HIV-related immune dysregulation during antiretroviral therapy in sub-Saharan Africa

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Chapter 7

General discussion

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The rollout of combination antiretroviral therapy (ART) has led to markedly improved well-being and increased life expectancy of people living with HIV (PLWH), globally and in sub-Saharan Africa. However, with more people initiating life-long ART, new challenges have arisen: PLWH on ART remain at an increased risk of AIDS and non-AIDS related complications. Incomplete $CD4^+$ T-cell recovery and persistent immune dysregulation during ART are among the main drivers of the increased risk of morbidity and mortality.

In this thesis, we sought to explore the extent and consequences of poor $CD4^+$ T-cell recovery and persistent chronic immune activation in PLWH on ART in sub-Saharan Africa. In this final chapter the key findings described in this thesis are discussed, with a specific focus on $CD4^+$ T-cell recovery. The key findings will be placed in the context of current literature to further dissect the possible underlying pathogenic mechanisms of poor $CD4^+$ T-cell recovery. Finally, future perspectives regarding long term clinical management of PLWH in sub-Saharan Africa are discussed.

Role of $CD4^+$ T-cells in the immune response

$CD4^+$ T-cells, also known as T-helper cells, are essential in mounting an effective humoral and cellular immune response. Activation of naïve $CD4^+$ T-cells through antigen recognition by the T-cell receptor (TCR) primes the naïve $CD4^+$ T-cell to proliferate and differentiate into effector cells [1]. In $CD4^+$ T-cell activation three signals are crucial for an appropriate immune response against pathogens. The first signal is induced by the TCR when recognizing antigens presented by MHC-II molecules on antigen presenting cells (APCs), such as dendritic cells. Second, co-stimulation by receptors-ligand interactions on the $CD4^+$ T-cell and APC is required to induce an immune response; absence of co-stimulation leads to an anergic and tolerogenic state. Lastly, cytokine production by the APC will provide a polarizing signal directing $CD4^+$ T cell subset differentiation and function (**Figure 1**).

The different $CD4^+$ T-cell subsets can be identified by transcription factor expression, cytokine production and their function in the immune response: T-helper 1 (Th1) are primarily induced in response to intracellular bacteria and viruses; Th2 in response to large extracellular parasites; Th17 in response to extracellular bacteria and fungi [2, 3]. Th9 and Th22 subsets are more prevalent during allergies and autoimmune diseases (**Figure 1**). The polarization of $CD4^+$ T-cells involves the expression of homing receptors to facilitate the migration to the site of inflammation and the production of cytokines to aid in the clearance of the pathogen by recruiting myeloid cells (e.g. macrophages) and stimulate B-cells to produce antibodies. In addition, T follicular helper cells (Tfh) located in the B cell follicles in lymphoid tissues, are

involved in the humoral immune response. Regulatory T cells (Treg) are another subset of effector CD4⁺ T-cells and are induced during nearly all types of infection. They are essential in balancing and controlling the magnitude of the immune response through the induction of immune regulatory cytokines [4, 5].

When a pathogen is cleared, inhibitory signals are required to terminate the immune response. The latter is regulated by immune checkpoint (IC) molecules such as programmed cell death-1 (PD-1), cytotoxic T-lymphocyte antigen-4 (CTLA-4), and lymphocyte activation gene protein (LAG3), which are expressed on the cellular membrane and upregulated at the end of pathogen clearance [6]. In the absence of antigen, IC molecule expression is downregulated to achieve homeostasis and effector T-cells will undergo apoptosis while only a small fraction will survive and become memory T-cells [7, 8] (**Figure 2**).

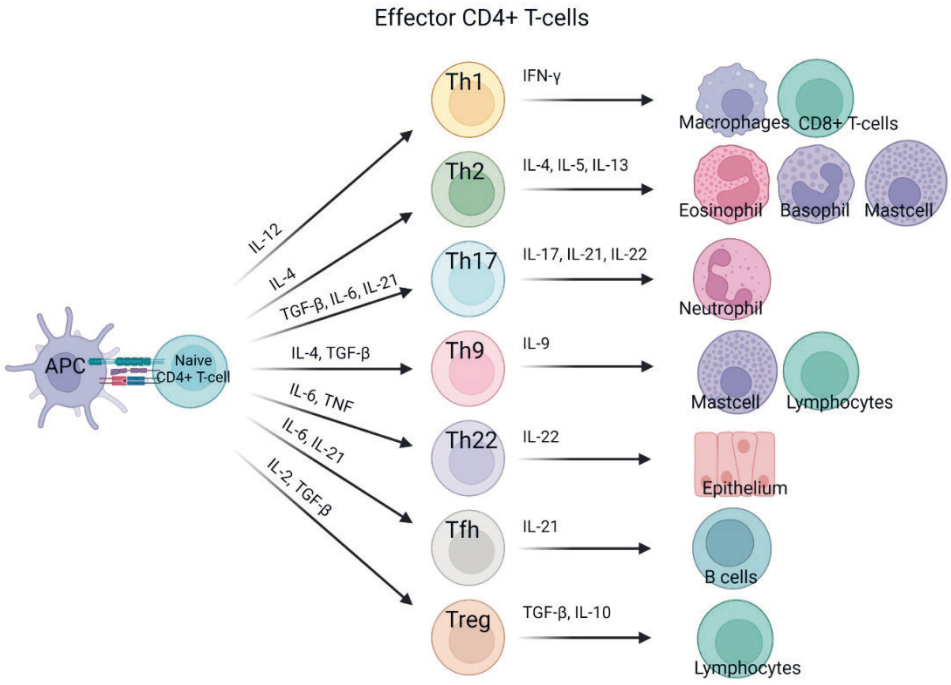

Figure 3 CD4⁺ T-cell polarization. Polarization of naïve CD4⁺ T-cells to specific effector CD4⁺ T-cells is driven by the presence of various cytokines. Each effector subset has a unique function and produces cytokines that have an effect on various other immune cells. In general, Th1 cells induce IFN-γ and activate macrophages and CD8⁺ T-cells; Th2 induce IL-4, IL-5, IL-13 and affect Eosinophils, Basophils and Mastcells; Th17 cells induce IL-17, IL-21, IL-22 and affect Neutrophils; Th9 cells induce IL-9 and affect mastcells and lymphocytes; Th22 cells induce IL-22 and affect epithelial cells; Tfh cells induce IL-21 and activate B cells; Treg induce TGF-β and IL-10 and suppress lymphocytes [9]. Created in Biorender.

CD4$^+$ T-cell depletion and immune dysfunction during HIV infection

During HIV infection massive CD4$^+$ T-cell depletion occurs. Given the central function of CD4$^+$ T-cells in the immune response, the loss of CD4$^+$ T-cells explains why AIDS is characterized by opportunistic infections and HIV-related neoplasms [10-14].

Upon infection, the majority of HIV infected CD4$^+$ T-cells die which accounts in part for the loss of CD4$^+$ T-cells. However, a small fraction of the HIV infected CD4$^+$ T-cells survive and become the HIV reservoir [15]. The HIV reservoir is established early during infection and is continuously replenished, which is one of the reasons for HIV persistence [16]. Moreover, the immune system is unable to clear the infection due to the high HIV replication and mutation rate which facilitates escape from the adaptive immune response. The persistence of HIV results in ongoing activation of the immune system, and high turnover, and apoptosis of immune cells including CD4$^+$ T cells. In addition, IC molecules which normally are down-regulated upon terminating the immune response, remain highly expressed and block T-cell function [6, 17] (**Figure 2**).

Besides the CD4 receptor, the chemokine co-receptor CCR5 or CXCR4 is required for viral entry and infection of the CD4$^+$ T-cells [18]. R5-tropic viruses (using the CCR5 co-receptor) are mostly responsible for HIV-transmission [19-22] and therefore predominate during early infection. In up to half of PLWH evolution within the envelope protein of HIV may result in the ability of the virus to use the CXCR4 co-receptor (X4-tropic) [21, 23, 24]. Different CD4$^+$ T-cell subsets have different expression levels of the chemokine co-receptors and are thus differently susceptible to HIV infection. Most CD4$^+$ T cells, including naïve CD4$^+$ T-cells, express CXCR4, while CCR5 is predominantly expressed on activated CD4$^+$ T-cells. The ability of X4-tropic HIV to infect naïve and quiescent memory CD4$^+$ T cells is thought to explain the more rapid CD4$^+$ T-cell depletion and accelerated disease progression associated with the emergence of X4-tropic variants. CCR5 is highly expressed on activated Th1 and Th17 CD4$^+$ T-cell subsets, of which the Th17 cells in particular are highly susceptible to HIV infection [25, 26]. It is thought that this is due to the lower secretion of CCR5 ligands MIP-1α and MIP-1β, which compete with HIV for binding to CCR5, by Th17 cells as compared to Th1 cells [26, 27]. The CD4$^+$ T-cell residing in the gut associated lymphoid tissues (GALT) are predominantly Th17 CD4$^+$ T-cells, and therefore the gut is severely affected already during early HIV infection [28, 29]. The Th17 CD4$^+$ T-cells play a sentinel role in the defense against bacterial/fungal pathogens. The depletion of CD4$^+$ T-cells in the GALT causes weakening of the local immune system, allowing structural changes to the gut and microbial translocation, which contributes to systemic immune activation and CD4$^+$ T-cell turnover (**Figure 2**).

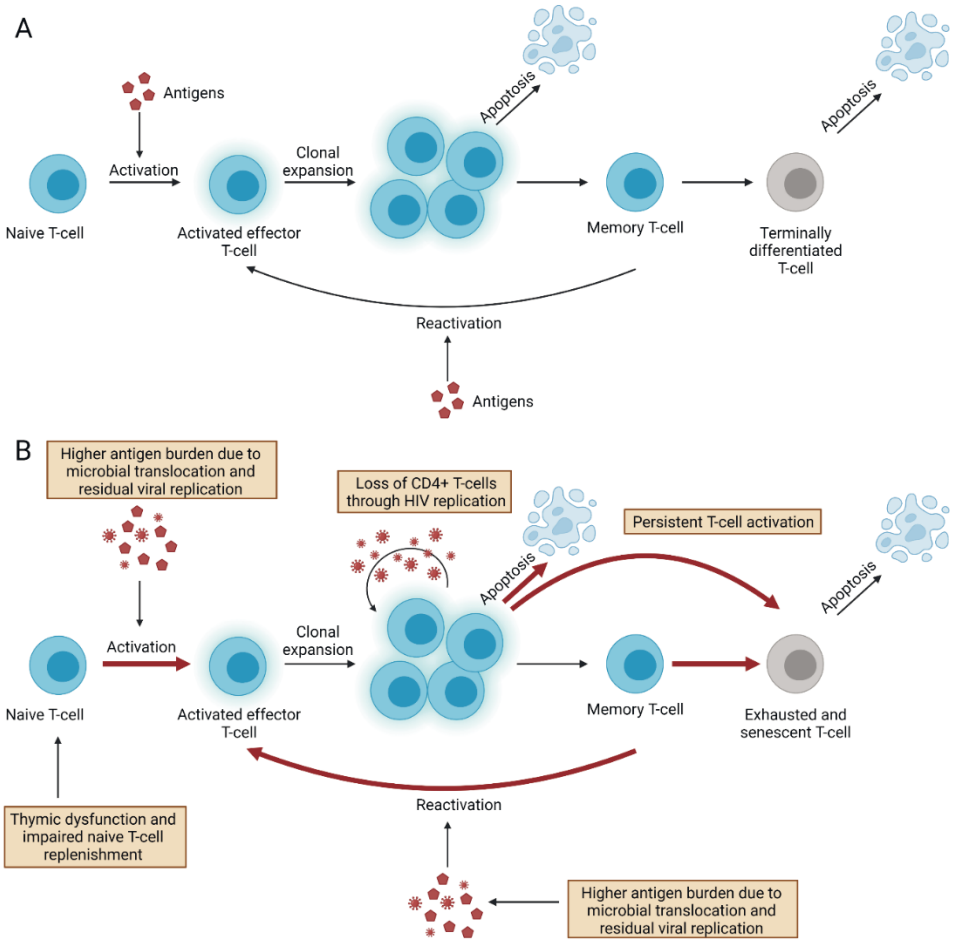

Figure 2 T-cell ($CD4^+$ and $CD8^+$) differentiation during antigen exposure. A. Naïve T-cells that encounter and recognize an antigen, differentiate and proliferate into effector T-cells. After clearance of the pathogen, the short-lived effector population will deflate through apoptosis and only a minor subset will survive and form a quiescent memory T-cell population. Upon repeated antigen encounters memory T-cells are rapidly reactivated to clear the pathogen. During a lifetime people encounter many pathogens and with age T-cells become senescent and exhausted. B. HIV infection is characterized by gradual loss of $CD4^+$ T-cells: HIV infection and replication in $CD4^+$ T-cells leads to excessive $CD4^+$ T-cell loss due to apoptosis and recognition by the immune system. Persistent antigen exposure due to microbial translocation and residual viral replication leads to activation, exhaustion and senescence of T-cells which will undergo apoptosis. Impaired naïve T-cell replenishment occurs due to thymic dysfunction.

Suboptimal $CD4^+$ T-cell recovery during antiretroviral therapy

ART suppresses HIV replication and allows for immune recovery as defined by the gain of $CD4^+$ T-cells. However, $CD4^+$ T-cell recovery is variable and PLWH, and especially those who experience limited $CD4^+$ T-cell recovery, remain at increased risk of morbidity and mortality (**Chapter 2 and 3**) [30]. In participants of the PASER cohort in sub-Saharan Africa who had predominantly severe $CD4^+$ T-cell depletion prior to ART initiation, we demonstrate that the

majority did not experience restoration of $CD4^+$ T-cells to levels observed in HIV-uninfected individuals (**Chapter 2 and 3**). These findings concur with other cohort studies in sub-Saharan Africa that demonstrated that 30-60% of PLWH on ART had incomplete $CD4^+$ T-cell recovery [31, 32]. Further understanding of characteristics and the underlying mechanisms of poor immune recovery may provide insight for therapeutic interventions and identifying PLWH at risk of complications.

In line with previous evidence, we demonstrated in the PASER cohort that pre-ART $CD4^+$ T-cell count and older age were associated with suboptimal $CD4^+$ T-cell recovery [31-37] (**Chapter 3**). The analysis of the differentiation state and functionality of $CD4^+$ T-cells, may provide insight in underlying mechanisms of suboptimal $CD4^+$ T-cell recovery, and why those of older age and those with low nadir CD4+ T-cell counts are at particular risk of suboptimal CD4+ T-cell recovery [38]. It has been reported that PLWH had lower naïve $CD4^+$ T-cells compared to uninfected individuals despite successful ART [39-43]. The lower number of naïve $CD4^+$ T-cells was mostly found in those who initiate ART at low $CD4^+$ T-cell counts [40]. Furthermore in PLWH of especially older age, recovery of naïve $CD4^+$ T-cells that recently emigrated from the thymus is impaired [33, 44], indicating that age-related thymus functionality play a role in $CD4^+$ T-cell recovery. In addition, PLWH on ART have impaired T-cell proliferation compared to uninfected individuals [45-47], which indicates that homeostatic proliferation capacity may also affect $CD4^+$ T cell recovery. Indeed, studies in African populations have shown that PLWH with suboptimal $CD4^+$ T-cell count recovery have impaired proliferation responses [48, 49]. Moreover, residual $CD4^+$ T-cell activation [41, 50-52], higher expression levels of exhaustion and senescence markers (e.g. PD-1, TIGIT, CD57) [53-55] and higher Treg frequencies [56] have been associated with suboptimal $CD4^+$ T-cell recovery. This indicates that the overall immune status as reflected by ongoing immune activation, dysfunction and imbalance prior and during ART is an important factor for $CD4^+$ T-cell recovery of PLWH, especially for those of older age and those who initiate ART at low nadir CD4+ T-cell counts.

In African populations, the higher life-time pathogen exposure may influence $CD4^+$ T-cell differentiation and functionality and thus immune recovery. Studies have shown that helminth infections influence $CD4^+$ T-cell count and activation prior to HIV and may also impact the recovery capacity in PLWH during ART [57-61]. Helminth infections skew the immune response profile towards a Th2 profile (**Figure 1**). Intracellular pathogens such as HIV and *Mycobacterium tuberculosis* (TB), require a Th1 response (**Figure 1**) [62], therefore Th2 skewing by helminth infections may attenuate Th1 responses against intracellular pathogens [63]. Indeed, it has been shown that deworming of individuals with HIV and helminth co-

infection leads to reduced HIV viral loads and increased CD4+ T-cell recovery [64, 65]. This suggest that treatment of helminth infections may contribute to better immunological responses in PLWH. In the PASER-cohort we did not measure helminth infections, however given the endemicity of helminth infections in the African continent [66, 67] it is likely that helminth infections are highly prevalent in our cohort.

We and others found that PLWH with CD4+ T-cell counts <200 cells/μL have a higher incidence rate of opportunistic infections like TB compared to those with higher CD4+ T-cell counts [68, 69] (**Chapter 3**). However, low CD4+ T-cell counts have also been described in patients with tuberculosis not infected with HIV [70-72], and anti-tubercular treatment has been shown to reverse this reduction [73]. This suggests that TB by itself influences CD4+ T-cell homeostasis. During TB-HIV co-infection, depletion and suboptimal restoration of CD4+ T-cells during ART may therefore be exacerbated. In our analysis, we did not find that TB diagnosis at ART initiation was associated with suboptimal CD4+ T-cell recovery during suppressive ART (**Chapter 3**). In contrast, incident TB during ART has been associated with impaired CD4+ T-cell recovery [74]. It is noteworthy that participants within the PASER-cohort had regular routine clinical monitoring and received TB treatment upon TB diagnosis, which may have mitigated negative effects on CD4+ T-cell recovery.

Immune activation and inflammation are also believed to be among the principal correlates of poor CD4+ T-cell recovery [52]. In the PASER-cohort, biomarkers of immune activation and inflammation, namely; interleukin (IL)-6, C-reactive protein (CRP), C-X-C chemokine ligand 10 (CXCL10), soluble CD14 (sCD14), soluble CD163 (sCD163), CXCL9, chemokine (C-C motif) ligand 2 (CCL2) and lipopolysaccharide binding protein (LBP), were elevated in PLWH prior to ART. During effective ART, levels of CXCL10, LBP, CRP, sCD163, and sCD14 remained elevated compared to uninfected controls, which is in line with various other reports [75-78]. Strong associations were found between the pre-ART biomarker levels and the on-ART biomarker level. This indicates that the magnitude of pre-ART immune activation is highly predictive of immune activation during effective ART (**Chapter 4**).

Immune activation during ART is driven largely by microbial translocation. One of the most common measured translocated microbial products is lipopolysaccharide (LPS; a gram-negative bacterial cell wall component) [79]. In PLWH LPS has been directly associated with activation of cytotoxic T-lymphocytes (CD8+ T-cells) and monocytes, residual viremia, and proinflammatory cytokines such as IFN-α, IL-6, and TNFα [79-84]. In **Chapter 4** we described the dynamics of two markers, sCD14 and LBP, before and during ART which are directly associated with LPS. LBP interacts with LPS and supports binding of LPS to its receptor CD14, expressed on monocytes, which is subsequently released as sCD14. We observed

that plasma levels of both sCD14 and LBP remained elevated in PLWH despite effective ART compared to HIV-negative controls, indicating that microbial translocation contributes to immune activation during ART. As helminths can cause significant damage to the intestine [85], microbial translocation may even be further exacerbated in PLWH from the African region.

We observed that higher levels of sCD14 during effective ART were associated with subsequent suboptimal $CD4^+$ T-cell recovery (**Chapter 5**), which is in line with findings from other studies [86-88]. Besides LPS, also other bacterial components can induce the release of sCD14 upon activation of monocytes by pro-inflammatory cytokines (e.g. IL-6 and IL-1β) [89]. These findings could suggest a link between microbial translocation and CD4+ T-cell recovery, but may also suggest the presence of other microbial and/or viral antigens. We also found that TB co-infection was associated with the pre-ART sCD14 level, but not the on-ART sCD14 level (**Chapter 4**). Similarly, a study conducted in South Africa found higher levels of sCD14 in PLWH with active TB, but not latent TB infection, compared to PLWH without TB [90]. It has been reported that replicating *Mycobacterium tuberculosis* induce IL-1β, which can induce the release of sCD14 [91].

We also found an association between on-ART CRP levels and subsequent suboptimal $CD4^+$ T-cell recovery (**Chapter 5**). CRP is mainly secreted by hepatocytes in the liver in response to the pro-inflammatory cytokines IL-6 and IL-1β. CRP has been associated with adverse outcomes during HIV in many other studies [92, 93]. CRP is also highly associated with infections and the height of the CRP level is indicative of viral or bacterial infections [94]. The detected association between CRP and subsequent suboptimal $CD4^+$ T-cell recovery may therefore be reflective of underlying pathogenic burden, including co-infections and residual viral replication.

In the PASER cohort elevated levels of CXCL10 during ART were predictive of subsequent viral rebound despite ART. In addition, CXCL10 levels during ART were associated with $CD4^+$ T-cell recovery in participants with sustained viral suppression during ART (**chapter 5**). CXCL10 has been shown to be strongly associated with HIV infection and viral load [95, 96] and CXCL10 levels can distinguish between PLWH on ART with and without a detectable viral load [97]. There is also evidence that CXCL10 is correlated with $CD4^+$ T-cell count [98, 99]. Monocytes are the main source of CXCL10, but it can also be secreted by various other cell types, such as T-cells, NK cells and endothelial cells [100]. CXCL10 secretion is induced by a variety of cytokines [101, 102], but predominantly by Interferon-gamma (IFN-γ) [103]. Studies have indicated that high levels of CXCL10 can suppress immune function, through suppression of T-cell responses to the HIV gag protein, and impairment of T-cell proliferation

capacity [104]. Therefore, high CXCL10 levels in PLWH pre- and on ART may be indicative of overall immunological damage caused pre-ART and residual immune dysfunction during ART.

We also observed that PLWH with a limited decline of sCD163 from pre-ART to 12 months on ART had an increased risk of subsequent viral rebound during ART (**Chapter 5**). Several reports have shown that sCD163 is a marker of poor outcomes during HIV infection [105, 106]. It has been shown that sCD163 levels in plasma reach levels comparable to uninfected people when ART is started during early ART, but not during chronic HIV infection [107]. CD163 is expressed by monocytes and macrophages and upon cleavage induced by proinflammatory stimuli is released as sCD163 [108, 109]. Persistent elevated levels of sCD163 are thought to be reflective of chronic macrophage activation [107]. Moreover, coinfection with hepatitis C virus attenuates sCD163 reduction during ART [110]. These findings reflect the importance of underlying inflammation as well as coinfections on immune recovery in PLWH on ART.

Drug toxicity is also among the contributors of immune activation. In the PASER-cohort, the first-line ART regimen were non-nucleoside reverse transcription inhibitor (NNRTI) based. Currently more treatment options are available and many countries, including in sub-Saharan Africa, are adopting the use of integrase inhibitor (INSTI) based regimen as the preferred first-line treatment option. The use of INSTIs has several benefits compared to NNRTIs, e.g. higher resistance barrier and less side effects [111, 112]. In addition, the roll-out of INSTIs in the African continent is also associated with better outcomes in terms of retention to care and viral suppression [113]. Similarly, at the time of enrollment the use of stavudine, a nucleoside reverse transcriptase inhibitor which is highly effective for the treatment of HIV but is associated with very severe side effects [114-117], was still among standard treatment options. Although shortly after the enrollment of participants in the PASER-cohort most countries started to phase-out stavudine [118, 119], a substantial proportion (~25%) of participants in the PASER-cohort initiated an ART regimen which contained stavudine [120]. The current improved treatment options may therefore also influence CD4+ T-cell recovery and immune activations in PLWH in sub-Saharan Africa.

In **Chapter 6**, we compared plasma microRNA levels in PLWH who experienced poor and good subsequent $CD4^+$ T-cell recovery. MicroRNAs play an important role in many cellular processes including the immune response and inflammation [121]. microRNA dysregulation has been observed in many diseases including HIV infection and therefore changes in circulating microRNAs may provide insight in the underlying mechanism of suboptimal $CD4^+$ T-cell recovery and immune dysregulation in PLWH on ART.

The pre-ART plasma levels of miR-199a-3p and miR-200c-3p were higher in PLWH with poor compared to those with good CD4$^+$ T-cell recovery (**Chapter 6**). In addition, we found that the levels of miR-17-5p and miR-501-3p were higher in plasma during ART in participants with poor CD4$^+$ T-cell recovery compared with good CD4$^+$ T-cell recovery. Two recent studies conducted in China found associations between microRNA miR-580, miR-627, miR-138-5p, miR-16-5p, and miR-323-3p, and let-7d-5p in the plasma of participants with poor CD4$^+$ T-cell recovery compared to those with good CD4$^+$ T-cell recovery [122, 123]. Another study in elite controllers and viremic PLWH from North America found correlations between CD4$^+$ T-cell counts and the expression levels of miR-150-5p, miR-29a-3p, miR-31-3p, miR-31-5p and miR-181b-5p in PBMCs [124]. Overall, these studies show no overlap in the identified microRNAs, which is likely due to differences between study populations of PLWH from different regions across the world, and highlights the effect of host genetics, differential pathogen exposure, and other environmental factors or exposures. These data do strongly indicate that no single microRNA can uniformly identify PLWH at risk of poor CD4$^+$ T-cell recovery.

Nonetheless, our analysis of microRNAs may provide relevant insights in immunopathology in PLWH. MicroRNAs have been shown to be involved in Th polarization. The miR-17-92 cluster is a cluster of microRNAs (MiR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1), that are located in the same open reading frame on chromosome 13 and has been intensely investigated [125-127]. It has been reported that this cluster regulates the generation of both Th1 and Treg cells [128]. Deficiency in the expression of this microRNA cluster leads to reduced expression of T-bet (a transcription factor for Th1 polarization) and IFN-γ (see Figure 1), and thus promotes polarization to Tregs rather than Th1 CD4$^+$ T-cells. In particular miR-17 have been shown to account for the pro-Th1 influence through targeting the TGF-β receptor II (TGFβII), and cAMP-responsive element binding protein 1 (CREB1), which are essential in Treg generation [128].

Also miR-200c-3p seems to play a role in the immune response. ZEB1, a target of miR-200c-3p, is required for anti-viral memory CD8+ survival and maintenance [129]. In addition, ZEB1 has been implicated to promote Th1 and Th17 polarization at the expense of transcription factors which guide cells to a Th2 phenotype, and thus is essential in creating a balance between the Th subsets [130]. Whether the elevated plasma levels of miR-200c-3p at ART initiation and miR-17-5p during ART in the PASER-cohort who have poor CD4$^+$ T-cell recovery, is reflective of the CD4$^+$ T-cell subsets or viral immune response through CD8+ T-cells is unknown. However modulation of the survival and maintenance of CD8 + T-cells, and skewing of Th subsets may attenuate immune responses against pathogens, including HIV and thus influence the disease course during ART.

Conclusion

Despite effective ART, suboptimal $CD4^+$ T-cell recovery is frequently observed in PLWH in sub-Saharan Africa. PLWH who have an incomplete $CD4^+$ T-cell recovery remain at increased risk of (non)-AIDS related complications and mortality. PLWH who are older or those who start ART late, when $CD4^+$ T-cells have been severely depleted are particularly at increased risk of incomplete $CD4^+$ T-cell recovery. Current guidelines on providing ART upon diagnosis (Test and Treat) should aid, at least in part, in overcoming the serious effects of severe $CD4^+$ T-cell depletion and suboptimal immune recovery in newly diagnosed PLWH. Nonetheless, a substantial fraction of PLWH still presents late in HIV care in many settings in sub-Saharan Africa [131-133].

Furthermore, we demonstrate that immune activation and inflammation persists in many African PLWH despite effective ART. Pre-ART immune activation and inflammation was highly associated with on-ART immune activation and inflammation. This further emphasizes the need for early HIV diagnosis and treatment. In our research we identified relevant biomarkers (sCD14, CRP, and CXCL10) that may aid in identifying PLWH at risk of poor treatment outcomes, such as treatment failure, poor $CD4^+$ T-cell recovery or co-infections. Further evaluation studies are needed to assess the clinical utility of these biomarkers in predicting adverse outcomes.

Future perspectives and recommendations

It is clear that initiating ART as early as possible is essential to avoid immune dysregulation, including inflammation and poor CD4+ T-cell recovery, and promote healthy aging with HIV. During recent years, test and treat strategies have been widely promoted. However barriers to get tested remain. Therefore strategies to reduce barriers to get tested (e.g. stigma and reachability of clinics) are needed to reduce the burden of late HIV diagnosis [131, 134].

However, the majority of PLWH today started treatment when CD4+ T-cell counts had already been depleted and immune activation and inflammation was prevailing. These people could benefit from therapeutic interventions mitigating the pathological effects of persistent immune activation and inflammation. Several anti-inflammatory agents (such as statins and anti-coagulents) have been evaluated, but none of such approaches to date has been proven to be beneficial [135, 136]. Targeting underlying mechanisms of immune activation may result in a more constructive solution. Specifically of importance for African populations is treating coinfections such as TB, viral infections and helminths. Reducing pathogen burden could aid in restoring immune function and attenuating immune activation and inflammation. In addition, restoring the intestinal barrier function could aid in reducing microbial translocation, and thus

reduce systemic immune activation and inflammation. Studies have shown that the use of probiotics can reduce microbial translocation and immune activation, these results are promising especially because most probiotics are generally well tolerated and inexpensive [137-141]. In addition, therapies targeting HIV persistence in the gut may aid in restoring the barrier function [142]. Finally, studies focusing on reducing the HIV reservoir, may contribute in reducing the burden of persistent residual viral replication. There are many ongoing studies, e.g using latency reversal agents, CRISPR-cas, or small interfering RNAs (siRNA), which aim to target the HIV reservoir. However, the successfulness up until now is limited and mostly pre-clinical [143-147]. Nonetheless, methods to detect and target the HIV reservoir needs to be adapted to comprise the different HIV subtypes and vast host genetic diversity in the African continent [148].

Screening of biomarkers of immune activation may also be useful in low-resource settings and could potentially aid in identifying PLWH at risk of poor outcomes. Development of point-of-care test (POC) for biomarkers, such as CRP, CXCL10 and sCD14, could provide an easy accessible tool in resource limited and rural settings. CRP POC tests have shown promising results in screening for TB in PLWH [149]. CXCL10 POC test has shown to be a low-cost and effective triage test to identify PLWH who experience viral failure [150]. POC diagnostic tests could be used by community health workers to monitor patients on ART. Positive results on the point-of-care test may aid in the decision to plan a clinic visit for further clinical examination, a viral load test and possibly therapeutic interventions, such as treatment switches, treatment of co-infections and prophylactic cotrimoxazole. In addition, other screening methods suitable for low resource settings could be explored to further investigate the extent of immune dysfunction in PLWH in low-resource settings. A common test currently used, predominantly in screening for immune deficiencies in infants, is the quantification of T-cell receptor excision circle (TREC) in DNA extracted from dried blood spot (DBS) [151]. This measurement allows for the quantification of naïve T-cells, and may be more practical and feasible than other methods using flow cytometry, in low-resource settings. In addition, DNA extraction from DBS could be used to measure telomere lengths to quantify the senescence status of T-cells [152]. These measurements could be specifically of interest for PLWH, since those with few naïve T-cells and those with senescent T-cells are at increased risk of poor outcomes.

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