

Review article

Barriers to HIV remission research in low- and middle-income countries

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Abstract

Introduction: HIV eradication and remission research has largely taken place in high-income countries. In low- and middle-income countries (LMIC), there may be factors that have a substantial impact on the size of the latent HIV reservoir and the immunological response to infection. If a curative strategy is to be available to all HIV-infected individuals, these factors must be understood.

Methods: We use a scoping review to examine the literature on biological factors that may have an impact on HIV persistence in LMIC. Three databases were searched without date restrictions.

Results: Uncontrolled viral replication and higher coinfection prevalence may alter the immunological milieu of individuals in LMIC and increase the size of the HIV reservoir. Differences in HIV subtype could also influence the measurement and size of the HIV reservoir. Immune activation may differ due to late presentation to care, presence of chronic infections, increased gut translocation of bacterial products and poor nutrition.

Conclusions: Research on HIV remission is urgently needed in LMIC. Research into chronic immune activation in resource poor environments, the immune response to infection, the mechanisms of HIV persistence and latency in different viral clades and the effect of the microbiological milieu must be performed. Geographic differences, which may be substantial and may delay access to curative strategies, should be identified.

Keywords: HIV eradication; HIV cure; HIV remission; resource limited; low- and middle-income countries; immune system

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Introduction

Access to antiretroviral therapy (ART) has improved the lives of millions affected by HIV, but HIV remains incurable. In 2015, there were an estimated 36 million people living with HIV (PLHIV), most of whom live in low- and middle-income countries (LMIC). Worldwide, approximately half of HIV-infected individuals accessed HIV treatment but there are wide differences between countries in the number of HIV-infected individuals on treatment [1]. Recent data have spurred the World Health Organization (WHO) to recommend ART for all HIV-infected individuals [2]; thus, there will be more individuals receiving treatment in the near to medium future. HIV treatment is lifelong and strict adherence to treatment regimens is required for successful therapy. The burden of care for treatment programmes in countries with limited resources is enormous and will continue to grow. Moreover, the immunological damage due to HIV infection and ongoing viral replication leads to a cascade of events that are associated with important non-infectious morbidities which will likely stress healthcare resources even further. Sustained remission of HIV without the need for ART, if attainable, will be critical to the overall health of the population and will allow for a broader distribution of resources.

The cure of the “Berlin patient” combined with prolonged remission in a relative large proportion of early treated individuals in the Visconti cohort and SPARTAC trial has led to enthusiasm for clinical research that aims to achieve HIV remission without continued need for ART. Total eradication of HIV remains a long term but challenging goal. Clinical research in HIV remission and cure research has largely occurred in resource-rich settings where the epidemic is driven by men who have sex with men. In many LMIC, women and children are especially affected by the epidemic. Worldwide, women comprise more than half of all adults living with HIV [3] and there are an estimated 1.8 million children living with HIV [4]. There are important differences in viral strains, HIV-infected populations and the clinical context between resource-rich settings and LMIC. These differences are poorly understood and may be an important barrier to successfully achieving sustained remission or eradication. The size of the pool of latently infected cells, the immune response and viral factors associated with different HIV subtypes may all be important. Indeed, a recent modelling exercise suggested that a cure might be cost-effective in LMIC [5]. If cure research is to reach all HIV-infected people, these differences need to be evaluated and assessed well ahead of any potential interventions. Sex differences that may be

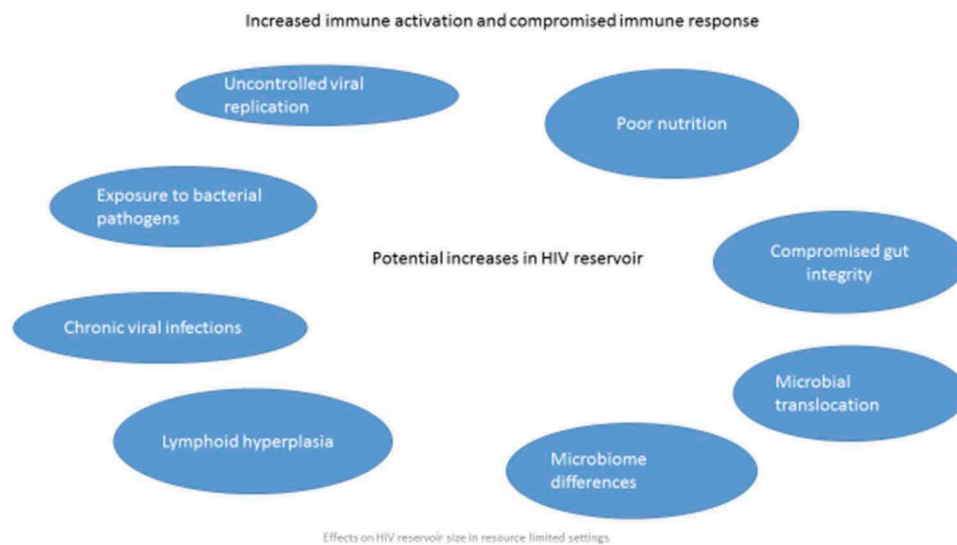


Figure 1. Effects on HIV Reservoir Size in Resource Limited Settings

important have already been reviewed in this journal [6]. This article focuses on biological factors that may be important in eradication research that are different in different geographic environments.

A critical barrier to achieving sustained remission of HIV is the presence of long-lived latently infected memory CD4+ T cells that contain replication competent HIV DNA, the “reservoir”. A first step towards remission is reducing the reservoir. Factors affecting the reservoir size have not entirely been elucidated, but in LMIC, late presentation to care, the presence of chronic infections causing immune activation and chronic inflammation, increased gut translocation of specific bacterial products and poor nutrition which attenuates the response to infections may be important determinants of the size of the HIV reservoir and may be different in resource-rich and resource-limited environments.

Methods

We used a scoping review to examine the literature using Arksey and O’Malley’s framework [7]. Scoping studies summarize key evidence on a topic but do not go through the process of a formal systematic review. We searched the following databases: MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards) and the Cochrane Library. We reviewed this literature to explore biological differences between resource rich and LMIC, focusing on potential differences in the HIV reservoir. No ethics approval was required because this was not human subjects research.

Immune activation and inflammation

Pre-ART immune activation and inflammation pose an important barrier to reservoir eradication, in several ways: First, inflammation may increase the size and persistence of

reservoirs. Patients with higher levels of inflammation and activation before ART initiation have long-lasting higher levels of persisting HIV-infected cells and HIV cell associated RNA [8]. Moreover, chronic inflammation, associated with T-cell activation and proliferation, may result in an increased number of cells harbouring replication competent HIV [9–11]. Immune stimulation also causes lymphoid hyperplasia locally, and the lymphoid follicle has now been shown to be critical to HIV persistence [12]. Integrated HIV DNA, localized in T follicular helper cells within the B-cell follicle, are inaccessible to immune killing at least in part because there are few cytotoxic T lymphocytes (CTLs) with a CD8 phenotype in this compartment. Second, inflammation may limit killing of infected cells, which is an important component of the “kick and kill strategy” for reservoir reduction. T-cell exhaustion, the progressive and irreversible loss of T-cell function, is characterized by decreased effector function, a change in the ability to respond to different antigens and sustained expression of inhibitory receptors such as the checkpoint molecules (for example PD1 and LAG 3) [13–17]. Chronic immune activation is associated with bystander killing of CD4 cells and exhaustion of HIV-1-specific CD4 and CTLs [16,18–20]. Importantly, expression of these markers of immune exhaustion is associated with higher levels of integrated DNA and a more rapid virological rebound in a cohort interrupting therapy suggesting that this process is also associated with an expanded HIV reservoir size [21]. Exhausted CD8 cells are unable to kill HIV-infected cells even when latency is reversed and infected cells express CTL epitopes [22]. While cytokine secretion improves and expression of PD-1 decreases in the presence of durable, suppressive ART [23], functional capacity is not fully restored and multiparametric T-cell pathology persists, despite early initiation of ART [24].

Uncontrolled HIV

Uncontrolled viral replication is probably the most important factor driving increased immune activation and inflammation in LMIC. Despite changes in guidelines which now recommend therapy for all HIV-infected individuals, full access to HIV treatment is limited. In South Africa, for example, of the estimated 7 million HIV-infected people, only approximately 3 million were on therapy in 2015 [25]. Even when guidelines recommend initiation of therapy, late presentation to care is common. A recent meta-analysis documented profound immunosuppression at ART initiation in many sub-Saharan African countries [26,27]. In addition, although viral load monitoring for clinical care is now recommended, it is not universally available and even when it is available, it is often not acted upon [28]. Uncontrolled HIV replication is associated with a heightened state of inflammation due to both HIV itself and the presence of other infections which are poorly controlled in the setting of immune suppression.

ART initiation at low CD4 counts is often indicative of a prolonged period of uncontrolled HIV and is associated with a greater degree of immune activation [29]. Immune reconstitution is incomplete even after ART has rendered the viral load undetectable, shown in both resource rich and resource poor environments, though there are no direct comparisons [30,31]. Importantly, very early therapy may protect mucosa-associated immune tissue, limit reservoir seeding, prevent CTL escape and retain functional CD4 and CD8 HIV-1 immune responses [32]. Recent work from Thailand has attempted to identify the specific immune lesions in the setting of acute infection and has found that acute HIV infection is associated with increased levels of the biomarkers of intestinal damage, inflammation, coagulation and fibrosis [31]. With suppressive ART initiated during acute HIV infection, the procoagulant state normalizes but gut damage persists and monocyte activation, systemic inflammation and fibrosis remain increased, albeit lower than in individuals starting ART in chronic HIV infection. Importantly, increases in CD4 T-cell populations correlate with decreases in specific markers of immune activation [33]. The notion that immune damage persists after the initial insult was confirmed in a study of biomarkers in the United States [34], and recent data from the AIDS clinical trials group suggest that HIV levels correlate with inflammation and activation before starting therapy but not during long-term suppressive therapy, suggesting that HIV reservoir size is related to factors present before ART initiation [8]. CD8 activation has an important impact on CD4 T-cell recovery [35] and a low CD4/CD8 ratio correlates with T-cell activation; those who start treatment with a low ratio are less likely to normalize over time [36]. The absence of immune exhaustion and premature immune senescence may increase the ability to respond to future therapeutic interventions designed to induce HIV remission [32].

Viral subtype

Viral subtype may be important in HIV persistence. Globally, subtype C represents about half of all infections and is by

far the most common subtype found in LMIC. Subtype B accounts for only approximately 12% of all infections worldwide [37] but the majority of remission-related research is done in patients infected with subtype B virus and there may be significant differences. The HIV tat protein is critical to transcriptional activity and may play an important role in establishing and maintaining latency [38]. Pronounced differences in clade B and C tat protein have been identified [39]; the significance of this to cure strategies has not yet been determined. Investigations into the differences in neurological complications of HIV have revealed that clade C virus is associated with a very different inflammatory environment in the central nervous system and is associated with the expression of different cytokines such as IL-33, leading to different rates of apoptosis of cells infected with clade C virus [40]. Ongoing studies are evaluating the cytokine profiles in patients infected with different subtypes and the differences may be profound [41]. HIV LTR promoters, which are different in different clades, affect transcription of cellular genes. Induction of HIV-1 subtype C viral production may be more responsive to a cellular inflammatory milieu, in particular TNF α production due to an additional NF- κ B element in its long terminal repeat (LTR) promoter region [42]. When studying various subtypes and inter-subtype recombinants in Tanzania, subtype C LTR was also associated with a sixfold increased risk of HIV mother to child transmission [43], CD4 cells with HIV DNA integrated near growth genes may proliferate more than other HIV-infected or uninfected cells [44,45]. Studies of cells with identical viral integration sites, indicative of clonal expansion, are currently limited to resource-rich settings and significant differences may exist in LMIC; this is an important knowledge gap that requires research. Importantly, after years on treatment, the integration site repertoire may be affected by the pervasive CD4 cell milieu which may differ in patients with high levels of chronic immune stimulation. It is unknown whether there is link between viral integration and a particular cytokine milieu that affects CD4 proliferation, stimulation and differentiation [46].

Chronic infections

Many bacterial, viral, and parasitic infections are diseases of poverty and are more common in LMIC. The immune response to these infections may influence the HIV reservoir size when pathogen-specific HIV-infected cells expand in response to antigen stimulation. Chronic antigenic exposure stimulates a broad adaptive and innate immune response. Bacterial infections stimulate reactive lymphoid hyperplasia and growth of local lymph nodes and viral pathogens cause a non-specific T-cell activation that may contribute to the proliferation of T cells containing replication competent HIV. Mounting evidence suggests that macrophages may be infected with HIV and serve as a critical HIV reservoir, most importantly in the central nervous system [47]. The macrophage has diverse roles in host immunity and may be important in the immunopathogenesis of chronic bacterial infections such as tuberculosis (TB).

Specific examples of pathogens that are more common in LMIC and affect the populations most impacted by the epidemic may be illustrative of mechanisms that are important in establishing and maintaining the HIV reservoir.

Tuberculosis (TB)

TB is the most clinically important coinfection for PLHIV in LMIC and is the leading cause of death among PLHIV worldwide [48]. The immune response to TB is characterized by a sustained inflammatory response that persists in many individuals even after therapy for TB has been completed [49]. T-cell activation, as measured by elevated CD38 and HLA-DR expression on CD4+ and CD8+ T-lymphocytes, is described in both latent TB infection and in active TB disease [50]. These lymphocytes can harbour replication competent HIV DNA but it is unknown whether the expansion of these cells in the setting of TB is associated with an increased reservoir size. TB overcomes the intracellular killing mechanisms of the host macrophage's defences and there may be a synergistic relationship between HIV and TB in which the autophagic pathways are disrupted leading to increased longevity of HIV-infected cells and increased replication of TB in macrophages [51].

Sexually transmitted bacterial infections

LMIC often have a higher burden of sexually transmitted infections (STIs) and it is plausible that STI coinfection may increase the size of the active HIV reservoir. WHO databases show that antenatal syphilis is more common in LMIC [52]. A systematic review found that 91% of incident infections of chlamydia, gonorrhoea and trichomonas were from individuals in LMIC [53], a finding confirmed by the Global Burden of Disease 2013 Study [54]. Similarly, trichomonas infection is disproportionately found in LMIC [53]. Coinfection with STIs is associated with HIV shedding even when HIV is adequately controlled [55]. One study identified approximately a 2.5-fold risk of HIV shedding in the setting of chlamydia and gonorrhoea in women and a specific cytokine profile in the female genital tract associated with HIV shedding that is also associated with recruitment of CD4 T cells in women seroconverting to HIV [56]. Several studies have found greater HIV shedding in individuals with HIV–trichomonas coinfection [57,58]. Among women with HIV–trichomonas coinfection, trichomonas treatment reduced vaginal HIV shedding at three months post-treatment [58]. Another study from Kenya found a fourfold decrease in vaginal HIV shedding after trichomonas treatment [59]. Both studies included coinfecting women who had undetectable HIV viral load in plasma but detectable viral shedding in the genital tract. Although we do not know the implications of these trends for latency, increased shedding may be reflective of an active mucosal reservoir which could pose challenges for immune control and reservoir reduction. Recent work in HIV-uninfected women documented recruitment of potential reservoir cells into the genital tract in women with concomitant STIs, lending credence to the notion that

local inflammation may be associated with an expansion of cells that could contain replication competent HIV [60,61]. This is an area that requires further study.

Other encapsulated bacteria

Streptococcus pneumoniae (pneumococcus) and *Haemophilus influenzae* are important pathogens and have a huge disease burden in both HIV-infected and uninfected children. Nasopharyngeal carriage of pneumococcus has been studied in the context of studies of the conjugated pneumococcal vaccine. The prevalence of pneumococcal carriage was as high as 93% in young children [62] in LMIC. In contrast, the highest rates described in resource-rich countries were 50.8% [63]. Higher rates of CD4 cells with memory and effector phenotypes have been described in adenoidal tissue of children colonized with pneumococcus [64]. Rates of *H. influenzae* carriage in HIV-infected children in India were double that of uninfected children [65]. As described above, lymphoid tissue plays a major role in HIV persistence as lymph node follicles are a major HIV reservoir site [14]. The relationship between oropharyngeal carriage of bacterial pathogens, including pneumococcus, and the size of the local HIV reservoir in oropharyngeal mucosa is entirely unknown but may be significant. Given the population structure of the HIV epidemic in LMIC is important to study.

Viral infections

Chronic viral infections are associated with immune activation and inflammation and are postulated to play an important role in HIV persistence. Modelling research suggests that Africa has a higher burden of both herpes simplex type 2 [66] and herpes simplex type 1 [67] compared to other regions of the world. Other herpesviruses, such as cytomegalovirus (CMV), Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), are endemic in many developing countries [68,69]. CMV seroprevalence approaches 100% in adults in sub-Saharan Africa [70] and EBV and KSHV infections are often acquired very early in life. The role of herpesviruses has been studied in the context of HIV persistence. Asymptomatic shedding of both EBV and CMV viruses is more common in individuals infected with HIV, and recent work has associated more frequent shedding of these viruses in virally suppressed HIV-infected individuals with a larger HIV reservoir [71]. The greatest burden of hepatitis B infection and hepatitis C infection is in LMIC [72–74]. The population attributable fraction for human papillomavirus is 6.9% in less developed regions of the world and 2.1% in more developed regions [75]. The relationship between the HIV reservoir and these viruses is unknown but recent work has shown an association with both inflammatory markers and expansion of T cells with activation and exhaustion markers in women who are dually infected with HIV and HPV [76,77].

Helminths and other parasites

There is significant geographic overlap between the communities most affected by HIV and helminthic infections. The immunopathology of helminthic infection is complex; they stimulate immune responses via tissue damage and egg production during acute infection but have evolved mechanisms of immune evasion, often using regulatory immune mechanisms. A common feature of all helminthic infections is Th1/Th17-mediated inflammation and Th2-dependent pathology [78,79]. Th2 cells orchestrate the protective immunity against worm infections but, at the same time, the T-regulatory cells induced by helminths appear to attenuate Th2 responses that mediate allergy and autoimmune diseases [80]. Increased levels of immunosuppressive cytokines such as IL-10 and TGF- β 1 have been demonstrated [81]. Helminthic infections are associated with the expression of markers of T cell exhaustion [82] and it is plausible that helminthic infestations may contribute to an expansion of the pool of latently infected cells.

Microbial translocation

One of the key drivers of immune activation in HIV infection is considered to be translocation of intestinal organisms because of early disruption of the intestinal epithelial barrier, due to preferential infection and depletion of CCR5-expressing CD4+ T lymphocytes in the gut associated lymphoid tissue. Portal circulation of these gut organisms may contribute to immune activation [83–87]. Binding of microbial-associated molecular patterns, such as peptidoglycan, lipopolysaccharide, flagellin and CpG DNA, to pattern recognition receptors, activates a signalling cascade in innate immune cells, resulting in the production of pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6, tumour necrosis factor and type 1 interferons [88,89].

Very early initiation of ART (during stage FI/II) has been shown to maintain the mucosal barrier and fully reverse the initial mucosal and systemic immune activation, most likely through preservation of mucosal CD4+ Th17 cells [90].

The role of intestinal microbiota in aggravating or ameliorating microbial translocation is increasingly recognized [91–93]. HIV has been associated with “bidirectional unbalances [sic]” in metabolic activity of the intestine, potentially affecting the integrity and function of the mucosal barrier as well as local and systemic inflammation [94]. More severe immunosuppression is associated with low diversity microbiota and increased immune activation [95,96] that is present even in people on ART. In LMIC, poor sanitation and hygiene, persistent diarrhoea, inadequate diet and severe acute malnutrition have been described [97] and are associated with inflammation of the small intestine, reduced absorptive capacity, increased intestinal permeability and subsequent increased microbial translocation. Very few studies have examined whether microbial translocation differs between persons living in resource-limited and resource-rich settings. In one study, products of gut translocation were found to be higher in

HIV-infected patients from Vietnam and Ethiopia compared to Swedish patients. The researchers proposed in addition to the factors described above that HIV subtype may play a role [98].

Nutrition

The contribution of nutrition to immunological health cannot be overstated [99]. Inadequate dietary intake leads to a loss of epithelial integrity in the skin, histologically characterized by atrophy and a cutaneous inflammatory response. Malnutrition-associated enteropathy compromises the intestinal barrier function and is accompanied by an inflammatory mucosal infiltrate [100,101]. A Th2 response has been noted and a state of heightened inflammation has been postulated, although a greater proportion of activated T cells has not been found. Intestinal infections, common in LMIC, contribute to micronutrient malabsorption. Vitamins and other micronutrients have been implicated in disease progression and may be important in the microenvironment in which cellular signalling occurs [102,103]. The specific defects that may affect HIV persistence are entirely unknown.

Laboratory evaluation of the reservoir

There is no universally agreed method for evaluating the size of the latent HIV reservoir [104–106] and different methods either under- or overestimate the pool of cells that contain replication competent DNA. Although often regarded as the gold standard for HIV reservoir assays, as it detects replication competence, the quantitative viral outgrowth assay (QVOA) has significant limitations. It is expensive, cumbersome and requires a large volume of blood and biosafety level 3 procedures. Cell viability is affected by variable processing and storage conditions, although these have improved with standardized procedures and quality management [107]. QVOA may underestimate the number of intact proviruses by 25–27-fold. Only a small proportion of intact proviruses are inducible after one round of stimulation, with additional proviruses being induced after a second round of stimulation [108,109]. Samples that are QVOA negative have been shown to bear infectious virus when using humanized mice for viral recovery [110] and patients who tested negative on QVOA had viral rebound after therapy cessation [111]. Importantly, assay sensitivity is dependent on drawing large volumes of blood to isolate a sufficient number of CD4 cells. Alternative, more practicable assays of inducible provirus are the total virus recovery assay [112] and Tat/rev Induced Limiting Dilution Assay [104]. Although these assays could be useful in monitoring patients receiving curative interventions, they require virus culture facilities and have the limitation that inducible virus does not prove replication competence. Molecular assays of HIV nucleic acid do not require culture facilities and may be more feasible to perform in LMIC: assays of total or integrated HIV DNA can be performed on smaller sample volumes but may severely overestimate the infectious or true reservoir

[108]. Low copy viremia detected by a single copy HIV-1 plasma RNA assay correlates well with QVOA but is undetectable in a large proportion of patients [113]. The evaluation of tissue, including lymph nodes and other pathological specimens, requires substantial clinical and laboratory infrastructure which are not available in many LMIC facilities [114,115]. In addition to the above, PCR-based assays require validation across various subtypes and uniform reporting against a reference standard such as the number of cells assayed is required.

Community response

Community acceptance is critical to a successful cure strategy [116]. Stakeholder engagement among a diverse group of individuals from different local contexts was identified as a priority by the International AIDS Society [117]; yet, communities in regions most affected by the epidemic do not have input into cure studies. Some of the concepts are complex, and messaging needs to be carefully tailored to the specific audience [118]; however, attitudes that suggest that the principles cannot be understood are divisive, presumptuous and unhelpful. HIV remission research will need to be integrated within existing HIV prevention and treatment strategies and prioritized within the context of the overall goal of ending the epidemic. It is likely that focus on immediate prevention and treatment goals is deemed more attainable and more practical than cure goals, but as the field moves forward, access to curative strategies may be delayed because of the need for local safety and efficacy data. Importantly, lack of meaningful participation through mechanisms such as advisory boards could ultimately affect the acceptability of remission strategies [119]. Preparatory research must be implemented in LMIC or the majority of PLHIV globally will not have access to this important strategy.

Conclusion

The inclusion of patients from high burden LMIC is essential for cure research. HIV subtype diversity, differences in inflammation and concurrent infections, may have an important impact on the size and cell and tissue distribution of the reservoir. The inclusion of patients from high burden LMIC is essential for cure research. Curative strategies that rely on immune function may be sensitive to differences in immune exhaustion, and the determinants of immune exhaustion in diverse settings need to be studied. We outline specific areas for research (Table 1), but it is clear that including patients from LMIC early in the process of clinical research might then accelerate the gradual process of scaling up an effective HIV cure in the future. In the SPARTAC study, a high proportion (22.7%) of early treated African patients achieved post-therapy control [120] demonstrating that treatment goals are feasible and acceptable. Early treatment would reduce the burden of opportunistic infections associated with delayed initiation and prevent onward transmission from early

Table 1. Knowledge gaps regarding HIV remission research in low- and middle-income countries

Role of HIV subtype in viral integration, transcriptional activation and CD4+ T cell growth
Effect of a particular cytokine milieu that affects CD4 proliferation, stimulation and differentiation on viral integration
Whether TB-induced expansion of T cells increases HIV reservoir size
Association between chronic viral infections, such as hepatitis B, hepatitis C and HPV, and the HIV reservoir
The relationship between oropharyngeal carriage of bacterial pathogens and the size of the local HIV reservoir in oropharyngeal mucosa
Whether viral shedding in the presence of coinfections, such as trichomonas, impacts of measures to achieve latency
Impact of poor nutrition on immune function, activation and the size of the HIV reservoir
Biomarker of HIV reservoir size available in LMIC

acute hyper-infectious individuals. Such an approach towards remission should be prioritized in LMIC.

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Competing interests

The authors have no competing interests. This paper was written by Catherine Godfrey in her capacity as an NIH employee, but the views expressed in this paper do not necessarily represent those of the NIH.

Authors contributions

All authors participated in the literature search and wrote the manuscript. All authors have read and approved the final version.

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