Immunologic Biomarkers, Morbidity, and Mortality in Treated HIV Infection

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Despite marked improvements in the modern treatment era, human immunodeficiency virus (HIV)–infected individuals, particularly those who initiated antiretroviral therapy (ART) at advanced disease stages, continue to have increased age-related morbidity and mortality, compared with the general population. Immune activation and inflammation persist despite suppressive ART and predict many of these morbidities. The goal of this review is to examine the evidence suggesting a link between the persistent inflammatory state and morbidity and mortality in this setting, to describe the impact of early ART initiation on these factors, and to highlight important unanswered questions for the field. We also advance a hypothesis to explain why some morbidities—and their root inflammatory drivers—may be prevented more than others by early ART initiation.

Keywords. immune activation; inflammation; biomarkers; morbidity; mortality; HIV-1 infection; antiretroviral therapy.

Although human immunodeficiency virus (HIV)–infected individuals are now living longer in the modern treatment era, they continue to have a shorter life expectancy than the general population; this is particularly so among older HIV-infected individuals and those who started ART at an advanced disease stage [1, 2]. Furthermore, many chronic morbidities commonly associated with aging are also increased in the HIV-infected population. While confounding lifestyle factors (eg, smoking, overconsumption of alcohol, and illicit drug use) may explain some of this increased risk, much attention has focused on the contribution of persistent immune activation and inflammation to this process. Indeed, levels of markers of innate and adaptive immune activation remain abnormal in many individuals maintaining durable ART-mediated viral suppression [3–9], and such levels are strong predictors subsequent morbidity and mortality [10]. While the root drivers of this inflammatory state are incompletely characterized, it is likely that HIV reservoirs [11], coinfections (eg, with cytomegalovirus [CMV]) [12], and microbial translocation all contribute to variable degrees [13] and represent important interventional targets in this setting. The goal of this perspective is to review the state of our knowledge regarding the link between biomarkers of immune activation and end-organ disease during treated HIV infection, to examine the impact of early ART initiation on both immune activation and non–AIDS-related and AIDS-related morbidity, and to highlight important unanswered questions for the field, particularly as more HIV-infected individuals around the world initiate ART at earlier disease stages.

BIOMARKERS OF INNATE IMMUNE ACTIVATION AND INFLAMMATION

Several studies have demonstrated that persistent innate immune activation predicts non–AIDS-defining morbidities, which remain increased among HIV-infected individuals in the modern treatment era. The first such study was an analysis of interleukin 6 (IL-6) and D-dimer data from the Strategies for Management of Antiretroviral Therapy (SMART) trial, which linked persistent inflammation and coagulation to subsequent non–AIDS-defining morbidity and mortality in treated HIV infection [10]. Since then, several studies have demonstrated that increased markers of inflammation (eg, interleukin 6 [IL-6], fibrinogen, soluble tumor necrosis factor receptor 1 (sTNFR1), sTNFR2, and high-sensitivity C-reactive protein [hs-CRP]), monocyte/macrophage activation (soluble CD14 [sCD14]), indoleamine 2,3-dioxygenase-1 (IDO) activity, and gut epithelial barrier dysfunction (zonulin and intestinal fatty acid binding protein [I-FABP]) predict increased mortality during treated HIV infection, even among those with high current CD4+ T-cell counts [10, 14–19]. In most of these studies, the mortality associations were much stronger than observed in HIV-uninfected cohorts, suggesting that inflammation was likely to be a more important contributor to morbidity and mortality in the context of HIV infection than it is in the general population. Levels of markers of coagulation (eg, D-dimer), which are increased with bacterial lipopolysaccharide-induced monocyte activation among other mechanisms during treated HIV infection [20], and inflammation were also strongly predictive of cardiovascular and thromboembolic events in several studies [21–23]. Plasma biomarkers of inflammation and lymphocyte activation were
BIOMARKERS OF ADAPTIVE IMMUNE DEFECTS

While biomarkers of innate immune activation, inflammation, and coagulation appeared to be the strongest predictors of morbidity and mortality in recent studies of North American cohorts, several biomarkers of adaptive immune defects have continued to predict morbidity and mortality, particularly in resource-limited settings. For example, our own group reported several years ago that T-cell activation (characterized by CD38 and HLA-DR coexpression on CD8⁺ T cells) predicted subsequent mortality in Ugandans with ART-suppressed HIV loads who were initiating their first ART regimen [36]. Similarly, CD4⁺ T-cell activation remained an important predictor of mortality in HIV-infected individuals starting their first ART regimen in resource-limited settings as part of a recent AIDS Clinical Trials Group study [37]. While higher T-cell activation also predicted mortality in participants with ART-suppressed HIV loads in the North American Study of the Ocular Complications of AIDS cohort [15], this effect was more modest than observed in resource-limited settings, where infectious complications remain a more important cause of death. The plasma kynurenine/tryptophan ratio, a biomarker of the immunoregulatory enzyme IDO, which confers adaptive immune defects, also appears to be a somewhat stronger predictor of mortality in resource-limited settings than in resource-rich settings [15, 16, 19]. A low ratio of CD4⁺ T cells to CD8⁺ T cells, which has also been associated with adaptive immune defects in treated HIV infection (and correlated strongly with both T-cell activation and IDO activity), also predicts increased non–AIDS-defining morbidity and mortality during treated HIV infection in many international cohorts, even among those who achieve CD4⁺ T-cell counts >500 cells/mm³ during suppressive ART [38, 39].

Interestingly, levels of classic markers of T-cell senescence that predict mortality in elderly HIV-uninfected populations (ie, shorter telomere length, higher percentage of CD28⁻ cells among CD8⁺ T cells, or higher percentage of CD28⁻CD57⁺ cells among CD8⁺ T cells), while often abnormal in treated HIV infection [40, 41], have failed to predict mortality during treated HIV infection in previous studies [15, 16]. Unlike innate immune activation markers or even T-cell activation, these classic senescence markers also fail to predict frailty in treated HIV infection [35]. Interestingly, while asymptomatic CMV infection and aging are associated with an expansion of terminally differentiated effector CD8⁺ T cells that have undergone many cell divisions and express CD57, HIV-infected individuals have an expansion of incompletely differentiated effector CD8⁺ T cells that actually express abnormally low levels of CD57 [42]. Indeed, abnormally low CD57 expression on effector CD28⁺CD8⁺ T cells is associated with increased mortality among treated HIV-infected participants [43]. This suggests that treated HIV disease may be characterized by a phenotypic defect in effector CD8⁺ T-cell proliferation and/or terminal differentiation that is distinct from the T-cell defects observed with aging or CMV infection in HIV-uninfected populations. While the root cause of this effector CD8⁺ T-cell defect during treated HIV infection is unclear, it appears to be correlated with markers of innate immune activation (and not T-cell activation), suggesting a possible link between innate and adaptive immune defects in HIV-infected individuals and a potential novel target for interventions [43].

VERY EARLY ART INITIATION REDUCES BUT FAILS TO NORMALIZE PERSISTENT IMMUNE ACTIVATION

While several studies have described persistently high levels of immune activation despite ART-mediated viral suppression, until recently, most of these studies have comprised HIV-infected individuals who initiated ART at relatively advanced stages of disease [3, 4, 6–8]. This is an important consideration because low nadir CD4⁺ T-cell counts have been consistently associated with higher immune activation during ART-mediated viral suppression [3, 5]. Nevertheless, more-recent observational studies from seroincident cohorts demonstrate that, while individuals initiating ART during very early HIV infection (ie, within 6 months of initial infection) achieve a lower immune activation set point than those who delay therapy by a few years, immune activation remains abnormally high upon ART-mediated viral suppression, compared with that among high-risk HIV-uninfected controls [44, 45]. One recent report from Utay et al suggests that individuals who start ART as early as the first 2–3 weeks after HIV infection continue to have increased levels of plasma markers of microbial translocation (eg, sCD14 and I–FABP), inflammation (eg, hs-CRP), and fibrosis (eg, hyaluronic acid) than matched highly exposed HIV-uninfected controls. Similarly, while some markers of adaptive immune function return to levels observed in HIV-uninfected controls (eg, the proportion of CD28⁻CD8⁺ T cells expressing CD57 [43]), others remain abnormal (eg, T-cell activation and ratio of CD4⁺ T cells to CD8⁺ T cells [38, 44]) despite ART initiation within the first 6 months after HIV infection. While levels of all of these markers improved in HIV-infected individuals initiating ART within the first 6 months after HIV infection, compared with levels in those who initiated ART >2 years after...
infection [38, 43–45], these studies highlight that abnormal immune activation likely persists even in those who initiate ART early.

WHY DO SOME BUT NOT ALL INFLAMMATION-ASSOCIATED MORBIDITIES DECLINE WITH EARLY ART INITIATION?

Despite the clear benefit of early versus delayed ART initiation on the persistent inflammatory state [38, 43–45], not all inflammation-associated morbidities were reduced by immediate ART in the recent Strategic Timing of Antiretroviral Treatment (START) trial [46]. In this study, most of the benefit of immediate (versus delayed) ART initiation were due to a reduction in infections and cancers (particularly those with an infectious etiology). There was little evidence for a reduction in cardiovascular events or even surrogate markers of vascular disease when comparing immediate versus delayed ART initiation [46, 47]. Similarly, immediate ART did not appear to decrease other noninfectious inflammation-associated morbidities, such as neurocognitive dysfunction, obstructive pulmonary disease, and osteoporosis (although the expected early ART-mediated increases in bone turnover may complicate the interpretation of data on osteoporosis) [48–50].

This differential effect of immediate ART initiation in the START trial in reducing some but not other non-AIDS-defining morbidities is in striking contrast to the clear benefits of ART in reducing noninfectious morbidities in the earlier SMART trial, which compared CD4+ T-cell count-guided intermittent ART to continuous ART strategies among individuals with later stage HIV infection [51]. In the SMART trial, not only did infectious complications decline with continuous ART, but the incidence of cardiovascular events was also significantly reduced when compared to that among participants interrupting ART. Granted, the SMART trial assessed the impact of ART interruptions, rather than immediate versus delayed ART, but a post hoc analysis among the treatment-naïve group at entry (ie, the so-called when-to-start substudy) provided similar inferences as the parent study [52]. Why did cardiovascular and other non-AIDS-defining complications clearly improve with ART in the SMART trial but not in the START trial? While the START trial participants were approximately 7 years younger than those in the SMART trial, this age difference does not seem to be large enough to account for the differences observed. It is also possible that much longer observation times would be needed to observe a benefit of a reduced immune activation set point on cardiovascular outcomes in the START trial, but this did not appear to be the case in the SMART trial, where the difference between intermittent and continuous ART arms became apparent within the first several months of observation. We believe that the most likely explanation for the lack of a difference in cardiovascular and other noninfectious events in the immediate versus delayed ART arms in the START trial is that these morbidities had not yet been established. Indeed, the incidence of cardiovascular events in both arms of the START trial was 4-fold lower than the incidence observed, even in the continuous ART arm of the SMART trial (Figure 1) [53]. This interpretation is further supported by recent observational studies demonstrating a strong link between lower nadir CD4+ T-cell count and cardiovascular disease risk in HIV-infected individuals [54–56] and other studies suggesting that the relative risk of cardiovascular disease among HIV-infected individuals as compared to the general population has been declining as the median CD4+ T-cell count at ART initiation has been rising [57, 58]. Other morbidities that did not appear to improve in the START trial, including neurocognitive dysfunction, obstructive pulmonary disease, and osteoporosis, have also been linked to low nadir CD4+ T-cell counts [29, 59, 60] and might have plausibly been prevented even in the delayed ART initiation arm of
Why might some inflammation-associated diseases fail to develop during the duration of the START trial if a persistent inflammatory state is established within the first few weeks of HIV infection? One possibility is that a certain threshold of systemic inflammation is required to drive some end-organ diseases and that those who start ART very early achieve levels below this threshold. While this hypothesis is certainly possible, we favor an alternative hypothesis related to the distinct putative drivers of immune activation during ART-mediated viral suppression, their relationship to the degree of pre-ART immunodeficiency (ie, nadir CD4⁺ T-cell count), and their anatomic localization (Figure 2). For example, viral reservoirs in central memory T-cell compartments, which are most abundant in inductive lymphoid tissues, are established within the first several days of systemic HIV or simian immunodeficiency virus (SIV) infection, with an exponential increase in the first few weeks of infection [61, 62] followed by slower increases over the next few years [44]. Once ART-mediated viral suppression is established, this primarily T-cell reservoir of HIV persists in lymphoid tissues and likely contributes to ongoing inflammation and lymphoid fibrosis [63–65], which may plausibly contribute to adaptive immune defects since these responses normally develop in the same anatomic compartment. Since immediate ART dramatically reduces HIV expression from T-cell reservoirs in lymphoid tissues, it is plausible that this mechanism explains why immediate ART reduced infectious and cancer events in the START and TEMPRANO trials. Conversely, the fact that immediate receipt of ART, even in recently infected individuals, fails to eliminate T-cell reservoirs of HIV in lymphoid tissues [66, 67] may explain the persistently high incidence of infectious and neoplastic complications (relative to those seen in the general population) even among individuals in the immediate ART arms of both the START and TEMPRANO trials [46, 68].

Other persistent drivers of the inflammatory state during treated HIV infection may require more-significant pre-ART immunodeficiency to be irreversibly established. For example, while some markers of microbial translocation are evident in plasma within the first few weeks of HIV infection (and within days of SIV infection) [45, 69], the degree to which these defects become irreversible after ART-mediated viral suppression is greater with more-advanced disease [70–72]. While translocated microbial products are concentrated in the liver and mesenteric lymphoid tissues, they are distributed widely throughout the circulation, so this driver of the systemic inflammatory state may plausibly contribute to several end-organ diseases, including cardiovascular complications, particularly in individuals with lower nadir CD4⁺ T-cell counts. Other potential drivers of the inflammatory state may require significant pre-ART immunodeficiency to be established and have distinct anatomic localization. For example, myeloid reservoirs of HIV in tissues (eg, brain microglia, adipose tissue, and liver [73–75]), if they do persist during suppressive ART, might not be established to a clinically meaningful extent until later stages of untreated HIV infection because it appears to take some degree of CD4⁺ T-cell depletion for the virus to evolve the capacity to efficiently infect myeloid cells [76, 77]. This might explain why irreversible neurocognitive dysfunction, which is thought to be driven in part by direct HIV infection of central nervous system myeloid cells, appears to be much less common in individuals who initiate ART at high nadir CD4⁺ T-cell counts [78]. Myeloid reservoirs of HIV in fat and liver might also plausibly contribute to metabolic disease and liver fibrosis in individuals with low nadir CD4⁺ T-cell counts [74, 75]. Similarly, asymptomatic CMV replication, which has been proposed to be a mediator of vascular disease in HIV-infected individuals, particularly since it replicates in the vascular endothelium [12, 79–82], may require a certain degree of immunodeficiency to establish sufficient CMV reservoirs in myeloid precursors or CMV-specific adaptive immune defects to drive cardiovascular disease [83].

Thus, not all putative root drivers of the inflammatory state in treated HIV infection are likely to be active in individuals who initiate ART at early disease stages. While this model might explain why individuals initiating ART during early disease stages may prevent the establishment of certain (eg, cardiovascular, neurocognitive, and pulmonary) morbidities but still have a measurably increased risk of infectious and neoplastic morbidities as compared to the general population, it may

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**Figure 2.** A theoretical model for the impact of nadir CD4⁺ T-cell count on the root drivers of immune activation in treated human immunodeficiency virus (HIV) infection and their disease manifestations is shown. While several putative drivers of persistent immune activation despite antiretroviral therapy (ART)–mediated viral suppression have been described (ie, HIV reservoirs in T cells and macrophages, microbial translocation, and cytomegalovirus [CMV] coinfection), not all of them are likely to be active in individuals who initiate ART at high nadir CD4⁺ T-cell counts (ie, very early disease stages), and many of them preferentially drive inflammation in distinct anatomic compartments. Consequently, both the root drivers of inflammation reflected by systemic biomarker measurements and the disease manifestations induced by inflammation are likely to vary on the basis of nadir CD4⁺ T-cell count.
also inform the interpretation of systemic immune activation and inflammatory markers. For example, if one measures levels of systemic immune activation markers in HIV-infected individuals with high nadir CD4⁺ T-cell counts (ie, those above the dotted line in Figure 2), the primary source of any measured abnormalities is likely to be HIV reservoirs in lymphoid tissues and, potentially, microbial translocation. Conversely, when one measures systemic immune activation and inflammatory markers in HIV-infected individuals with very low nadir CD4⁺ T-cell counts, a much greater diversity of sources and anatomic sites likely contributes to any elevations observed. If such a model were true, one would expect that systemic inflammatory markers measured in individuals with high nadir CD4⁺ T-cell counts might predict infectious and neoplastic conditions but not necessarily cardiovascular, pulmonary, and neurocognitive morbidities. Conversely, systemic inflammatory markers measured in individuals with low nadir CD4⁺ T-cell counts might predict all of these morbidities of interest. While no biomarker studies to date have had sufficient numbers of clinical events among participants with high nadir CD4⁺ T-cell counts to address these hypotheses, this could be explored in future studies.

CONTRIBUTION OF LIFESTYLE FACTORS AND COINFECTIONS

Other coinfections and lifestyle factors might independently contribute to persistent immune activation in HIV-infected individuals despite ART-mediated viral suppression. For example, hepatitis C virus (HCV) coinfection is quite common [84] and associated with increased T-cell activation, increased innate immune activation, and a decreased ratio of CD4⁺ T cells to CD8⁺ T cells despite suppressive ART [85–90]. Coinfection with HIV and hepatitis C virus (HCV) has also been associated with cardiovascular disease risk over and above that seen with HIV monoinfection [91–93]. Although some studies demonstrated that HCV treatment decreases inflammation [94, 95], others have also demonstrated persistent immune defects despite HCV eradication [85]. Other lifestyle factors, such as smoking [96], hazardous alcohol consumption [97], methamphetamine abuse [98], and obesity [99], have also been associated with increased immune activation in treated HIV infection and may further contribute to persistent immune activation despite ART suppression. However, several studies have detected persistently abnormal immune activation despite suppressive ART, even after adjustment for these factors [8, 100].

TARGETING IMMUNE ACTIVATION IN CLINICAL TRIALS

The initial, low-lying fruit approach to decreasing immune activation in treated HIV infection has been to test commonly prescribed medications with antiinflammatory properties. For example, statins decreased levels of inflammation, immune activation, and surrogate markers of cardiovascular disease in several clinical trials of individuals with ART-suppressed HIV loads [101–104], prompting a large clinical end point trial that is currently enrolling (clinical trials registration NCT02344290). Conversely, other commonly used antiinflammatory medications, such as aspirin, have failed to reduce levels of immune activation and/or surrogate markers of cardiovascular disease in placebo-controlled trials [105]. Other studies evaluating commonly used medications are underway, with some showing preliminarily promising results, such as angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers [106] and dipeptidyl peptidase 4 inhibitors [107]. Nevertheless, it is unlikely that these interventions are going to be potent or targeted enough to fully reverse persistent immune activation in treated HIV infection.

Thus, there is continued interest in targeting the potential root causes of inflammation in treated HIV infection. The most successful of these approaches to date was a trial of valganciclovir, which showed that treating asymptomatic CMV replication significantly reduced T-cell activation in treated HIV-infected individuals with poor ART-mediated CD4⁺ T-cell recovery [12]. While valganciclovir toxicity precludes its long-term use, other novel inhibitors of CMV replication are in development and may be promising. On the other hand, interventions designed to reverse microbial translocation (rifaximin [108] sevelamer [109], and mesalamine [110]) largely failed to reduce microbial translocation to any meaningful extent, suggesting the need for novel strategies to reverse gut barrier defects in this setting. Finally, novel strategies to reduce expression of HIV in inductive lymphoid tissues (and, potentially, macrophages in the periphery) may hold promise in the future [111].

Last, potent immune-based therapeutics are also being pursued in many ongoing trials of treated HIV-infected participants. Downstream inflammatory pathways that strongly predict disease are being inhibited directly (eg, IL-6 inhibition with tocilizumab; clinical trials registration NCT02049437), although it remains to be seen whether inhibiting a downstream pathway will provide benefit if the root causes of inflammation and other parallel inflammatory pathways are not also blocked (ie, the so-called whack-a-mole theory). Nevertheless, it is possible that there are common early immunologic pathways that are activated by most of the putative root drivers of inflammation while also giving rise to several downstream parallel inflammatory pathways (ie, the trunk of the tree) and/or exhibit positive feedback loops (eg, the IDO pathway’s potential causal role in promoting adaptive immune defects, as well as microbial translocation [112]). Current examples include Toll-like receptor antagonists (clinical trials registration NCT02443935), based on some preliminary promising results from a weak Toll-like receptor agonist, chloroquine [113, 114]; methotrexate (clinical trials registration NCT0000834), interleukin 1β inhibition (canakinumab; clinical trials registration NCT02272946), and JAK1/JAK2 inhibition (ruxolitinib; clinical trials registration NCT02475655).
CONCLUSION

While the life expectancy of HIV-infected individuals has extended dramatically in the modern treatment era, non–AIDS-defining morbidity and mortality remains higher than in the general population, particularly among individuals who initiate therapy at low nadir CD4+ T-cell counts [1,2]. Abnormal immune activation and inflammation persist despite ART-mediated viral suppression and are associated with several non–AIDS-defining morbidities, but most of these data linking inflammatory biomarkers to morbidity have been generated in cohorts of HIV-infected individuals with relatively low nadir CD4+ T-cell counts. It remains to be seen whether the inflammatory state, which persists even among those who initiate ART in the first few weeks of HIV infection, will continue to predict all morbidities or a more narrow spectrum of infectious and neoplastic conditions in HIV-infected individuals who initiate ART early in the disease course. If this were the case, it would have important implications for the target populations (and end-organ diseases of interest) in need of immune-based interventions, as well as the specific root drivers of the inflammatory state to be targeted. Nevertheless, despite changing international guidelines supporting ART initiation at any CD4+ T-cell count, the majority of HIV-infected individuals around the world will have (now and in the foreseeable future) initiated ART at low CD4+ T-cell counts and remain at risk for multiple end-organ diseases as a consequence of the systemic inflammatory state [115]. In addition, given the continued marked infectious morbidity among HIV-infected individuals initiating ART at high CD4+ T-cell counts in these resource-limited settings [68], immune-based interventions to reverse adaptive immune defects will play an important role in this population, as well.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org/. Consisting of data provided by the author to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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