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Does Persistent HIV Replication Explain Continued Lymphoma Incidence in the Era of Effective Antiretroviral Therapy?

Does persistent HIV replication explain continued lymphoma incidence in the era of effective antiretroviral therapy?

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Running title: Lymphoma and HIV suppressive therapy

Highlights

- Control of HIV with cART fails to reduce the incidence of many lymphomas.
- Replication of HIV in lymph nodes may affect lymphoma risk during therapy.
- Replication of HIV in adipose tissue may create a lymphoma-promoting metabolic state.
- HIV pathology in reservoirs may inform mechanisms of cART-resistant lymphoma risk.

Abstract

Non-Hodgkin lymphomas are highly increased in incidence in individuals infected with HIV, and this continues to be the case in spite of highly effective combined antiretroviral therapy (cART). New evidence has demonstrated that while successful virtual recovery of CD4 counts and elimination of HIV from peripheral blood can be achieved with cART, viral replication can still occur in lymphoid tissues. In addition, recent studies have suggested that adipose tissue provides an additional reservoir for HIV-infected macrophages and T lymphocytes even in the context of successful cART therapy. In this review article, we discuss possible mechanisms leading to the development of lymphoma in the cART era.

Introduction

The incidence of Kaposi Sarcoma (KS) and other AIDS-defining cancers has decreased significantly since combined antiretroviral therapy (cART) became widely available [1], but overall cancer incidence is increasing in patients living with HIV (PLWH) [2]. Indeed, the D:A:D study recently concluded that malignancy is now the leading cause of non-AIDS-related mortality in the PLWH [3]. Although some studies attribute this trend to increased survival rates in the cART era producing an aging HIV-infected population [4], standardized incidence ratios for several cancers including anal, liver, and lung malignancies are demonstrably higher in the HIV-positive population compared to the general population [5]. Importantly, the incidence of malignancy in this cohort can be correlated with lower CD4+ T cell count at cART initiation [5], suggesting that HIV-mediated immunosuppression predisposes patients to malignancy even when HIV disease is later controlled. Malignant lymphoproliferation is common in HIV positive patients regardless of treatment status [6], and non-Hodgkin lymphomas (NHL) are still considered an AIDS-defining cancer. Although incidence of some types of NHL have decreased in the cART era, other manifestations of NHL are increasing and NHL remains a significant source of mortality in HIV-infected individuals [7,8]. In fact, lymphomas have become the most common AIDS-related cancer in the developed world, constituting over 50% of all AIDS defining cancers and the most common cause of cancer-related death in HIV-infected individuals [9]. Interestingly, KSHV co-infection further increases the risk of NHL in HIV positive patients [10], and rates of KSHV-associated PEL and MCD remain rare but are unaffected by the widespread use of cART [5,11]. Similarly, incidence of EBV-associated lymphomas including Burkitt lymphoma and classical Hodgkin's Lymphoma (cHL) remains high. Moreover, aggresive histological subtypes of cHL occur more frequently in PLWH and these cases are often characterized by increased numbers of pathogenic Reed-Sternberg cells that are EBV infected, suggesting that EBV has an

unusual role in cHL pathogenesis in the context of HIV infection [6,12]. Taken together, these data suggest that (1) HIV infection predisposes patients to malignant lymphoproliferation in general and (2) cART-mediated immune reconstitution is not always sufficient to prevent disease progression in PLWH. In this review, we will highlight some of the recent reports which may shed light on the persistence of lymphoma in PLWH in the context of successful cART treatment.

Persistent replication of HIV in lymph node reservoirs

Despite the success of cART at preventing the progression of HIV infection to AIDS, it is now apparent that more subtle HIV-mediated pathology, including increased susceptibility to malignancy, persists even in well-controlled patients with high CD4 counts and undetectable levels of circulating HIV. The concept that latent reservoirs of HIV infected cells persist in anatomical sites where penetration of cART drugs is low has received considerable attention in recent years as a significant barrier to achieving an HIV cure [13,14]. Indeed, viral persistence in lymph nodes and gut-associated lymphoid tissues (GALT) under cART therapy has been documented in both HIV [15] and SIV [16,17] infection. However, the definition of these reservoirs as “latent” has recently been challenged in an elegant study by Lorenzo-Redondo et al, which demonstrates that HIV continues to replicate in lymphoid tissue and traffics between the lymph nodes and blood even in the context of effective cART-mediated suppression of plasma viremia. Moreover, the authors posit that viral evolution in these protected niches may be independent of selective pressure from cART therapy [18]. This provides us with a theoretical context in which the persistent immune dysfunction found in PLWH successfully treated with cART may be due to residual viral pathogenesis restricted to the lymph nodes rather than aberrations caused by dysregulated immune reconstitution. Indeed, recent evidence suggests that germinal center resident follicular helper T lymphocytes (Tfh) may be a site

of HIV persistence [17,19,20] and that the lack of CD8+ T cell surveillance of the germinal center [21] may contribute to the escape of HIV-infected T cells from elimination by CTL in this niche. Moreover, these results create a framework in which we can hypothesize HIV-infected T lymphocytes in germinal centers contribute to the development of lymphoma in PLWH and that this pathology persists during cART.

Contribution of HIV to a lymphomagenic microenvironment during cART

Given the novel finding that HIV continues to replicate in lymph nodes during cART therapy, and that germinal center-resident Tfh cells are a particularly critical cell type for HIV persistence, we will now consider possible mechanisms, other than the obvious possibility of chronic antigenic stimulation, by which HIV might contribute to lymphoma risk in this context.

Effects of follicular T cell subsets in the germinal center

Recent findings suggest that Tfh from HIV-infected individuals are phenotypically [20] and functionally [22] distinct from those of uninfected controls, raising the possibility that the significant aberrations in B cell development and function observed during chronic HIV infection [23,24] might stem from effects of HIV-infected Tfh on the germinal center reaction. Appropriate Tfh function is also critical to the control of chronic viral infection [25,26], suggesting that HIV-mediated dysfunction in this cell type may also play a role in lymphoma pathogenesis driven by viral co-pathogens such as EBV and KSHV. Interestingly, Cubas et al demonstrated that Tfh from HIV-infected subjects displayed increased IL-21 production after *ex vivo* stimulation [22]. Given the well-documented effects of IL-21 on EBV latency and LMP1 expression [27-29], it is interesting to speculate that HIV-driven changes in Tfh function might enhance B cell transformation by EBV. Similarly, it was recently shown that expanded Tfh cell populations in individuals chronically infected with HIV are associated with an increase in germinal center B cells

and plasma cells, decrease of memory B cells and IgG1 hypersecretion [30]. Moreover, the expansion of T follicular regulatory cells (Tfr) in HIV disease adds a layer of complexity to this milieu [31]. Taken together, the expansion of both Tfh and Tfr would have the expected outcome of diminishing B cell activation. Instead, these expansions are coincident with excessive B lymphocyte activation [32] and hypergammaglobulinemia [33], underscoring the fact that, although follicular T lymphocyte subsets increase in frequency, their normal lymph node functions are perturbed in the context of chronic HIV infection leading to poorly-understood alterations in B cell physiology (Figure 1). Certainly, additional mechanisms underlying lymphoma development in this context will continue to evolve along with our understanding of the normal and pathological role of follicular T cell subsets in the germinal center reaction.

Chronic B cell stimulation

It is well known that HIV-induces chronic B cell stimulation, which may be critical for the development of lymphoma [32,34,35]. In particular germinal center expansion, as seen as a florid follicular hyperplasia at the early stages of AIDS, is thought to contribute to lymphomagenesis. This is the site where B cell receptor rearrangements occur (i.e class switching) and also somatic hypermutation through the effects of activation-induced cytidine deaminase (AID), and these events can result in mistakes involving oncogenic genetic alterations. In addition, lymphomas in HIV-infected patients are most frequently of germinal center cell origin (including Burkitt lymphoma and GC-subtype of diffuse large B cell lymphoma). These B cell abnormalities were still present in cART-treated patients, albeit somewhat diminished when compared to untreated patients. This indicates that even in the context of good peripheral HIV control through cART, there are B cell abnormalities that could play a role in lymphoma development.

Direct effects of HIV virions and proteins on lymphomagenesis

If HIV maintains active replication in lymph nodes during cART treatment, we must consider that direct functions of HIV virions and/or HIV proteins on B cell functionality may also persist even if HIV does not directly infect the B cells that give rise to lymphomas. A number of potential mechanisms by which HIV proteins might directly contribute to lymphoma have previously been characterized [1,36], and specifically a number of these may contribute to B cell hyperplasia, including Tat [37], Nef [38,39], Env gp120 [40], Env gp130 and Gag p17 [41]. Tat has inhibitory effects on DNA repair mechanisms in B cells [42], and can activate DNMT1 resulting in aberrant hypermethylation of target genes [43]. HIV viral proteins have been shown to interact with other oncogenic herpesviral proteins, even if expressed in different cells, through secretion or transmission of HIV proteins by structures like nanotubules or exosomes (reviewed in [44,45]). Two studies have documented interactions between HIV Nef and KSHV: (1) Nef could synergize with KSHV vIL-6 and activate the AKT pathway, which lead to angiogenesis and tumor formation in a model of Kaposi sarcoma [46] and (2) Nef was shown to regulate KSHV latency by inducing cellular miRNA 1258 (hsa-miR-1258) that directly targets a seed sequence in the 3' untranslated region of the mRNA encoding the major lytic switch protein of KSHV (RTA) [47]. HIV Nef was shown to be secreted in exosomes [48] and to penetrate bystander, uninfected B cells [38]. Thus, it may have an effect in maintaining latency of EBV or KSHV-infected B cells that eventually become transformed. Transgenic mice expressing the HIV protein Tat develop B cell lymphoma, a phenotype that might be linked to the ability of Tat to induce production of IL-6 and IL-10. Using another transgenic mouse model, Curreli et. al. recently described the association of pre-lymphoma lymphadenopathy associated with increased expression of the HIV proteins p17 and gp120 [49]. Indeed, high levels of p17 persist in lymph nodes of PLWH, even under the influence of cART [50,51]. Working from these observations, Dolcetti and colleagues recently demonstrated that particular p17 sequence variants are expressed in HIV patients

presenting with NHL, and that these altered p17 proteins have direct effects on B cell proliferation and signaling to Akt and PTEN [52]. It is particularly interesting to consider that these lymphomagenic p17 variants may arise during HIV sequence evolution in lymph nodes during cART, thus providing a source of lymphoma risk independent of treatment status. A different mechanism by which HIV itself may affect lymphoma development is suggested by the observation that host-derived CD40L can be incorporated into virions and the binding of these virions to B lymphocytes can potentiate activation and proliferation mimicking the endogenous CD40-CD40L interaction [40].

Adipose tissue reservoirs and implications for lymphoma

An increasing number of population studies are linking both infection and cART treatment with increased incidence of obesity in PLWH [53,54]. Although there is no consensus in the clinical literature as to whether there is a direct association between obesity and risk of NHL in the general population [55], recent studies suggest that in the context of HIV infection in general, and PLWH on cART specifically, obesity coupled with specific defects in adipose tissue metabolism could provide a context in which lymphoma risk is increased. In particular, recent studies demonstrate that adipose tissue provides an additional reservoir for HIV-infected immune cells (macrophages and T lymphocytes) even in the context of successful cART therapy [56,57], and studies using SIV infection of non-human primates (NHP) conclusively demonstrate that HIV infection of adipose tissue contributes to metabolic abnormalities and systemic inflammation during chronic infection [56,58], similar to what is observed in PLWH. Moreover, a significant proportion of PLWH on cART therapy have lipodystrophy, characterized by a loss of subcutaneous adipose tissue coupled with an increase in visceral adipose tissue [59-61] and these modifications are associated with chronic inflammation and, specifically, increased levels of serum leptin and IL-6 [62]. Increased expression and circulation of leptin was also reported in chronic SIV infection in NHP [58]. Aside from the obvious contribution of chronic inflammation in

lipodystrophy to lymphoma risk, a number of recent studies specifically link leptin signaling to pathogenesis in lymphoma via induction of PI3K/Akt signaling [63-66]. Thus, it seems that adipose-specific metabolic defects in PLWH undergoing cART treatment could directly contribute to lymphoma risk via increases in circulating leptin. Moreover, a number of direct effects of HIV accessory proteins on adipocyte functions have been described in animal models [67,68] and human cells [69-71] many of which could contribute to the induction of a lymphoma-promoting chronic inflammatory state.

Conclusions

A great deal of progress has been made controlling HIV infection and preventing the progress to full-blown AIDS. This has led to a marked decrease of some AIDS defining malignancies like Kaposi sarcoma, but surprisingly not their disappearance and some cancers may even be rising in incidence as people with HIV infection are living longer. Of note, lymphomas are occurring in people with controlled HIV infection and no obvert immunodeficiency. While lymphoma is considered an “AIDS defining condition”, these individuals may not have other evidence of AIDS, such as opportunistic infections, and respond to lymphoma treatment with survival comparable to that of HIV-negative individuals. Thus, one could argue that lymphoma should no longer be considered an “AIDS-defining cancer”, but rather an “HIV-associated malignancy”.

HIV is considered an oncogenic pathogen, although it does not directly infect the cancer cells [1], and an indirect role related to inflammation and HIV proteins that may be secreted has been implicated for some time. However, control of HIV should have eliminated these factors responsible for lymphoma development. A possible explanation has been that HIV infection results in upregulation of AID, which induces gene rearrangements and somatic hypermutation in B cells and could induce aberrant genetic alterations resulting in permanent damage that leads to lymphoma down the road,

regardless of subsequent control of HIV. However, this phenomenon has not been formally demonstrated experimentally. The recent evidence demonstrating that HIV continues to replicate in lymph node reservoirs even in the context of successful cART suppression of plasma viremia creates a new scenario wherein the effects of HIV on follicular T cell subsets as well as direct effects of HIV proteins may induce lymphoma development, even in when HIV disease is well-controlled, and may explain the continued prevalence of HIV-associated lymphomas in the era of effective antiretroviral therapy.

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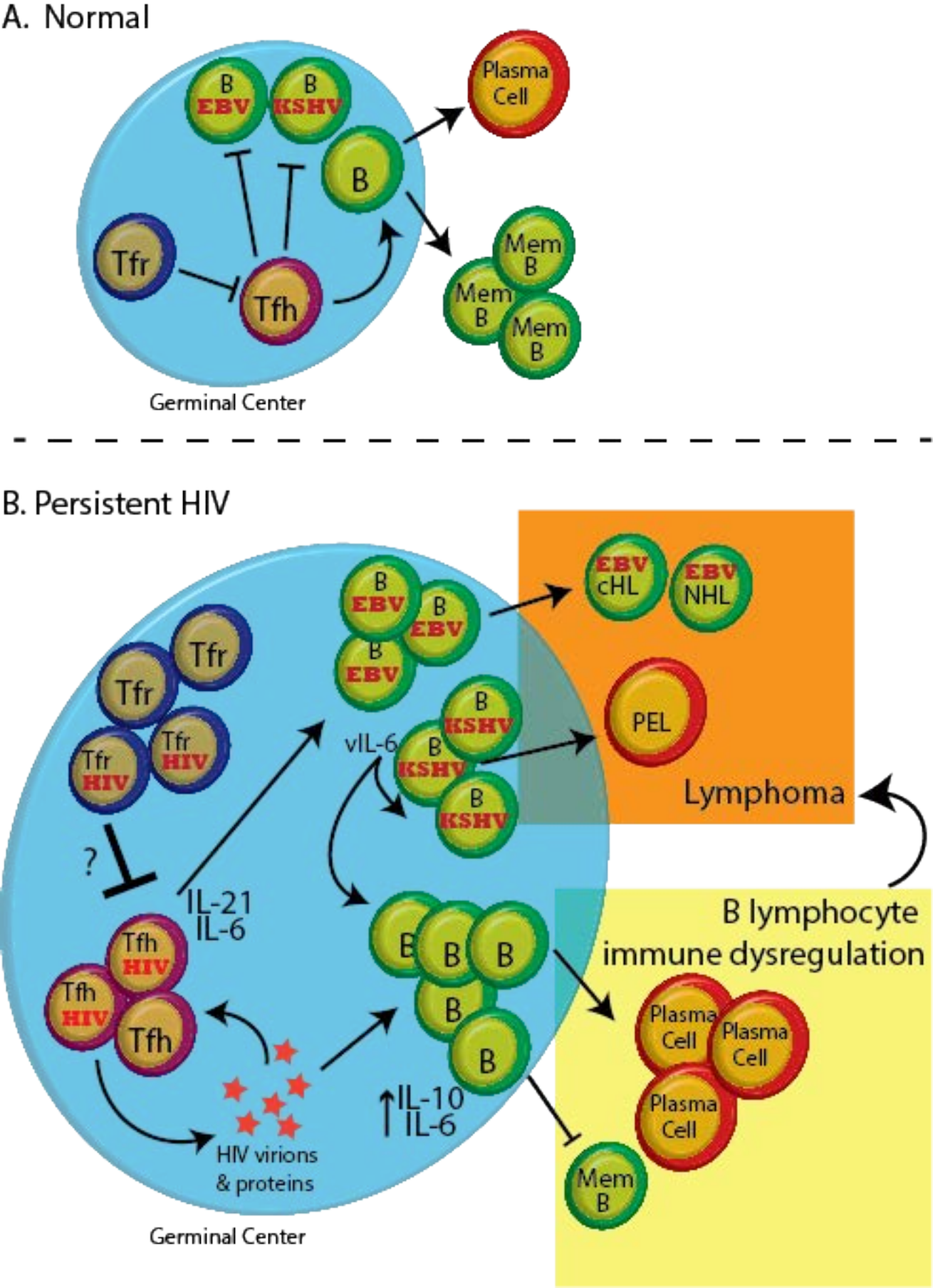
Figure Legend

Figure 1: Model of intra-follicular dysfunction leading to lymphoma in chronic HIV infection. (A) In normal lymph nodes, Tfr regulate the proliferation and function of Tfh, which, in turn, suppress replication of lymphotrophic pathogens KSHV and EBV and regulate the germinal center reaction and production of memory B lymphocytes and plasma cells. (B) HIV infection results in expansion of both Tfr and Tfh subsets, but dysregulates their

interaction as well as the ability of Tfh to provide appropriate help to B cells, creating a decrease in memory B cell production and dysregulation of plasma cell differentiation. Suppression of Tfh function could allow proliferation of lymphotrophic pathogens KSHV and EBV and allow direct lymphomagenesis by viral mechanisms. Additionally, HIV gene products and virions accumulating in lymph nodes contribute directly to both B cell dysfunction and viral lymphomagenesis.


A. Normal
B
EBV
B
KSHV
B
Plasma Cell
Tfr
Tfh
Mem B
Mem B
Mem B
Germinal Center
B. Persistent HIV
B
EBV
B
EBV
B
EBV
EBV cHL
EBV NHL
PEL
Lymphoma
Tfr
Tfr
Tfr HIV
Tfr HIV
vIL-6
B
KSHV
B
KSHV
B
KSHV
?
IL-21
IL-6
B lymphocyte immune dysregulation
Tfh HIV
Tfh HIV
Tfh
B
B
B
B
B
Plasma Cell
Plasma Cell
Plasma Cell
Mem B
↑IL-10
IL-6
HIV virions & proteins
Germinal Center