

Impact of alemtuzumab on HIV persistence in an HIV-infected individual on antiretroviral therapy with Sezary syndrome

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Objective: To study the effects of alemtuzumab on HIV persistence in an HIV-infected individual on antiretroviral therapy (ART) with Sezary syndrome, a rare malignancy of CD4⁺ T cells.

Design: Case report.

Methods: Blood was collected 30 and 18 months prior to presentation with Sezary syndrome, at the time of presentation and during alemtuzumab. T-cell subsets in malignant (CD7–CD26–TCR–VBeta2+) and nonmalignant cells were quantified by flow cytometry. HIV-DNA in total CD4⁺ T cells, in sorted malignant and nonmalignant CD4⁺ T cells, was quantified by PCR and clonal expansion of HIV-DNA assessed by full-length next-generation sequencing.

Results: HIV–hepatitis B virus coinfection was diagnosed and antiretroviral therapy initiated 4 years prior to presentation with Sezary syndrome and primary cutaneous anaplastic large cell lymphoma. The patient received alemtuzumab 10 mg three times per week for 4 weeks but died 6 weeks post alemtuzumab. HIV-DNA was detected in nonmalignant but not in malignant CD4⁺ T cells, consistent with expansion of a noninfected CD4⁺ T-cell clone. Full-length HIV-DNA sequencing demonstrated multiple defective viruses but no identical or expanded sequences. Alemtuzumab extensively depleted T cells, including more than 1 log reduction in total T cells and more than 3 log reduction in CD4⁺ T cells. Finally, alemtuzumab decreased HIV-DNA in CD4⁺ T cells by 57% but HIV-DNA remained detectable at low levels even after depletion of nearly all CD4⁺ T cells.

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Conclusion: Alemtuzumab extensively depleted multiple T-cell subsets and decreased the frequency of but did not eliminate HIV-infected CD4⁺ T cells. Studying the effects on HIV persistence following immune recovery in HIV-infected individuals who require alemtuzumab for malignancy or in animal studies may provide further insights into novel cure strategies. Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Despite the great advances in antiretroviral therapy (ART), treatment is life-long, and there is no cure. HIV can persist on ART as a latent infection in multiple long-lived T-cell subsets, including central memory and naïve T cells [1]. In addition, latently infected CD4⁺ T cells can undergo homeostatic proliferation [2] and clonal expansion [3–6].

Sezary syndrome is a leukaemic form of cutaneous T-cell lymphoma (CTCL) and is considered a malignancy of CD4⁺ central memory T cells (Tcm) [7]. Treatment options for Sezary syndrome have recently included low-dose alemtuzumab [8], a humanized anti-CD52 mAb licensed for B-cell chronic lymphocytic leukaemia and relapsing-remitting multiple sclerosis (RRMS) [9,10]. CD52 is expressed not only on all T-cell subsets, including long-lived Tcm and naïve T cells, but also on B cells, macrophages, natural killer (NK) cells and dendritic cells [11,12]. Alemtuzumab depletes CD52 positive circulating cells but has minimal effect on noncirculating skin resident effector memory T cells (Tem), required to maintain mucosal immunity and protection from opportunistic infections. Although associated with some risk, complete depletion of Tcm and naïve T cells followed by immune reconstitution on ART, may potentially perturb or even eliminate HIV persistence on ART.

Here, we describe the effects of alemtuzumab treatment in an HIV-infected individual on ART with Sezary syndrome. We aimed to characterize whether malignant CD4⁺ T cells in Sezary syndrome were a consequence of clonal expansion of HIV-infected cells; the effect of alemtuzumab on malignant and nonmalignant T cells including memory subsets; and how alemtuzumab impacted the frequency of latently infected CD4⁺ T cells.

Methods

Sample collection

We collected blood and isolated peripheral blood mononuclear cells (PBMCs) and plasma at regular intervals prior to and following the diagnosis of Sezary syndrome and prior to and during alemtuzumab

treatment (Fig. 1). The protocol was approved by the Ethics Committee of the Alfred Hospital and the patient provided written informed consent for participation and for use of stored PBMC collected from a previous study.

Flow cytometry for malignant and nonmalignant CD4⁺ T cells

We used flow cytometry and fluorescence activated cell sorting (FACS) to quantify and isolate malignant CD4⁺ T cells, defined by lack of expression of CD7 and CD26 [13] and expression of T-cell receptor (TCR)-Vβ2. In some experiments, we used a less stringent definition of nonmalignant cells as CD3+CD4+TCR-Vβ2- (Fig. S1, <http://links.lww.com/QAD/B104>). Memory T-cell subsets were defined as naïve (CD45RA+CCR7+), terminally differentiated (CD45RA+CCR7-), Tcm (CD45RA-CCR7+), Tem (CD45RA-CCR7-CD27-) and transitional memory (CD45RA-CCR7-CD27+) T cells.

HIV-DNA quantification

Cryopreserved PBMCs were sorted into total CD4⁺ T cells, malignant (CD3+CD4+TCR-Vβ2+CD7-CD26-) or nonmalignant (CD3+CD4+TCR-Vβ2-CD4⁺ T cells using FACS. Cells were then lysed and cell-associated HIV-DNA was measured by quantitative PCR using primers and probes as previously described [14].

HIV-DNA full-length sequencing

To address whether there was clonal expansion of HIV-infected cells, we employed a full-length HIV sequencing method based on next-generation sequencing techniques (Hiener *et al.*, CROI 2017). Proviral sequences were diluted to a single genome and a near full length 9 kb region of HIV-DNA amplified using a nested PCR protocol. Each full-length HIV genome was fragmented and a sequence index or tag was added to the representative fragments of each genome for unique identification in the final data analysis. After sequencing, the individual proviruses were *de novo* assembled using a specifically designed workflow in CLC Genomics. Proviruses were characterized as defective (containing insertions/deletions, stop codons or APOBEC3G hypermutation) or intact (full-length; lacking defects).

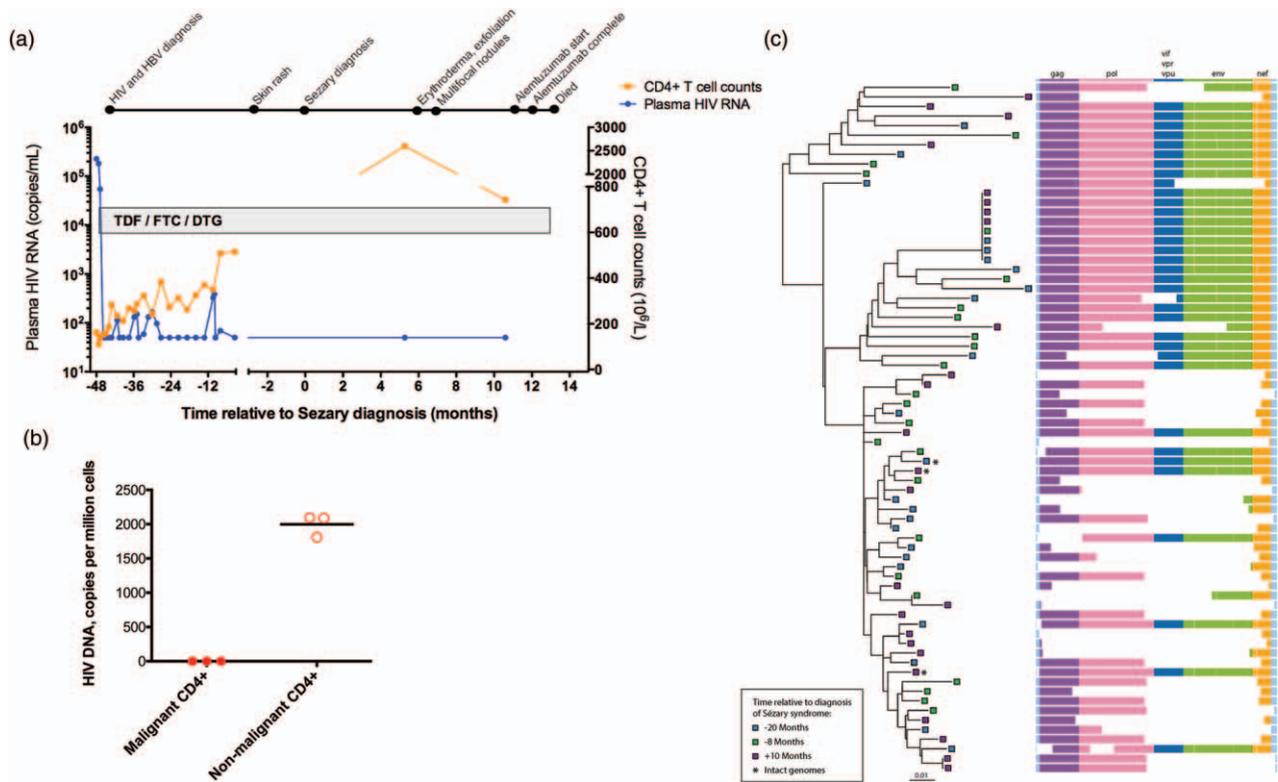


Fig. 1. Clinical course and HIV-DNA in malignant and nonmalignant cells. (a) Chronological overview of key clinical events, plasma HIV RNA and CD4⁺ T-cell counts since start of antiretroviral therapy. (b) HIV-DNA in sorted malignant (CD3⁺CD4⁺CD7⁻CD26⁻TCR⁻Vbeta2⁺, shown as closed circles) and nonmalignant CD4⁺ T cells (CD3⁺CD4⁺TCR⁻Vbeta2⁻, shown as open circles) collected 2 weeks before starting alemtuzumab. The DNA PCR was done in three replicates, lower limit of detection was 1 copy per well, and a total of 220 000 malignant CD4⁺ T cells were analysed. (c) Phylogenetic tree of full-length HIV-DNA sequences obtained from CD4⁺ T cells isolated from blood collected prior to (blue and green squares) and following (purple squares) the diagnosis of Sezary syndrome. The majority of sequences were defective and contained major deletions in HIV genes as illustrated by the coloured bars in the right hand side of the figure. ART, antiretroviral therapy; DTG, dolutegravir; FTC, emtricitabine; GFP, green fluorescent protein; PHA, phytohaemagglutinin; TDF, tenofovir disoproxil fumarate.

Gp120-specific antibodies and antibody-dependent natural killer cell activation assay

As previously described, we used an enzyme-linked immunosorbent assay to determine the concentrations of gp120-specific antibodies in plasma [15] and performed a plate-bound antibody-dependent NK cell activation assay to measure intracellular NK cell IFN γ and CD107a expression [16].

Results

Clinical course

A 62-year-old man with chronic hepatitis B virus (HBV) and HIV coinfection on ART presented with generalized rash and was diagnosed with Sezary syndrome. He also had coexistent primary cutaneous anaplastic large-cell lymphoma (pcALCL), another separate CTCL well

recognized to occur in patients with coexistent mycosis fungoides/Sezary syndrome [17]. Both HBV and HIV infections were diagnosed 4 years prior to presentation with Sezary syndrome at age 58 years. At HIV diagnosis, HIV RNA was 228 100 copies/ml and nadir CD4⁺ T-cell count was 110 cells/ μ l. Combination ART with tenofovir disoproxil fumarate, emtricitabine and dolutegravir was commenced immediately after HIV diagnosis. Suppression of plasma HIV RNA to less than 50 copies/ml was achieved after 1 month on ART but, over the following 4 years, frequent low-level plasma HIV RNA was detected, in the range of 50–387 copies/ml, while CD4⁺ T cells reached more than 500 cells/ μ l after approximately 3 years on ART (Fig. 1a).

Three months prior to diagnosis with Sezary syndrome, the patient initially observed an eczematous-like rash over his trunk, which rapidly progressed to generalized erythroderma with pruritus, scaling and acral keratoderma

(Fig. 1a). Skin biopsies and peripheral blood flow cytometry were consistent with a diagnosis of Sezary syndrome. He also had multifocal tumours over his neck, axilla and trunk and biopsies of these demonstrated coexistent CD30+ pcALCL. Five months after diagnosis of Sezary syndrome, his CD4⁺ cell count had risen to 2599 cells/ μ l from 515 cells/ μ l 3 months prior to diagnosis. The rise was assumed to be an increase in the malignant CD4⁺ clone (Fig. 1a).

Treatment of the Sezary syndrome was initiated 10 months after diagnosis with low-dose alemtuzumab combined with extracorporeal photophoresis and tumour-directed radiotherapy. The patient received 10 mg of alemtuzumab subcutaneously three times per week for 4 weeks. After an initial excellent symptomatic treatment response with marked reduction of pruritus and improvement in skin integrity and erythroderma, he subsequently experienced worsening liver function followed by multiorgan failure with progressive lymphadenopathy and died 6 weeks after commencing alemtuzumab.

HIV-DNA was detected in nonmalignant but not malignant CD4⁺ T cells

Given recent reports of clonal expansion of latently infected CD4⁺ T cells in individuals on ART [3,6,18], we first asked whether the presentation of Sezary syndrome was associated with malignant clonal expansion of a latently infected CD4⁺ T cell. We quantified HIV-DNA in malignant (CD7⁻CD26⁻TCR⁻Vbeta2⁺) and non-malignant (CD7⁺CD26⁺TCR⁻Vbeta2⁻) CD4⁺ T cells collected 10 months after the diagnosis of Sezary syndrome but prior to alemtuzumab. HIV-DNA was detected in nonmalignant CD4⁺ T cells (1998 copies/million cells) but not in the malignant CD4⁺ T cells (<4 copies/million cells) (Fig. 1b). These findings were consistent with expansion of a CD4⁺ T-cell clone not infected with HIV.

We next asked whether there was tumour-antigen driven expansion of HIV-infected CD4⁺ T cells, similar to a recent case report of metastatic squamous cell carcinoma in an HIV-infected individual [4]. We performed full-length sequencing of HIV-DNA in CD4⁺ T cells collected before and after presentation with Sezary syndrome. We obtained and sequenced 25–26 single HIV genomic sequences and found 8–10 full-length HIV genomes from each time point; however, the majority of these genomes contained deletions prior to the HIV gag gene region and did not represent replication-competent virus. We identified only one clonal expansion and this sequence contained hypermutant genomes from all time points (Fig. 1c). Taken together, we demonstrated that the malignant CD4⁺ T cells were not infected, and there was no evidence of clonal expansion of HIV-DNA in the nonmalignant cells.

Effect of alemtuzumab on T cells, T-cell subsets and HIV persistence

Alemtuzumab led to a dramatic depletion of total T cells within 18 days, including a more than 1 log reduction in total CD3⁺ T cells and more than 3 log reduction in CD4⁺ T cells (Fig. 2a). There was a greater decline in malignant compared with nonmalignant CD4⁺ T cells (Fig. 2b). We quantified memory CD4⁺ and CD8⁺ T cells in cells enriched for malignant (CD7⁻) and nonmalignant (CD7⁺) markers prior to and 4 and 11 days during alemtuzumab. We observed depletion of central memory but not effector memory CD4⁺ T cells not expressing CCR7. This pattern was not seen for CD8⁺ memory T cells or for CCR7⁺ cells (Fig. 2c and d). We did not quantify T-cell subsets 18 and 25 days post alemtuzumab due to very low T-cell numbers at these time points.

We next quantified HIV-DNA in total CD4⁺ T cells prior to and following alemtuzumab. We observed a 57% reduction in HIV-DNA per million cells from pre-alemtuzumab (day 0) to day 25 on-alemtuzumab (Fig. 2e) but, even when the total lymphocyte count was as low as 80 cells/ μ l on-alemtuzumab, we were still able to detect HIV-DNA. To exclude the possibility that the HIV-DNA was a contaminant, we sequenced the virus and found no alignment with any laboratory strains of HIV (data not shown). The rapid decay of malignant CD4⁺ T cells after starting alemtuzumab allowed for quantification of HIV-DNA in sorted nonmalignant CD4⁺ T cells at only one time point 4 days after starting alemtuzumab. We did not detect a decrease in HIV-DNA in nonmalignant CD4⁺ T cells at this early phase of alemtuzumab treatment (1998 and 2098 copies per million nonmalignant CD4⁺ T cells at days 0 and 4, respectively).

Finally, as CD52 is also expressed on NK cells, we investigated whether alemtuzumab affected HIV-specific antibody-mediated NK cell activation. We found no changes in anti-gp120 IgG concentrations during the 4 weeks of alemtuzumab treatment and also did not see any change in the ability of the patient's plasma to induce NK cell expression of IFN γ or CD107a (Fig. S2, <http://links.lww.com/QAD/B104>).

Discussion

In an HIV-infected individual on ART with Sezary syndrome, the malignant CD4⁺ T cells were not infected with HIV and full-length sequencing of HIV-DNA demonstrated no clonal expansion in the nonmalignant HIV-infected CD4⁺ T cells. Alemtuzumab caused extensive depletion of T cells and decreased the frequency of latently infected CD4⁺ T cells, but HIV-DNA remained detectable even after extensive CD4⁺ T-cell depletion.

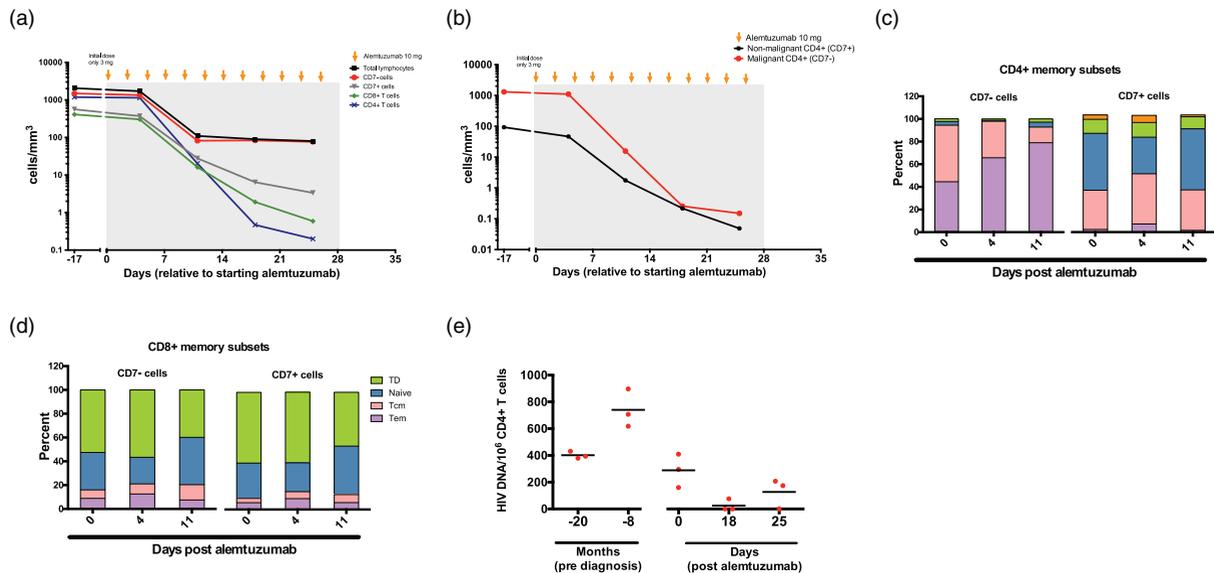


Fig. 2. Depletion of T cells and HIV-DNA during alemtuzumab. (a) Absolute counts of total lymphocytes and T-cell subsets in blood prior to and following alemtuzumab treatment. (b) The change in frequency of CD7⁻ (malignant) or CD7⁺ (nonmalignant) CD4⁺ T cells following alemtuzumab. (c) The distribution of memory T-cell subsets, stratified by CD7 expression, shown for both CD4⁺ T cells and (d) CD8⁺ T cells; T-cell subset analysis was only performed during the first 2 weeks of alemtuzumab because of extensive T-cell depletion at subsequent time points. (e) Levels of HIV-DNA in total CD4⁺ T cells for samples obtained before diagnosis with Sezary syndrome, before starting alemtuzumab and at several time points during alemtuzumab. Horizontal bars show mean values of three replicate measurements at each time point. Tcm, central memory T cells; TD, terminally differentiated; Tem, effector memory T cells; Ttm, transitional memory T cells.

Alemtuzumab is rarely used in HIV-infected individuals and no previous study has analysed its effect on HIV persistence. In a case series of HIV-infected patients receiving alemtuzumab as part of renal transplant conditioning, there were no opportunistic infections despite a slow recovery of CD4⁺ T cells but effects on latent HIV were not investigated [19]. Our data confirm the extensive depletion of multiple T-cell subsets also seen in other studies [12,20] and we also detected a decrease in the frequency of HIV-DNA in total CD4⁺ T cells during alemtuzumab. The detection of HIV-DNA, even when the total CD4⁺ T-cell count was less than 1 cell/ μ l, was surprising and could potentially be explained by persistence of virus in other long-lived cells such as T memory stem cells. These have been shown to be preferentially infected with HIV in individuals on ART [21], have an extremely long half-life, and it is unknown if these cells are depleted by alemtuzumab.

Lymph node T follicular helper (Tfh) cells were recently shown to be an important source of persistent HIV in patients on suppressive ART [22], but the direct effects of alemtuzumab in lymph node tissue, including effects on Tfh cells could not be evaluated in our study. However, as CD52 is ubiquitously expressed on all mature T cells [11,12], we assume CD52 is also expressed on Tfh cells as well as other long-lived CD4⁺ T cells that serve as important reservoirs for HIV. Notably, in malignant transformation of Tfh cells, as seen in

angioimmunoblastic T-cell lymphoma, high levels of CD52 expression in both lymph node aspirates and peripheral blood were demonstrated [23–25]. Given that alemtuzumab-mediated depletion of CD52 positive cells occurs via antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity [11], which is dependent on the presence of immune effector cells such as NK cells and neutrophils [26], tissue-resident cells like Tfh, may potentially avoid depletion in the absence of those effector functions. For example, in a study of mycosis fungoides, alemtuzumab was shown to potently deplete circulating long-lived central memory and naïve T cells but had minimal effect on noncirculating skin resident Tem [27]. Similarly, in cynomolgus monkeys who received alemtuzumab following cardiac transplant, there was a much more profound reduction of CD4⁺ T cells in peripheral blood (99% reduction) than in lymph nodes (70% reduction) [28].

We hypothesized that, following immune reconstitution after alemtuzumab, the frequency of latently infected CD4⁺ T cells could be substantially reduced if there was preferential recovery and expansion of uninfected cells. Unfortunately, the patient died 6 weeks postalemtuzumab, which prevented analyses following T-cell reconstitution. We are currently testing this hypothesis in a simian immunodeficiency virus-infected rhesus macaque model.

In conclusion, alemtuzumab extensively depleted multiple T-cell subsets and also decreased the frequency of but did not eliminate CD4⁺ T-cells harbouring HIV-DNA in blood. Studying the effects of alemtuzumab in blood and lymphoid tissue as well as following immune recovery in HIV-infected individuals who require alemtuzumab for the treatment of malignancy or RRMS or in animal studies may provide further insights into novel HIV cure strategies.

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S.R.L., T.A.R., P.U.C., H.M.P., J.M., J.S., M.R. and A.O. conceived of and designed the study and its analyses; H.M.P., C.V.D.W., J.M., S.R.L. and T.A.R. were responsible for study visits and all clinical interaction; J.J.C. and A.D. performed cell sorting and flow cytometry analyses; T.A.R. performed PCR analyses; B.H. and S.P. performed HIV-DNA full-length sequences and related analyses; S.K. and W.S.L. designed and performed analyses of antibody-mediated cellular toxicity; M.R. performed HIV-DNA sequencing to rule out contamination; S.R.L., P.U.C., T.A.R., J.M., M.R., J.J.C. and J.S. performed data analyses and interpretation; S.R.L., P.U.C., T.A.R., J.J.C., S.P., B.H. and A.O. contributed to the graphical presentation of study results; S.R.L., T.A.R. and C.V.D.W. drafted the article; all authors reviewed, edited and approved the final article.

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Conflicts of interest

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