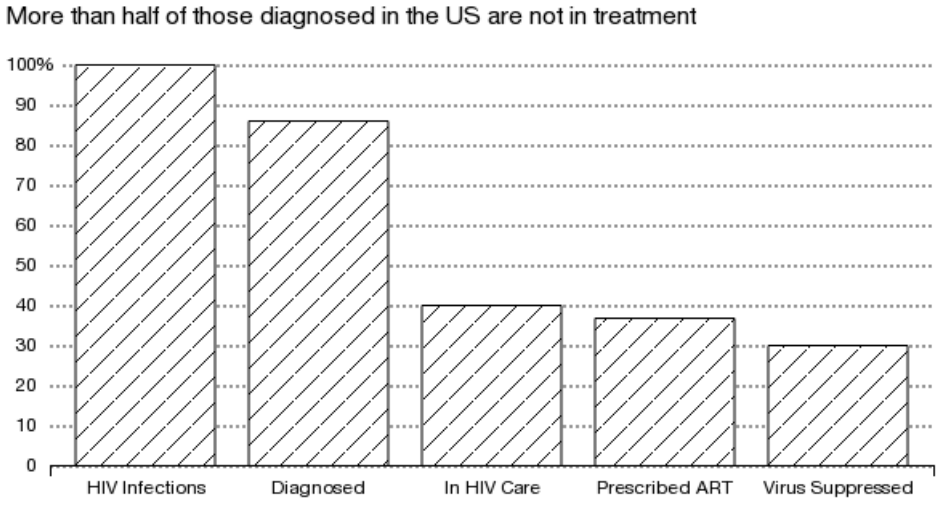
Today, the picture of HIV and AIDS in the United States is much different. Advances in the fields of molecular biology, virology, epidemiology, and clinical medicine, have transformed HIV and AIDS from a perceived a death sentence to a preventable, diagnosable, and treatable chronic disease. HIV and AIDS are among the most studied diseases of all time and, per Robert Gallo, one of the discoverers of HIV as the cause of AIDS: "we know as much about HIV as we do of any pathogen and about AIDS as we do any human disease.[10]" This knowledge has lead to the development of highly sensitive and specific blood tests for HIV, capable of providing readouts in 20 minutes. Over 30 antiretroviral medications are on the market that target HIV biology, disrupting its life cycle and reducing its replication to levels below the limit of

detection. And now, individuals living with HIV have the ability to live longer and healthier lives, a vision unimaginable to those who first saw the epidemic in 1981.

Yet nearly 35 years after the original MMWR report, more than 1.2 million people in the US are still living with HIV, with one in seven of those individuals completely unaware of their infection[11]. New HIV infections also continue at a high level, with approximately 50,000 HIV diagnoses each year.[12] The CDC's HIV Surveillance Report states that 13,712 people with AIDS died in 2013, adding to the approximately 658,507 people in the United States with an AIDS diagnosis that have died overall[13]. Epidemiological surveys have pinpointed the lack of availability of HIV testing and antiretroviral drugs as chief concerns for many developing countries combatting HIV/AIDS, especially in sub-Saharan Africa where the greatest global burden of HIV exists[14]. However, in the resource-rich environment of the United States, nearly all individuals with health insurance can access antiretroviral treatment. And for those uninsured and underinsured, antiretroviral coverage is offered through public assistance programs such as Medicaid, Medicare, and funding provided by the Ryan White Comprehensive AIDS Resource Emergency Act. These programs are made possible by a steady commitment by the US government to ending the HIV/AIDS epidemic at home, by renewing efforts in increasing access to care and prevention funding. In fact, the US government has steadily increased federal funding for domestic HIV/AIDS programs every fiscal year since the epidemic was first identified. According to President Obama's newly released Fiscal Year 2016 federal budget request, the domestic HIV budget for care and treatment, prevention, research, and cash and housing assistance was proposed at $25.3 billion – the highest ever[15]. Nevertheless, according to a study published last year by the CDC, among those who know of their HIV positive status in the US,

fewer than half are getting the care they need to stay healthy[16]. Despite having the highest concentration of specialized HIV clinicians, and in addition to being the manufacturing and research base for all existing HIV drugs on the market, this discordance continues to persist in the United States.

This literature review will explore the barriers and limitations to treatment that exist for people living with HIV/AIDS (PLWHA), especially those from vulnerable populations in the United States. Three key areas, where significant new literature has emerged, has furthered our understanding of the challenges to HIV treatment that prevent our eradication of this disease. Understanding how HIV treatment-specific stigma, social and structural barriers to HIV treatment, and urban poverty disproportionately affects vulnerable populations will be instructive in developing solutions to ending the HIV/AIDS epidemic in the US.



THE STIGMA OF A PILL

"I often hear my friends speak negatively about people being HIV-positive. They always have degrading or negative remarks to make. What I dislike most is when they call people names (e.g., fagot, whore, and junkie). Whenever I go out with them or they come over to visit, I don't take my medications. I could never let them know I'm positive."

– HIV-positive African-American woman living in Baltimore, U.S.[17]

Stigma is not a new concept to medicine and public health, but it is nonetheless a significant barrier to treatment and compliance despite its physical intangibility. Early work by Erving Goffman in the 1960s, provided an application of stigma theory to the realm of health and illness. He defined stigma as a discrediting attribute that reduces the bearer "from a whole and usual person to a tainted, discounted one.[18]" Stigma has been evident throughout human history, discrediting, diminishing, and justifying discriminating those with who are perceived to be deformed and diseased. Those with leprosy were once viewed as receiving "divine punishment for moral misconduct in centuries past" and individuals with cholera as "intemperate, lazy, and vice ridden"[19].

Stigma and fear surrounding HIV and AIDS emerged nearly simultaneously with the birth of the epidemic in the US in the 1980s. Beginning with the first reported cases of unusual infections and cancer in young gay men, the lack of understanding of what this new illness was and how it was transmitted invoked a fear of contagion amongst the public. Early efforts to quell public consternation can be seen in the July 3rd, 1981 *New York Times* article "Rare Cancer Seen

in 41 Homosexuals," where medical investigators described, incorrectly, that there was "no apparent danger to nonhomosexuals from contagion" as "no cases have been reported to date outside the homosexual community or in women.[20]" This fear intensified with other coinciding beliefs, that HIV/AIDS led directly to death, was the result of moral fault and "deviant sex", and was passed through homosexuality, drug use, and sex work, and other undesirable behaviors[21]. These sentiments persisted, with studies conducted in early 2000, 20 years after the first cases, revealing that one in six Americans admitted to feelings of "disgust" related to persons with AIDS[22].

More than 30 years after the first identified cases, HIV/AIDS-related stigma continues to be a persistent problem and major barrier for effective responses to the epidemic, despite significant advances in our understanding of the disease. This stigma, which is tied so intimately to a patient's lived experiences of HIV, has disastrous effects when internalized. It is associated with negative health outcomes, poor adherence to treatment, and increased participation in behaviors that increase risk of viral transmission[23]. Most importantly of all, HIV stigma adversely impacts the most vulnerable individuals living with the disease.

A recent study published earlier this year, examined HIV-related stigma among patients receiving treatment at an outpatient clinic in the Midwestern US[24]. Stigma scores among a sample of 201 individuals engaged in HIV care were higher among women, African Americans, younger patients, and patients with lower levels of educational attainment. This study revealed that HIV related stigma may affect specific disadvantaged communities disproportionately compared to other communities that may have greater social advantages, and that even among

patients from vulnerable populations already engaged in HIV care, stigma remains a penetrating aspect of their disease experiences. Further understanding of the driving forces behind stigma and how it impacts both acquisition of and treatment adherence in HIV disease is needed, especially with regards to disadvantaged populations already facing stigma born of racial or socio-economic discrimination.

To understand how the impact of stigma around HIV may specifically impact treatment adherence, Katz et. al. undertook a systemic review of literature that reported data on this relationship between ART adherence and HIV-related stigma[25]. Their review of qualitative and quantitative studies conducted among 26,715 persons living in 32 countries, found that HIV-related stigma compromised ART adherence by negatively affecting patient's psychological coping strategies. Their study highlighted that stigma impairs HIV-positive persons engaged in treatment from accessing social support and adaptively coping. They note these findings are not novel and are consistent with prior investigations that reflect the importance of social ties in promoting adherence to therapy. Moreover, Katz et al. underlined that social connections may be more important in settings where patients live in extreme poverty and barriers to receiving treatment are highly prevalent.

Paradoxically, Cama et. al. found that people living with HIV/AIDS appeared to experience greater stigma related to taking HIV treatment than general stigma associated with HIV[26]. They suggested that PLWHA may have "heightened concern about treatment specific stigma," which in part may be due to concerns that engagement in treatment and the side effects associated with ART medications visually identifies them as HIV+ individuals. They noted that

while their study results did not find that HIV treatment-related stigma negatively impacted willingness to start ART itself, it could potentially have an adverse effect on ART adherence, which was not assessed. Lastly, reflecting a common theme regarding stigma in the HIV community, Cama et al. noted that their online survey design may not have appropriately captured a representative sample of individuals from marginalized communities, such as injection drug users and immigrants. Such individuals may face pre-existing stigma regarding their behaviors and identities, and thus it is important to understand how HIV-related stigma may exist as an additional, isolating burden in these vulnerable populations and how it may further negatively impact their engagement with medical care.

HIV treatment stigma is a unique and complex social phenomenon. Stigma faced by HIV+ individuals, especially those from vulnerable populations, may translate into adverse experiences in the way these patients cope with their diagnosis, assess social support, and potentially engage with care[27]. Ultimately, when seeking to eradicate treatment stigma, we must recognize that much of what perpetuates this stigma comes from societal and structural sources. On a societal level, stigma can be targeted at its origins in fear and insensitivity due to ignorance and misinformation. Furthering HIV/AIDS education in secondary schools and teaching up-to-date knowledge about how HIV in transmitted, prevented, and specifically treated, will equip young adults with the understanding of what HIV has now become: a chronic, treatable disease. On a structural level, lawmakers and public health leaders need to promote new legislation that prohibits the discrimination of people living with HIV/AIDS as well as fight against policies that criminalize sex work, needle exchange programs, and homosexuality. Deacon et al. makes the argument that public health programs have traditionally focused on prevention, helping HIV-

negative people "stay that way"[28]. They contend that equal efforts should be made to create interventions directed at HIV-positive people to target the forms of stigma and discrimination most concerning to PLWHA. Such interventions can include bridging clinicians and community health workers with PLWHA to craft public relations messages about the significance and benefits of the newest forms of HIV treatment. In this way, PLWHA can act as their own advocates instead of an outside authoritative agency or figure, addressing HIV treatment-related stigma in their own way and with their own unified voice. And while tackling HIV stigma will undoubtedly require a multipronged approach, the first step in our battle will be to universally recognize that HIV/AIDS stigma continues to exist, not only in diagnosis but also in treatment.

STRUCTULLARY UNSOUND: HIV/AIDS INEQUALITY AROUND US

"There were problems such as people who denied the existence of AIDS, others who believed HIV did not exist, groups who believed HIV existed but didn't cause AIDS, and those who believed HIV existed, caused AIDS, and was developed in a U.S. laboratory to kill African Americans and gay men."

- Robert Gallo, *A Reflection on HIV/AIDS Research after 25 Years*[29].

"Structural competency" was theorized as a new approach to medical education that emphasized the need for physicians to be able to differentiate clinical symptoms and presentations, such as hypertension in a young African American man, as downstream manifestations of upstream forces, such as the results of urban infrastructures, zoning laws, and food delivery practices. In essence, this type of understanding is aimed at working in concert with "cultural competency" to mitigate an awareness for practitioners to how both culture and

structure are both joint forces in producing stigma and inequality[30]. Such a framework provides a more in-depth understanding that the conditions associated with culturally distinct groups may not only be a result of the attitudes and actions of said groups but also a consequence of injustices in the social systems that impact them. Just as many forms of HIV stigma and discrimination are disseminated through our legal and political system, many other significant barriers to treatment are also structurally incorporated into to the lives of marginalized people living with HIV. It is in this lens that we might be able to re-examine how institutionalized forces can lead to disparities in the disproportionate impact of HIV disease among vulnerable populations.

In 2010, African American women accounted for two-thirds of new AIDS diagnoses and are the largest proportion of women who are diagnosed with HIV[31]. As a whole, HIV-infected women belonging to racial and ethnic minorities are more likely to miss medical appointments and have late access to and more infrequent use and discontinuations of ART, compared to other women. A recent study by Toth et al. interviewed women-of-color with HIV using the Barriers to Care Scale, which was originally developed to specifically understand the severity of barriers that persons living with HIV face[32]. Toth et. al. found that women reported that the most common barriers they faced in accessing HIV care in order of prevalence were stigma, lack of personal financial resources, lack of adequate and affordable housing, lack of knowledge among people in their community about HIV/AIDS, breaches of confidentiality, and lack of supportive and understanding work environments as well as employment opportunities for people living with HIV/AIDS. While some of these barriers are well known social determinants of health, such as stigma and a lack of HIV/AIDS literacy, many other barriers are structurally based, such as

lack of affordable housing and of tolerant work environments. These authors concluded that specific interventions which targeted self-determination and increased social support for HIV-positive women of color will be necessary for addressing these barriers.

Black and Hispanic men also have some of the highest rates of new infections in the United States. Complicating this problem, an estimated 20-26% of Americans living with HIV were reported to have passed through a correctional facility, especially HIV-infected black men[33]. As jail and prison populations have tripled since the first cases of the HIV/AIDS epidemic in the US were reported, correctional facilities have now become a major factor in the control and treatment of HIV infection[34]. These facilities house populations with considerably higher HIV prevalence rates than the general population and also represent a greater proportion of at-risk groups – ethnic and racial minorities and injection drug users. Additionally, African Americans are incarcerated at nearly six times the rate of their white counterparts. Studies have found that antiretroviral treatment within jails and prisons does lead to significant viral load suppression and increased CD4 T-cell counts in HIV-infected inmates when appropriate clinical care is provided. Between 1995 and 2006, AIDS-related deaths in prisons dramatically declined from 34.2% down to 4.6%[35]. However, despite the demonstrated efficacy of provision of ARVs in correctional facilities, a study of the Texas prison system found that only a third of prisoners that met the Department of Health and Human Services criteria for treatment initiation were actually on therapy. Moreover, treatment regimens may not be continued for those already living with HIV at the onset of incarceration, and frequent transitions among court appearances, detention facilities, and corrections officers may all result in interruption to HIV care[33].

Incarcerated men of color who live with HIV also face the challenge of maintaining their HIV care during the transition from correctional facility back into their communities. A 2009 study found that only 5.4% of newly released prisoners filled their ARV prescriptions within 10 days of their release and 17.7% within 30 days[36]. Treatment interruption can have profound effects on the health outcomes of inmates after their release, as discontinuation of ARVs results in viral rebound, immune decompensation, and clinical progression of HIV/AIDS disease[37]. One cause of this phenomenon is that newly released prisoners face significant social and structural barriers upon release that contribute to treatment termination. Major barriers include drug relapse, untreated mental illness, homelessness, and poverty. Many state laws also require inmates to forfeit their Medicaid benefits upon incarceration, and hence they are without coverage upon their release. Moreover, in accordance with the Welfare Reform Act and Anti-Drug Abuse Act of 1988, many inmates convicted of drug-related crimes are denied food stamps or federal assistance as well as housing from public housing authorities. Such punitive legislation barring them from low income assistance and housing increases the instability HIV-positive inmates face when they transition back into society.

With the difficulties in access and maintenance of HIV treatment faced by patients from marginalized racial groups and with histories of prior incarceration, one proposed method of systemically eliminating barriers to care is through the creation of distinct, federal anti-discrimination legislation[38]. The Americans with Disabilities Act of 1990 does not mention HIV or AIDS as a disability specifically, although the US Supreme Court and Equal Employment Opportunity Commission have both named that the disease as a qualifying disability under the ADA. However, Elliott et al. argue that the creation of an explicit anti-discrimination law can be

used to challenge HIV-based discrimination and human rights violations as a form of discrimination based on disability. Such classification of HIV as a medical disability would not only prevent discrimination of those who live with HIV/AIDS, but also have the potential to assist in the provision of the necessary financial support for individuals who are already suffering co-existing morbidities and social disadvantages. Elliot et al. argue that HIV causes disability through its disease progression, which results in mental and physical conditions that severely debilitate. Even for those on successful antiretroviral treatment, the side effects can also be disabling. The physical damaging effects of HIV and HIV treatment are in addition to the disabling effects of discrimination and prejudice towards people living with HIV – through loss of employment, housing, and other services by employers, providers, and insurance companies. Individuals in the criminal justice system are of particular concern, as they already have limited autonomy and may be subjected to institutionalized discrimination based on their HIV status. It was only less than two years ago that the Alabama and South Carolina Department of Corrections finally abolished their policies of segregating HIV-positive prisoners from the general prison population after a decades-long campaign by the ACLU[39]. Still, PLWHA who enter the Alabama and South Carolina prison systems are excluded from certain in-prison jobs, limited or barred from participation in work release programs, and prohibited from participation in rehabilitative, educational, skills and vocational training programs - leading to longer and inequitable incarceration terms[40,40]. Federal law states that HIV transmission is a criminal offense only in the donation or sale of blood or other potentially infectious fluids or human tissues[41]. But based on a revised report from the Center for HIV Law and Policy, thirty-six states have reported at least 180 prosecutions from 2008 to 2013, where HIV-positive people have been arrested and/or prosecuted for consensual sex, biting, and spitting due to exaggerated fears about HIV

exposure[42]. Undeniably, much work still needs to be done to eradicate existing discriminatory policies and practices where they still exist. But more importantly, substantial efforts must be made at the federal level to enforce current antidiscrimination legislation to ensure protection of the civil liberties of PLWHA.

IS HIV A DISEASE OF POVERTY?

"You think AIDS is a problem? No way. I got real problems."

- A poor black woman and HIV positive mother in New Orleans[43]

In 2010, a new analysis from the CDC prominently revealed that poverty "is the single most important demographic factor associated with HIV infection among inner-city heterosexuals[44]." Their study suggested that many low-income cities across the US now have generalized HIV epidemics, with prevalence highest in those with low socioeconomic status. Contrary to the racial disparities that characterize the US epidemic, the study found no difference in HIV prevalence by race or ethnicity when looking at high-poverty areas of 23 inner-cities nationwide. Essentially, prevalence among African Americans, Hispanics, and Non-Hispanic whites existed uniformly at high rates in low income urban areas. This is contradictory to national numbers, which place the HIV prevalence rate of blacks at 8 times that of whites and among Hispanics, 3 times that of whites. And while the study did not examine HIV prevalence among high-risk groups for HIV infection, such as injection drug users, prior literature suggests a strong connection between high rates of substance abuse and AIDS among communities in poverty. These recent findings are hardly surprising, as public health leaders in the field have long believed that poverty was a key driver of the HIV epidemic. However, this study, which is the first National HIV Behavioral Surveillance System survey among heterosexuals, reveals that

poverty is a driving factor in generalized HIV epidemics outside of traditionally recognized high-risk groups.

Recently, studies have further examined how poverty affects adherence in individuals already engaged in treatment. In a study conducted at the University of Connecticut, Kalichman and Grebler matched patient's measures of extreme poverty with HIV treatment adherence, which was assessed through prospectively conducted, but unannounced pill counts. What they found was that in contrast to social and health stressors such as having "experienced discrimination" or "having a serious illness," which were not significantly associated with adherence, poverty experiences were clearly and consistently correlated with non-adherence. Decreased levels of medication adherence were significantly related to nearly every indicator of food insufficiency such as "having to choose between food and medications" and "running out of food"[45]. In fact, nearly one in four participants with poverty experiences dropped adherence to below 50%, increasing their risk of treatment failure and HIV disease progression. Kalichman and Grebler eventually concluded that although reducing depression, stigma, and social and health-related stressors has demonstrated adherence improvement in less impoverished communities, individuals in poverty are faced with astounding food insecurity and insufficiency that overshadows all other stressors.

Kalichman followed this initial study with a report of the health outcomes of food insecure patients who are prescribed antiretrovirals (ARVs) that should be taken with food. Access to a reliable and sufficient food source can be critical to maximizing the effects of ARVs, as food increases the bioavailability of the drugs, some by as much as 38%[46]. Building on their

previous method of unannounced pill counts, the new study also looked at abstracted HIV viral load and CD4 T-cell counts from medical records to assess viral suppression and disease progression. Their analysis found that greater than half of their participants who were food insecure were prescribed ARVs that should be taken with food. Consequently, these individuals were found to have lower CD4 cell counts and unsuppressed HIV viral loads. In the context of poverty, where many individuals live without a stable and dependable food access, the authors point out that food access must be addressed by clinicians and providers as food maybe the only aspect of poverty that plays a key role in the pharmacokinetics of antiretroviral medications[47].

In addition to food insecurity, a lack of permanent housing also provides a major barrier to consistent HIV medical care and treatment. Aidala et al. analyzed interviews of 1,661 individuals living with HIV/AIDS in New York City from 1994 to 2006 to determine a relationship between housing need and access to HIV care. Their study found that housing was a significant barrier to regular and appropriate care, and conversely, housing assistance had a positive, independent impact on access to and retention in HIV treatment[48]. In agreement with the conclusions by Kalichman, Aidala et al. stressed the need for prioritization of food and housing assistance to people living with HIV/AIDS and poverty as critical missing components in our current view of HIV treatment and management.

Because the lack of these basic needs has deleterious effects on both treatment adherence and on the clinical markers of disease progression and health outcomes, these are areas where effective interventions must be implemented to provide adequate food and shelter for this disadvantaged population. Such interventions are not only feasible, but can also have substantial

benefits in increasing the clinical outcomes of PLWHA. Hawk et al. used the "The Open Door," a nonprofit harm reduction housing program, to examine the impact of stable housing on the viral loads of previously chronically homeless individuals living with HIV/AIDS[49]. The Open Door, based in Pittsburg, PA, operates as a supportive housing program whose main mission is to serve individuals living with HIV that are not eligible for more traditional housing programs. These individuals, who may be active drug users, have criminal histories, and/or mental health diagnoses, are prioritized for admittance as they are less likely to be served by traditional housing models or have experienced limited success through such models. In order to assess the effectiveness of this housing approach on the health outcomes of the residents living with HIV, Hawk et al. utilized viral load data that were collected by The Open Door program staff from residents' existing clinical providers. Amazingly, after 22 months of residency in the program study, over 69% of residents achieved undetectable viral loads. This figure impressively surpasses the 39% of participants found to have undetectable viral loads in the landmark, CDC-sponsored Housing and Health Study, which investigated the effects of providing immediate rental housing assistance to PLHA who were homeless[50]. As such, these two studies provide evidence that not only is access to housing an effective structural intervention for homeless PLWHA, but also an important future approach to helping this marginalized population be retained in treatment and achieve undetectable viral loads.

CONCLUSION

Persons living with HIV and AIDS from vulnerable populations in the US experience considerable barriers to treatment engagement. From an additional strain of internalized HIV treatment stigma to the overwhelming burdens of food insecurity, homelessness, and poverty,

many of these impediments faced by the most marginalized members of our society disproportionately affect their retention in HIV care. Hence, specific strategies must be taken to target the obstacles that these patients experience on a societal, structural, systems-based, and individualized level. Mediation by clinicians and social workers to increase self-determination and self-motivation can provide the social support needed for those experiencing greater effects from the stigma of being treated for HIV. The creation and enforcement of national anti-discrimination legislation for PLWHA, especially those with current or past histories of incarceration, can help eliminate the punitive legislation and practices enforced out of fear and antiquity by isolated authoritative bodies. And increased federal funding and support for public assistance programs that provide supportive housing services to PLWHA such as The Open Door and the National AIDS Housing Coalition can address the homelessness, hunger, and poverty that complicate HIV treatment and lead to improved health outcomes. Ultimately, eliminating barriers faced by underserved and vulnerable populations in the US necessitate a prioritization of these social and structural factors as key areas for targeted intervention. It is only through this approach that we can improve the engagement of consistent HIV care for such patients, by learning to recognize and address the most intimate and pertinent challenges to their well-being that they face on a day-to-day basis.

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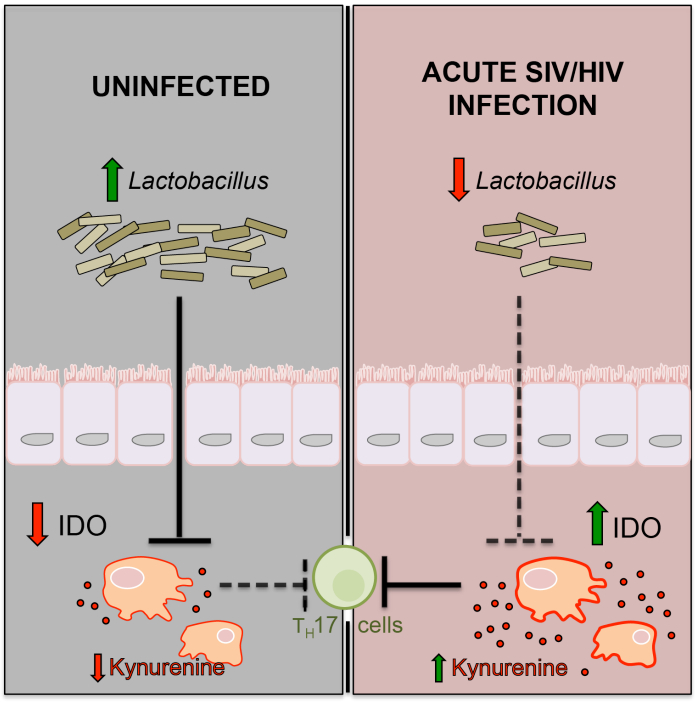
Part Two: Elucidating the Function of *Lactobacillus* in Modulating Tryptophan Catabolism and Maintaining Gastrointestinal Mucosal Integrity in SIV Pathogenesis



Graphical Abstract by Chu, Vujkovic-Cvijin
Vujkovic-Cvijin et al., 2015, Cell Reports *13*, 1-9

INTRODUCTION

The development of the first antiretroviral medications significantly improved the prognosis of people living with HIV/AIDS, transforming what once was seen as a death sentence to a now treatable, chronic condition. Nevertheless, despite the advent of Highly Active Antiretroviral Therapy (HAART) nearly 20 years ago, HIV-infected individuals today still experience higher rates of morbidity and mortality even while on treatment. Many treatment-experienced patients continue to lack complete immune reconstitution and exhibit signs and/or symptoms of systemic and chronic inflammation in spite of adequate control of viral loads. Such ongoing activation of the immune system has been hypothesized to induce the development of non-AIDS defining complications such as obesity, atherosclerosis, neurodegenerative disease, and autoimmune disease (Miedema et al., 2013).

Considerable evidence in the field has lead to the widely accepted understanding that chronic immune activation plays a key role in HIV and SIV pathogenesis and progression to AIDS. This chronic inflammatory process appears to persist indefinitely and does so in a heterogeneous manner that appears independent of historical and surrogate measures of HIV infection such as CD4 T cell count and viral load (Ipp et al., 2014). However, markers of immune activation have been shown to be the best predictors of progression to AIDS and death, independent of viral load (Miedema et al., 2013). A growing number of studies has pinpointed monocyte and macrophage-related inflammation instead of T cell activation as the primary drivers of disease progression, as elevations of IL-6, sCD14, and sCD163 in HIV disease have been shown to be better prognostic indicators of morbidity and mortality (Deeks et al., 2013; P. Hunt et al., 2012; A. Tenorio et al., 2013). Despite sustained control of viral replication, HAART-treated individuals continue to

demonstrate higher levels of immune activation and incomplete immune restoration, indicating a basis for immune insult outside of direct viral pathogenicity (Hunt et al., 2003).

The macaque model of SIV infection has been a major driver of advances in our understanding of the role of chronic immune activation in the setting of HIV disease. SIV infection of rhesus macaques provides a well-controlled model of HIV infection, as SIV infection of macaques leads to a disease syndrome indistinguishable from that seen in HIV infection of humans (Beer et al., 1998; Goldstein et al., 2005, Lackner and Veazey 2007). However, natural SIV infection does not result universally in progression to AIDS in all non-human primate species. Notably, African green monkeys (AGMs) (*Chlorocebus sabaeus*) and Sooty mangabey monkeys (*Cercocebus atys*), which are disparate species from the rhesus macaque (*Macaca mulatta*), exhibit nonpathogenic SIV infection that does not progress to AIDS. A distinguishing feature of pathogenic infection from nonpathogenic infection is impaired mucosal barrier epithelial permeability that is believed to prompt bacterial translocation leading to immune activation and inflammation (Nigam et al., 2011). As such, it is now believed that a major source of sustained inflammation is microbial translocation, as HIV and SIV infection have been found to directly compromise the epithelial barrier of the gastrointestinal tract, increasing its permeability (Estes et al., 2010).

The mucosal columnar epithelium of the gastrointestinal tract acts as both a physical and functional physiological barrier to exposure of both our commensal gut bacterial flora as well as harmful microbial species. HIV infection results in significant disruptions in epithelial tight junction protein expression, precipitating increased permeability, barrier functional impairment,

and increased translocation of bacteria and their microbial products (Nazli et al., 2010). This translocation through the epithelial barrier permits the interaction and activation of innate immune cells that contribute to systemic immune activation. This was first described when circulating lipopolysaccharide, a major component of the gram-negative bacterial cellular membrane, was found to be significantly increased in both chronically HIV-infected individuals as well as in pathogenic SIV infection of rhesus macaques (Brenchley et al., 2006). These microbial products can then activate macrophages and dendritic cells via toll-like receptors to produce pro-inflammatory cytokines and oxidative damage to lead to further activation of the immune system.

The pathogenesis of gut epithelial barrier destruction and microbial translocation may originate in an early preferential depletion of a specific subset of CD4+ T cells in HIV and pathogenic SIV infection. The loss of IL-17-producing Th17 cells, which provide protection against bacterial infection at mucosal sites (Mills KH, 2008), have been proposed to account for breakdown of the gut epithelial barrier and disruption of mucosal immunity (Brenchley et al., 2008). This selective depletion of Th17 cells is seen only in pathogenic SIV infection of macaques but not natural SIV infection of African Green monkeys, the latter of which maintain gut Th17 cells at levels comparable to uninfected animals and lack evidence of microbial translocation (Favre et al., 2009; Klatt et al., 2013). On the other hand, SIV infection has been shown to induce a loss of Th17 cell populations in the ileal mucosa of macaques, which has been shown to promote *Salmonella typhimurium* dissemination from the intestinal mucosa to mesenteric lymph nodes and the spleen due to impaired mucosal barrier function (Raffatellu et al., 2008). This depletion is also predictive of systemic and sustained T cell immune activation, mucosal immune

dysfunction, and is correlated with disease progression in such animals (Favre et al., 2009; Paiardini 2010). Antiretroviral treatment also fails to reverse a loss of mucosal Th17 cells even in long-term HAART-treated patients and is associated with ongoing microbial translocation (Chege et al., 2011).

Despite their selective depletion, however, there is no evidence that Th17 cells are preferentially infected during pathogenic SIV and HIV infection (Brenchley et al., 2008). Instead, it is believed that their destruction is a result of induction of the enzyme indoleamine 2,3-dioxygenase 1 or IDO1, which is found in macrophages and dendritic cells and up-regulated by interferons and toll-like receptor activation during early infection (Mellor et al., 2004). IDO1, which is elevated in progressive HIV infection, is the rate-limiting enzyme for catabolism of tryptophan into kynurenine. Kynurenine, as well as other tryptophan catabolites, can impede the differentiation of Th17 cells through the induction of FoxP3 expression, up-regulation of T regulatory cell function, and inhibit expression of the RORc gene, the key transcriptional regulator of Th17 differentiation (Favre et al., 2010). In animal models, elevated kynurenine levels are significantly higher in pathogenic SIV infection with high viremia compared to low viremia. Additionally, an elevated ratio of kynurenine to its parent compound tryptophan (a ratio used as a surrogate measure of IDO1 activity) is seen in both untreated and treated HIV infection and can predict disease progression independently of other inflammatory pathways (Boasso et al., 2007). More recent investigations have discovered that the initial disruption in mucosal immunity may precipitate the development of gut dysbiosis that enriches for gut-resident bacteria which also have a capacity to catabolize tryptophan through the kynurenine pathway, supplementing host-IDO1 production. Gut microbiota in HIV-infected individuals as well as HAART-treated patients

were found to be distinct from uninfected controls, with enrichment of species such as *Pseudomonas*, which were demonstrated to exhibit a direct capacity for kynurenine production in isolated culture (Vujkovic-Cvijin et al., 2013).

The presence of commensal gut bacteria has been shown to be a requirement for the induction of steady-state Th17 cells in the intestinal lamina propria. Antibiotic treatment or the complete absence of commensal bacterial flora has been shown to reduce Th17 cell populations in the intestine (Ivanov et al., 2008). Emerging studies have attempted to improve intestinal Th17 reconstitution in antiretroviral-treated, SIV-infected macaques through the administration of oral probiotics. Animals that were treated daily with the probiotic VSL#3, which is composed of predominantly live *Lactobacillus* species[1], showed improved gastrointestinal integrity and immunity (Klatt et al., 2013). Additional supplementation with IL-21 enhanced this affect, promoting intestinal Th17 recovery with reduction in markers of microbial translocation and intestinal dysbiosis (Ortiz et al., 2015). Probiotic and IL-21 supplementation with ARVs also reduced intestinal IDO1 and circulating kynurenine levels in chronically SIV-infected animals.

The studies described here-in report that the specific depletion of gut-resident *Lactobacillus* is observed during both acute and chronic stages of SIV infection in macaques. This depletion is correlated with increased IDO1 activity and a loss of the Th17 reservoir. Moreover, the addition of cell-free supernatants of *Lactobacillus* species to interferon-activated human monocyte-derived macrophages show the ability to suppress IDO1 catabolic activity *in vitro*. This effect is not maintained after progressive passage of *Lactobacillus* isolates in cell culture. These findings

[1] VSL#3 contains *Bifidobacterium breve, longum, and infantis*, *Lactobacillus acidophilus, plantarum, paracasei, and bulgaricus*, and *Streptococcus thermophilus* species

provide evidence that gut-resident *Lactobacilli* are critical for maintaining the Th17 reserve and repressing tryptophan catabolism and production of kynurenine by IDO1.

EXPERIMENTAL PROCEDURES

Sample collection and *Lactobacillus* isolation

Eight female rhesus macaques (*Macaca mulatta*) were infected with $SIV_{mac251\text{-}2010}$ on July 16, 2013 with 100 $TCID_{50}$ administered I.V. Stool was collected and stored at -80°C at the following days relative to the dates of infection for all rhesus macaque animals: -7, 3, 7, 10, 14, and 56 days. *Lactobacillus* primary isolates were isolated by plating frozen stool onto MRS selection agar (Becton, Dickinson and Company). Growth assessment was performed in MRS liquid media at 37° C using an automated spectrophotometer with OD_{600} readings taken at regular intervals. CFS was obtained by incubating *Lactobacillus* cultures at 37°C for 3 hours, centrifuging for 5 minutes, and then collecting the supernatant and filtering with 0.22 μm filters.

In vitro Experimentation

Peripheral blood mononuclear cells were isolated from whole blood using Ficoll-Paque reagent (GE Healthcare) and density-gradient centrifugation. Monocytes were isolated using positive selection with CD14+ microbeads (Miltenyi Biotec). Purified CD14+ monocytes were cultured in RPMI medium supplemented with 10% FBS, L-glutamine (2mM), penicillin (50U/mL), and streptomycin (50μg/mL), and macrophage colony-stimulating factor (50ng/mL) to promote macrophage differentiation. Macrophages were subsequently activated with interferon gamma (100ng/ul) to drive IDO1 production in cell culture. Cell-free supernatant cultures of various species of *Lactobacillus* spp. were added to IDO1-producing macrophages and IDO1 activity was measured at various time intervals.

IDO1 activity measurements

IDO1 activity was measured as the abundance of the IDO1 enzymatic product, kynurenine, in culture supernatant through the use of Ehrlich's reagent. Colorimetric determination was performed using an automated spectrophotometer.

Viability/Cytotoxicity assays

For all experiments, cell viability was confirmed in parallel. Cellular adenosine triphosphate levels were quantified using CellTiter-Glo® Luminescent Cell Viability Assay according to the manufacturer's instructions. Luminescence was assessed using an automated spectrophotometer. 0.1% saponin was used as a positive control for cell death.

RESULTS

Depletion of gut-resident *Lactobacillus* associates with increased IDO1 activity and decreased Th17 cell abundance

Two temporally independent cohorts of six female rhesus macaques were each experimentally infected with SIV_{mac251} and followed longitudinally via stool sampling before infection and at five time points through the acute and chronic infection phases. We profiled fecal bacterial microbiota by sequencing of the V4 region of the 16S rRNA gene and collapsing reads with 97% sequence homology into operational taxonomic units (OTUs). Using a linear mixed effects model approach (that accounts for inter-individual variation in longitudinal time-series data) to compare abundances of all bacterial genera detected in all rhesus animals at all time points to respective plasma Kyn:Trp ratios, we found that fecal abundance of the Lactobacillus genus correlated significantly and inversely with IDO1 activity ($p=0.00151$, Figure 1A). Moreover, IDO1 activity was found to be tightly correlated with peripheral blood Th17 cell loss across acute and chronic SIV infection ($p=0.0000052$, Figure 1B). The abundance of the Lactobacillus genus also exhibited a significant positive correlation with Th17 cell abundance ($p=0.00187$, Figure 1C).

Cell-free supernatant from *Lactobacillus*, but not *Streptococcus*, suppresses IDO1 activity in interferon-activated human monocyte-derived macrophages

To determine whether the gut microbial community belonging to the *Lactobacillus* genus can modulate IDO1 activity *in vitro*, we set out to assess whether *Lactobacillus* spp. can suppress tryptophan catabolism in our rhesus macaque model of HIV infection. *Lactobacillus* bacteria were isolated from rhesus macaque stool as described in the Methods. Cell-free supernatants of

cultured *Lactobacillus* primary isolates were added to cultures of monocyte-derived macrophages that were isolated from whole blood and activated with interferon gamma. The use of cell-free supernatants in our assay allow for observation of the effects of bacterial products on IDO1 activity, in the absence of direct immune recognition of bacterial cell surface components by macrophages. The choice of interferon gamma as an exogenous stimulus to this *in vitro* system was made to enhance our capacity to measure IDO1 activity, as interferon gamma is a known inducer of IDO1, and also to mimic *in vivo* conditions encountered by macrophages in HIV-infected subjects, as interferon gamma is upregulated in viral infections and specifically in HIV. Isolates from *L. animalis* and *L. reuteri*, which are the predominant species isolated from the stool samples of experimental animals, showed marked ability to inhibit tryptophan catabolism (Figure 2A, 2B). This effect persisted when CFS was filtered through a 3kDa filter, suggesting the active compound(s) of this inhibition are smaller than 3kDa. As a negative control, we isolated bacterial species from the *Streptococus* genus, which belong to the same taxonomic order as *Lactobacillus* but were not found to correlate with IDO1 activity in our cohort of rhesus macaques. Cell-free supernatants of these isolates belonging to the *Streptococcus* genus, namely *S. infantarius* and *S. equinus* spp., which are also high in abundance in macaque feces, did not demonstrate IDO1 suppression activity in contrast to *Lactobacillus* spp. The IDO1 suppressing effects of *Lactobacillus* spp. are not due to cell death as cell viability assays performed in parallel demonstrate equal viability as compared to untreated control cells (Figure 2B).

***Lactobacillus* cell-free supernatant suppression of IDO1 activity declines after repeated passage in culture**

The IDO1 suppressing activity of *Lactobacillus* spp. CFS compared to "no CFS" and *Streptococcus* spp. CFS controls as seen in Figure 2B, is not replicated in multiple isolates as demonstrated in 3A. On further examination, *Lactobacillus* isolates showed an abrogation of suppressant activity only after 2-3 consecutive passages as shown in comparison of figures 3B and 3C. The IDO1 suppressing activity of original *Lactobacillus* spp. primary isolates also possess an abrogation of suppressant activity after 3 consecutive passages as shown in 3D.

FIGURES

Figure 1*. IDO1 activity and Th17 cell dynamics associate with loss of gut-resident *Lactobacillus

A) The relative abundance of *Lactobacillus* correlates strongly with IDO1 activity as measured the peripheral blood Kyn:Trp ratio. (p=0.00151)

B) IDO1 activity as measured by Kyn:Trp ratio correlates strongly with Th17 cell abundance in peripheral blood. (p=0.0000052)

C) The relative abundance of *Lactobacillus* correlates strongly with Th17 cell abundance in peripheral blood. (p=0.00187)

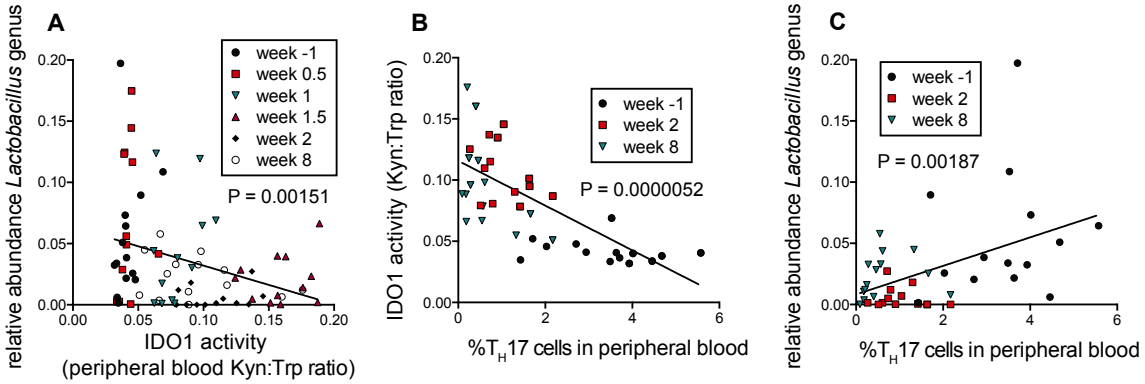


***Figure 2*. Cell-free supernatant from *Lactobacillus*, but not *Streptococcus*, suppresses IDO1 activity in interferon-activated human monocyte-derived macrophages**

A) *L. animalis* and *L. reuteri* are the predominant species of *Lactobacillus* isolated from stool specimens of experimental animals.

B) Cell-free supernatant from *Lactobacillus (L. animalis* and *L. reuteri*), but not *Streptococcus*, isolated from rhesus macaques suppress IDO1 activity in interferon-activated human monocyte-derived macrophages.

C) Cell-free supernatants from *Lactobacillus* and *Streptococcus* do not affect cell viability.

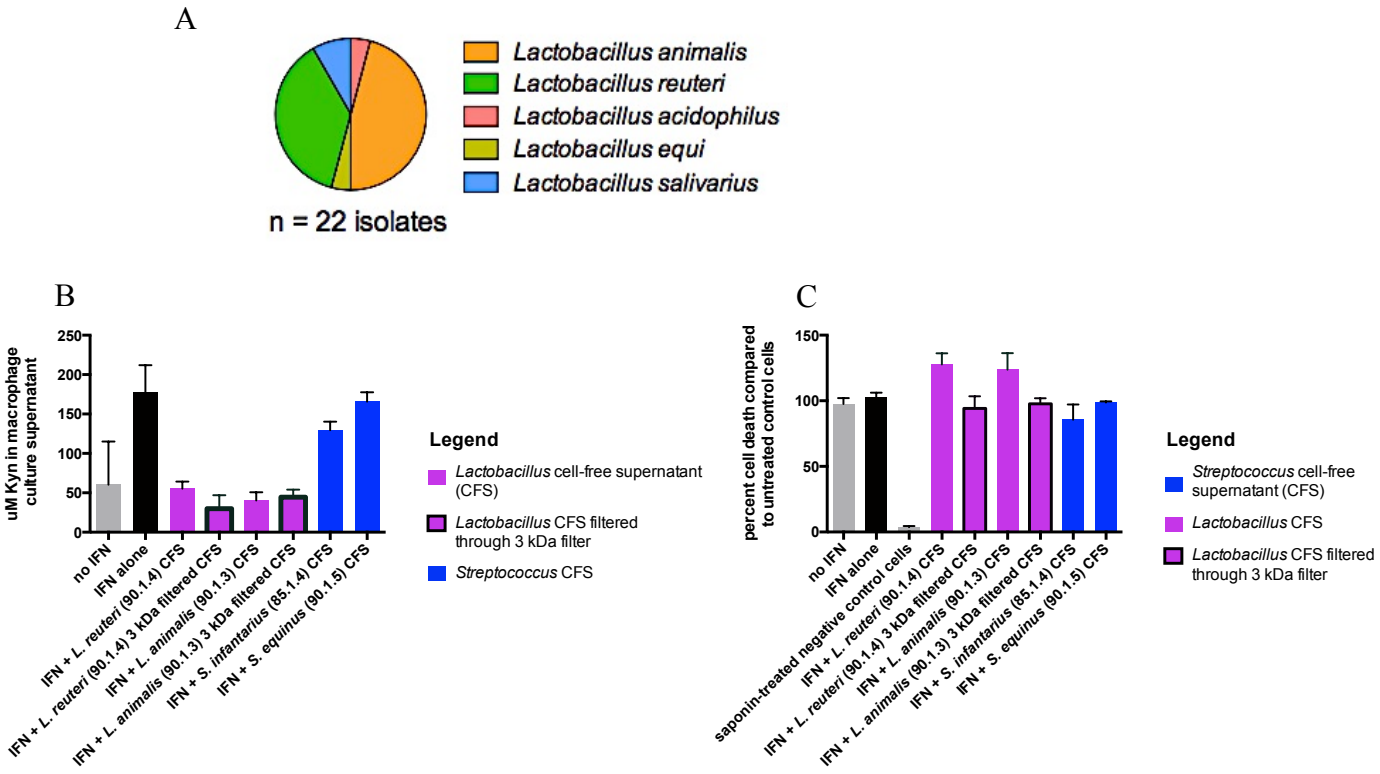


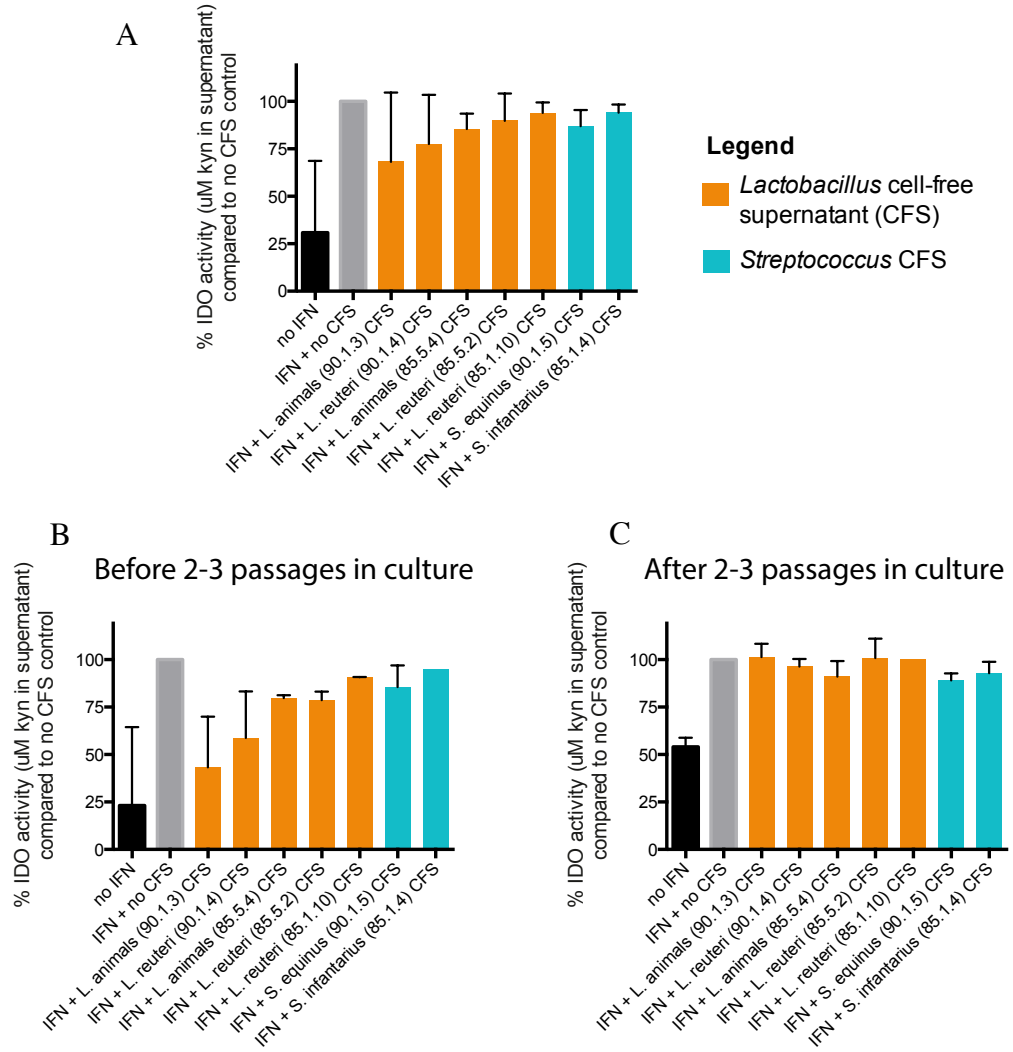
***Figure 3*. Suppression of IDO1 activity by *Lactobacillus* cell-free supernatant and primary isolates decline after repeated passage in culture**

A) Aggregate data from three experiments demonstrate loss of *Lactobacillus* cell-free supernatant suppression of IDO1 activity over time

B) *Lactobacillus* CFS suppression activity is maintained before 2-3 passages in culture

C) *Lactobacillus* CFS suppression activity is lost after 2-3 passages in culture

D) *Lactobacillus* spp. primary isolates also possess an abrogation of suppressant activity after 3 consecutive passages



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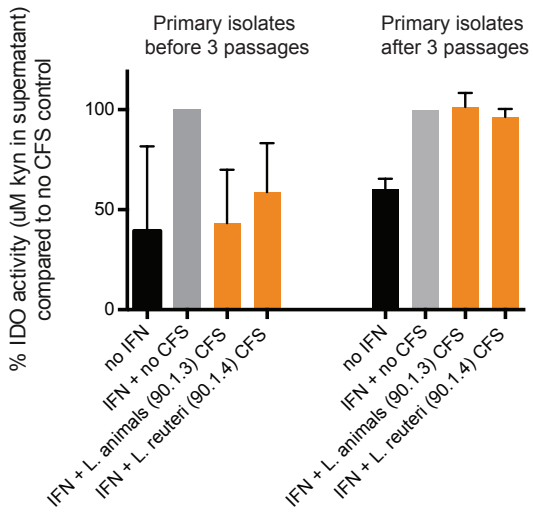

DISCUSSION

Chronic immune activation precipitated by the loss of Th17 cell populations in the gut and resultant disruption of the epithelial barrier and microbial translocation in the pathogenesis of SIV has been well described in the literature. Recent reports suggest that the absence of commensal bacterial flora reduces Th17 cell populations, while the administration of certain oral probiotics improves Th17 cell recovery and gastrointestinal mucosal integrity and immunity in SIV-infected animals (Ivanov et al., 2008, Klatt et al., 2013, Ortiz et al., 2015). However, direct relationships of gut microbiota members to the IDO1-mediated kynurenine pathway of tryptophan catabolism and depletion of Th17 cells in SIV infection have not been explored. In this work, we report the association of *Lactobacillus* species abundance with IDO1 activity and Th17 cell dynamics in SIV-infected macaques as well as the ability of *Lactobacillus* species to modulate tryptophan catabolism in an *in vitro* model of viral infection.

We observed that the loss of *Lactobacillus* species during acute through chronic SIV infection was correlated strongly with an increase in IDO1 activity as measured by plasma Kyn:Trp ratio and inversely with Th17 cell populations as measured in peripheral blood. Given the established importance of the Th17 pathway in maintaining mucosal barrier integrity, this finding further strengthens the growing body of evidence of the importance of the gut microbiota in maintaining intestinal immune homeostasis. More specifically, it is likely that only specific gut genera such as *Lactobacillus* inhibit IDO1 activity and that the distinct perturbations in the gut microbiota as a result of SIV/HIV infection are needed for the manifestation of microbial translocation and immune activation. Although further characterization of this relationship in larger cohorts would strengthen these findings, this report supports the clinical use of probiotic supplementation with *Lactobacillus*-rich species as a possible therapeutic intervention for Th17 reconstitution in patients with HIV.

In examining the direct effect of *Lactobacillus* species on IDO1, we initially observed an inhibition of IDO1 activity when cell-free supernatants from *Lactobacilli*, but not *Streptococci* isolated from rhesus macaques, were added to cultures of primary human monocyte-derived macrophages. Cell viability assays performed in parallel demonstrated no loss in viability compared to macrophages not treated with cell-free supernatants. Therefore, we believe that the loss of gut-resident *Lactobacillus* species that is seen throughout the course of SIV infection may play a direct role in the increase in IDO1 activity and loss of Th17 cell abundance in peripheral blood as observed in our experimental animals. The loss of similarly abundant gut species such as *Streptococcus* may not have an equally appreciable effect. While these results suggest a beneficial role for *Lactobacillus* gut species in maintaining intestinal immune homeostasis

during infection, subsequent experimentation failed to reproduce these robust initial findings. Further examination found an abrogation of IDO1-suppressing activity in *Lactobacillus* isolates after multiple consecutive passages in culture. The mechanism by which *Lactobacillus* inhibits IDO1 catabolic activity is unclear, and does not appear to be maintained after repeated passage in culture. It is possible that the selective pressures in culture with nutrient rich media are not only distinct from those *in vivo* in the rhesus macaque intestinal environment, but also may in turn select for optimum growth *in vitro* at the expense of preserving IDO1 inhibition machinery. Previous studies performed by Valladares et al. in which *Lactobacillus johnsonii* were fed to rat experimental animals identified hydrogen peroxide as the mechanism by which IDO1 is rendered inactive by this *Lactobacillus* species, through oxidization of IDO1 cysteine residues and heme center. Yet efforts to confirm this finding through the addition of both exogenous hydrogen peroxide and *Lactobacillus*-derived hydrogen peroxide did not replicate these findings in our hands. Because of the challenges in identifying a responsible mechanism of action, we were unable to assess the mechanistic functionality of our *Lactobacillus* isolates in culture and hence could not predict or determine why our isolates lost IDO1-suppressing activity in culture.

Further endeavors to develop an *in vitro* model that can accurately replicate the complex intestinal environment where microbiota-host cell dynamics take place would be substantially informative in increasing our fundamental understanding of how these relationships change in the setting of viral infection. Our *in vitro* model may be an inappropriate or insufficient model to examine the role of *Lactobacillus* in influencing gut homeostasis during viral infection. The use of cell-free supernatants may not reflect the complex gut environment of host-microbiota interactions *in vivo*. Moreover, dendritic cells perhaps may play a greater role in immune

activation and inflammatory response than originally appreciated and perhaps may be the better cell type to examine. Although the use of a humanized mouse model would be preferable, the poor colonization of exogenous bacterial species into the murine gut as well as the substantial technical effort needed to conduct such studies present their own challenges.

Nevertheless, this early *in vitro* exploration of gut microbiota and immune cell dynamics during SIV infection provides a precedent for which further investigations in the field of gut mucosal immunology and HIV pathogenesis can be established. Although we were unable to definitively establish a causal role for *Lactobacillus* to modulate tryptophan catabolism through IDO1 inhibition *in vitro*, our observations implicate the loss of gut *Lactobacillus* species as strongly correlated with IDO1 activity and a loss of the Th17 cell reservoir. These findings provide a foundation for future investigations and clinical trials that would examine the clinical importance of maintaining protective gut bacterial species during progressive HIV disease.

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