Enhanced T-Cell Maturation and Monocyte Aggregation Are Features of Cellular Inflammation in Human T-Lymphotrophic Virus Type 1–Associated Myelopathy

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**Background.** Human T-lymphotropic virus type 1 (HTLV-1)–associated myelopathy (HAM) is an inflammatory condition characterized by severe disability and high levels of infected white blood cells. The circulating cellular inflammatory changes that distinguish this condition from asymptomatic infection are not well understood.

**Methods.** To investigate the immune characteristics of individuals with low or high HTLV-1 proviral load (pVL), symptomatic disease, and the impact of immunosuppressive therapy, 38 women living with HTLV-1 infection, at a median age of 59 (52–68) years, were studied. Nineteen were asymptomatic carriers with low or high pVL; 19 were diagnosed with HAM, with 10 receiving anti-inflammatory therapy. Peripheral blood mononuclear cells were stained and analyzed for frequency distribution and activation of innate and adaptive immune cell subsets using multiparameter flow cytometry.

**Results.** Inflation of the CD4:CD8 ratio (>2) was observed among all groups irrespective of pVL. The frequency of naive CD4+ T cells correlated inversely with HTLV-1 pVL (rs = –0.344, P = .026). Mature T effector memory T EM CD4+ T cells were expanded in patients with untreated HAM compared with asymptomatic carriers (P < .001) but less so in those on therapy. High levels of exhausted (PD-1+) and senescent (CD28null) CD4+ and CD8+ T cells were observed in all individuals, particularly in those with HAM, while monocytes showed increased aggregation and CD14+CD56− monocytes were less frequent.

**Conclusions.** CD4:CD8 ratio inflation is a feature of HTLV-1 infection, whereas enhanced CD4+ T cell maturation and monocyte aggregation are features of HAM, reflecting widespread inflammatory change, which may be detectable presymptomatically and be amenable to anti-inflammatory treatment.

**Keywords.** HTLV-1; HAM/TSP; HTLV-1–associated myelopathy; inflammation; cellular maturation.

Human T-lymphotropic virus type 1 (HTLV-1) is an ancient blood-borne pathogen that can be transmitted sexually or from mother to child, establishing chronic infection. Limited screening and prevention programs have failed to tackle the high prevalence in some endemic communities [1, 2]. HTLV-1 is the etiological agent in several inflammatory conditions, including the debilitating inflammatory disease HTLV-1–associated myelopathy (HAM), also called tropical spastic paraparesis, and the frequently fatal adult T-cell leukemia/lymphoma (ATLL) [3, 4]. HAM is a degenerative condition that can involve motor, sensory, and bladder pathology, with progressive weakness, spasticity, numbness of the legs, and urinary incontinence [5].

Current evidence indicates that neither HTLV reverse transcription nor integration inhibition is effective in the management of established HTLV-1 infection, limiting treatment options for those with symptomatic infection [6, 7]. HAM is characterized by inflammation of the brain and spinal cord mediated by T cells, occurring in 1–4% of individuals with HTLV-1 [8]. HAM predominantly affects females, with a peak onset in the fourth and fifth decades. HAM is managed with immune suppression using corticosteroids or steroid-sparing agents. The most widely used treatment for HAM in the United Kingdom is weekly methotrexate with folic acid rescue.

Viruses that persist throughout the human life span, including the retroviruses HTLV-1 and human immunodeficiency virus type 1 (HIV-1) and the viruses of the human herpes family such as cytomegalovirus (CMV), will continue to increase in prevalence and become more widespread with aging and migration of the global population. Where individuals are at risk, prevalence of HTLV-1 infection increases significantly with age, with half of Indigenous Australian men aged 50–64 years infected in one study [9]. Chronic immune activation is observed in persistent viral infections and is associated with mortality in CMV infection and non-AIDS morbidities in HIV-1 infection [10].

The relationships between pathogen burden, viral or proviral load (pVL), inflammation, and development of symptoms, where persistent viral infection has been under immune or pharmacological control, is not well understood. The majority of individuals with HTLV-1 infection are asymptomatic carriers with a low pVL. Poor viral control can occur silently, leading to high pVL without clinical manifestation. HAM occurs exclusively in individuals who have a high pVL, which can drive expansion of high frequencies of inefficient virus-specific T cells [12]. Some data that compare pVL in HAM with that of asymptomatic carriers are available [13]. However, it has previously been challenging to assemble cohorts of asymptomatic carriers with high pVL in order to discriminate the contribution of pathogen burden from disease status on immune competency.

HTLV-1 and treated, suppressed HIV-1 infection share several key features. Both viruses employ persistence mechanisms that avoid adaptive cellular immunity through integration of proviral DNA, including clonal proliferation and cell-to-cell transmission, generating the hard-to-eradicate viral reservoir. Both viruses also propagate a clinical spectrum of disease that is contingent, at least in part, upon the potency of the cytotoxic T-cell response and on pathogen burden. Changes in T-cell subset distribution, including inversion of the CD4:CD8 ratio and alteration of T-cell receptor signaling pathways with accumulation of CD28null cells, have been described in CMV and HIV-1 infection and linked to maladaptive changes that occur with aging [10, 14, 15]. Dissection of cellular immunity in nonmalignant manifestations of chronic HTLV-1 infection could provide insight into the relationships between persistent retroviral integration, chronic inflammation, and disease that requires anti-inflammatory treatment.

We therefore studied a cohort of individuals with HTLV-1 infection with predefined clinical phenotypes representing a spectrum of pathogen burden and clinical disease manifestation. These were asymptomatic carriers with low pVL (AClo), asymptomatic carriers with high pVL (AChi), and symptomatic individuals with untreated HAM (uHAM). These groups were chosen to discriminate between the effects of high pVL and symptomatic disease. To evaluate the effect of anti-inflammatory treatment, we also studied a group of individuals receiving methotrexate. We used multiparameter flow cytometry to measure the frequency and phenotype of circulating cell subsets of the innate and adaptive immune system in these individuals. We hypothesized that differences in the frequency and phenotype of these cell subsets, similar to those described in other persistent viral infections, would be observed. Here, we show widespread inflammatory change in circulating cell subsets in symptomatic, untreated HTLV-1 infection, particularly affecting bulk T cells but also involving monocytes. These changes were only partially associated with pathogen burden (HTLV-1 pVL), and may be amenable to treatment with anti-inflammatory therapy.

METHODS

Study Design

Inclusion criteria were women aged ≥18 years with virological evidence of HTLV-1 infection and with sufficient sample for analysis. Samples for study were selected from the following individuals using a cross-sectional study design: AClo, AChi, uHAM, and treated HAM (tHAM). These were matched for sex (female), ethnicity (black African or Caribbean), and age. AChi were included to distinguish asymptomatic from symptomatic disease in high pVL states, while AClo were included as a control for risk of infection. Participants were assessed for neurological signs and evidence of HTLV-1–associated disease, including uveitis, sicca syndrome, and coinfections, and classified as asymptomatic carriers where absent. This classification is based on the guidance issued by the Centre for Human Retrovirology for monitoring and treating HTLV-1 infection. HAM was diagnosed according to World Health Organization criteria [16].

Diagnosis of HTLV-1 Infection and Proviral Load Measurement

HTLV-1 infection was diagnosed using commercially available enzyme-linked immunosassays and confirmed using Western blot (Genelabs HTLV 2.4, Singapore) according to the manufacturer’s instructions. This was undertaken by the Virus Reference Division of Public Health England. HTLV-1 pVL quantification is routinely performed as part of the standard of care according to published methods [17]. Low HTLV-1 pVL was defined as <1 and high pVL as ≥1 DNA copies/100 peripheral blood mononuclear cells (PBMCs).

Immunophenotyping

Briefly, PBMCs were thawed and washed in 10% fetal calf serum. Viable cells were aliquoted into 1 x 10⁶ cells per well. Fc blocking buffer human immunoglobulin G in phosphate-buffered saline (PBS) containing 0.5% sodium azide (BD Biosciences) was added prior to staining at 4°C with a preoptimized 14-color panel of fluorochrome-conjugated monoclonal antibodies against CD3, CD4, CD8, and CD278, human inducible costimulator (ICOS) CD275, ICOS-ligand (ICOS-L; BD, Biosciences, Oxford, UK), and CD28, CD14, CD19, CD56, CXCR5, CD279 (PD-1), CD45RA, and CCR7 (Biolegend, London, UK). Cells were fixed in Cytofix Buffer (4.2% paraformaldehyde [w/w] in PBS; BD Biosciences) before immediate acquisition using a Fortessa-A flow cytometer (BD Biosciences); 100 000 live lymphocyte events were acquired from each sample. Appropriate controls, including fluorescence minus one (FMO) controls, were included in every experiment.

Gating Strategy

All data were analyzed using FlowJo software, version 10 (TreeStar Ltd, OR). Major lineage subsets were selected for forward and light scatter properties followed by single, live cells.
Doublet discrimination was achieved by plotting forward scatter-width vs forward scatter-area and side scatter-width vs SSC-A [18]. For bulk T-cell analyses, live, single CD3+CD4+ and CD3+CD8+ cells were selected. The CD4:CD8 ratio was derived mathematically from the frequencies of CD3+ live T cells measured using flow cytometry. Live T cells were gated for expression of CD45RA, CCR7, and PD-1. An FMO was used to set the gate for continuous populations. CD4+ and CD8+ T cells that did not express CD28 (CD28null) were selected from bulk CD4+ and CD8+ T cells. Circulating T-follicular helper-like cells (cTFH) were gated on T cells bearing CXCR5 and negative for CD45RA and then gated on CCR7 and PD-1 prior to measurement of the frequency of ICOS⁺ cells. B-cell frequency and activation was measured by selecting live CD19⁺ cells and then measuring ICOS-L expression. CD14⁺CD56⁻ cells were selected from among live, single monocytes, and the frequency of cells expressing ICOS-L was measured [19]. For natural killer (NK) cell selection, CD56⁺CD14⁻ cells were selected from live, single NK cells that had been gated on light and scatter properties.

Statistical Analyses

Graphical presentation and statistical analyses were performed using GraphPad Prism 7.0b (GraphPad Software, San Diego, CA), SPSS v22.0, and Excel for Windows. Data were analyzed using the appropriate nonparametric, 2-tailed test with \( P < .05 \) considered significant. All four groups were compared using the Kruskal-Wallis test with Dunn’s posttest comparison using the AClo group as a control for comparison. Use of this healthy HTLV-1–infected group as a control provided comparison from a genetically similar background of participants with high pVL with or without symptoms. Correlations were analyzed using the Spearman rank correlation coefficient. Visual bisection of continuous data was used to generate categorical subsets; categorical analyses were calculated using the Fisher exact test. Where data were available (n = 4), data from those with tHAM were analyzed in matched paired pre- and post-treatment analyses using the Wilcoxon signed rank test.

RESULTS

Participants

Thirty-eight women with HTLV-1 infection and median (interquartile range [IQR]) age of 59 (52–68) years were studied, with no difference in age between groups (\( P = .69 \)) (Table 1). Nineteen individuals had asymptomatic HTLV-1 infection (n = 9 AClo, n = 10 AC hi), and 19 had HAM. As per the study design, HTLV-1 pVL, measured in HTLV-1 copies per 100 PBMCs (%), was significantly higher in AC hi (median, 11.46%; IQR, 6.60–18.20), uHAM (median, 13.90%; IQR, 7.57–19.36); and tHAM (median, 10.10%; IQR, 6.31–12.24) than in the AClo controls (median, 0.02% IQR, 0.00–0.03; \( P < .0001 \); Figure 1A).

CD4:CD8 Ratio

The frequency of CD3⁺, CD4⁺, and CD8⁺ T cells was similar in the 4 groups studied (Supplementary Figure 1). Table 1 shows the CD4:CD8 ratio for each group. The median CD4:CD8 ratio was higher than normal (≥1) at >2 for all those studied, with no difference irrespective of pVL, disease state, or anti-inflammatory treatment (Figure 1B). Absolute CD4 and CD8 counts in cells per microliter, performed by the clinical pathology laboratory, had similarly high values (Table 1).

Table 1. Demographics and Treatment History of Participants

| Demographic                        | Asymptomatic Carriers With Low pVL, Median (IQR) | Asymptomatic Carriers With High pVL, Median (IQR) | Symptomatic Patients With Untreated HAM, Median (IQR) | Treated Patients With HAM, Median (IQR) |
|------------------------------------|--------------------------------------------------|--------------------------------------------------|------------------------------------------------------|----------------------------------------|
| Gender                             | Female                                           | Female                                           | Female                                               | Female                                 |
| Ethnicity                          | Black African or black Caribbean                 | Black African or black Caribbean                 | Black African or black Caribbean                     | Black African or black Caribbean       |
| Age, y                             | 58 (55, 64)                                     | 58 (55, 64)                                     | 67 (54, 72)                                         | 59 (52, 61)                            |
| Diagnosis                          | Asymptomatic                                     | Asymptomatic                                     | HTLV-1 associated myelopathy                          | HTLV-1 associated myelopathy           |
| Proviral load classification       | Low                                              | High                                             | High                                                 | High                                   |
| HTLV-1 DNA copies/100 peripheral blood mononuclear cells | 0.02 (0.00–0.03) | 11.46 (6.60–18.20) | 13.90 (7.57–19.36) | 10.10 (6.31–12.24) |
| CD4 cells/μL                       | 1052 (750, 1284)                                 | 9700 (670, 1166)                                 | 1117 (868, 1181)                                    | 892 (678, 1048)                        |
| CD8 cells/μL                       | 451 (310, 666)                                   | 330 (220, 430)                                   | 565 (381, 693)                                      | 481 (440, 526)                         |
| CD4:CD8 ratio                      | 1.87 (1.82, 3.17)                                | 2.71 (2.03, 3.22)                                | 2.00 (1.74, 2.23)                                   | 2.01 (1.52, 2.17)                      |
| Treatment                          | Methotrexate                                     | Other (azathioprine or hydroxychloroquine)       | NA                                                   | NA                                     |

Abbreviations: HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; IQR, interquartile range; NA, not applicable; pVL, proviral load.

a Includes n = 4 individuals with pre- and posttreatment samples available for study.

b Data unavailable for 1 individual.
T-Cell Maturational Subsets

T-cell subsets were defined as naïve (CD45RA⁺CCR7⁻), central memory (T_{CM} [CD45RA⁺CCR7⁺]), effector memory (T_{EM} [CD45RA⁻CCR7⁻]), and CD45RA⁺ effector memory (T_{EMRA} [CD45RA⁺CCR7⁻]; Figure 2A). The frequency of naïve CD4⁺ T cells correlated inversely with HTLV-1 pVL (r_s = -0.344, P = .026; Figure 2B). Compared with AC_lo, CD4⁺T_CM were depleted in those with uHAM but not in those on therapy (Figure 2C). There was enrichment of CD4⁺T_EM in all those with high pVL compared with AC_lo, particularly in uHAM (P = .001), with attenuation in those on therapy (Figure 2D). The ratio of CD4⁺T_CM:T_EM was therefore lowest in uHAM (median, 0.58; IQR, 0.48–1.34) compared with AC_lo (median, 3.92; IQR, 2.77–5.76; P < .0001). The ratios of CD4⁺T_CM:T_EM in AC_hi and tHAM were similar (median, 1.63; IQR, 1.28–2.16 and median, 1.47; IQR, 0.94–2.74), and both were slightly similarly lower than AC_lo (P = .045 and P = .045, respectively). Only patients with HAM, whether treated or untreated, had >5% of CD4⁺ T cells that were T_{EMRA} (Figure 2E). High frequencies of mature CD8⁺ T_{EMRA} cells were observed in all individuals with HTLV-1 infection (median, 27.45%; IQR, 18.86%–40.83%). Individuals with ≥30% T_{EMRA} CD8⁺ T cells were observed only in groups with high pVL (Figure 2F).

PD-1⁺ and CD28null CD4⁺ and CD8⁺ T Cells

A high frequency of CD4⁺ T cells expressing PD-1 was observed (median, 24.80%; IQR, 19.39–29.55; Figure 3A). The proportion of those with >20% of CD4⁺ T cells expressing PD-1 was 4/9 (44.4%) in AC_lo, 7/10 (70%) in AC_hi, 12/13 (92.3%) in uHAM, and 8/10 (80.0%) in tHAM. Although the frequency distribution was not different across all 4 groups, those with uHAM were more likely to have >20% of CD4⁺ T cells expressing PD-1 than AC_lo (P = .02; Figure 3B). There were similarly high frequencies of CD8⁺ T cells expressing PD-1 (median, 31.03%; IQR, 21.16, 42.66; Figure 3C). CD28null T cells were gated using FMO controls (Figure 3D). CD4⁺CD28null T cells were relatively infrequent, with wide variance (median, 2.60%; IQR, 1.01, 6.48). There was a stepwise increase in the frequency of CD4⁺CD28null T cells across all 4 groups, with a trend for greater frequency in those with tHAM compared with AC_lo (P = .082; Figure 3E). The frequency of CD8⁺CD28null T cells was high in all individuals (median, 36.98%; IQR, 26.69, 59.95; Figure 3F). The frequency of CD4⁺ and CD8⁺ T-cell maturational subsets and the frequency of PD-1 positive and CD28null T cells showed no consistent direction of change in pre- and on-treatment analyses in 4 individuals where samples were available (Supplementary Table 1).

Circulating T-Follicular Helper Cells

A median (IQR) of 9.01% (6.67–11.69) CD3⁺CD4⁺CXCR5⁺CD45RA⁻ T cells were observed in the circulation of all individuals (Figure 4A). These cells were T_{CM} (Supplementary Figure 2), in accordance with previous descriptions [20, 21]. There was a trend toward a negative correlation of cTFH frequency and HTLV-1 pVL r_s = -0.303 (P = .051; Figure 4B). A median (IQR) of 12.38% (8.34–16.68) of these cells expressed the activation marker ICOS (Figure 4C). The absolute frequency and proportion of activated CD19⁺ B-lymphocytes was similar across all 4 groups studied (Supplementary Figure 3).
Figure 2. Frequency of maturational CD4+ and CD8+ T-cell subsets and relationship with HTLV-1 pVL in individuals with asymptomatic and symptomatic HTLV-1 infection. **A**, The gating strategy and FMO controls to identify CD4+ and CD8+ maturational T-cell subsets (upper left panel). CD4+ and CD8+ T cells were selected from single, live CD3+ T cells. T-cell subsets were identified based on expression of CD45RA and CCR7 on both CD4+ and CD8+ T cells using FMO controls. **B**, An inverse correlation of log_{10} HTLV-1 pVL DNA copies/100 PBMCs with the frequency of CD4+ T cells with a naive phenotype (CD3+CD4+CCR7+CD45RA+) across all individuals studied (upper right panel). The Spearman Rho correlation coefficient and associated P value are depicted. **C**, The comparison of the frequency of CD4+ T_{CM} (middle left panel). **D**, The comparison of the frequency of CD4+ T_{EM} (middle right panel). **E**, The comparison of the frequency of CD4+ T_{EMRA}, dotted line depicts 5% threshold (lower left panel). **F**, The comparison of the frequency of CD8+ T_{EMRA}, dotted line depicts 30% threshold (lower right panel). All graphs show, from left to right, AClo, AChi, uHAM, and tHAM. Median values are depicted. The P value below each graph was calculated using the Kruskal-Wallis test. Multiple group comparisons were calculated using the AClo group as the control group with Dunn's multiple comparisons test. Significant differences between AClo and the other groups studied are indicated above the graph; *P < .05, **P < .01, ***P < .001, ****P < .0001. Abbreviations: AClo, asymptomatic HTLV-1 infection with low pVL; AChi, asymptomatic HTLV-1 infection with high pVL; FMO, fluorescence minus one; HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; PBMC, peripheral blood mononuclear cell; pVL, proviral load; T_{CM}, central memory; T_{EM}, effector memory; T_{EMRA}, CD45RA+ effector memory; T_{NA}, naive; tHAM, treated HAM; uHAM, untreated HAM.
The gating strategy to identify CD28null live CD3+CD4+ and CD3+CD8+ T cells (lower left panel). *** Abbreviations: AChi, asymptomatic HTLV-1 infection with high pVL; AClo, asymptomatic HTLV-1 infection with low pVL; FMO, fluorescence minus P<.001, **** P<.0001. The comparison between the proportion of individuals with >20% of CD4+ T cells expressing PD-1, depicted by the dotted line, in the AClo vs the uHAM group is indicated above the graph, P value calculated using Fisher exact test; * P<.05, ** P<.01, *** P<.001. C. The comparison of the frequency of CD8+ T-cells that expressed PD-1 (upper right panel). D. The gating strategy to identify CD28null live CD3+CD4+ and CD3+CD8+ T cells (lower left panel). E. The comparison of frequency of CD4+CD28null T cells (lower middle panel). F. The comparison of frequency of CD8+CD28null T cells (lower right panel). All graphs show, from left to right, AClo, AChi, uHAM, and tHAM. The multiple group comparison with the AClo control group was calculated using the Kruskal-Wallis test with Dunn’s multiple test comparison. The effect of HTLV-1 infection previously reported in CD14++CD16- monocytes. Several of these changes, particularly among T-cell maturational subsets, were less evident in those taking methotrexate, suggesting partial amelioration could be possible with treatment; this is a response that was not predicated on reducing pVL.

Alterations in both innate and adaptive immunity were observed in patients with HAM. CD14+ monocytes were less frequent and more likely to occur as doublets, consistent with an effect of HTLV-1 infection previously reported in CD14++CD16 classic monocytes [22]. The exclusion of monocytes as doublets has two potential consequences—first, that the true monocyte count will be underestimated and, second, that these monocytes might be more activated, leading to aggregation. Restricting

**DISCUSSION**

We report a unique cell signature in the circulation of African and Afro-Caribbean women in the sixth to seventh decade diagnosed with HAM, a debilitating progressive inflammatory condition that occurs in 1–4% of those with HTLV-1 infection. Untreated HAM was characterized by expansion of mature CD4+ and CD8+ T cells, high proportions of T cells with a senescent or exhausted phenotype, and aggregation of monocytes. Several of these changes, particularly among T-cell maturational subsets, were less evident in those taking methotrexate, suggesting partial amelioration could be possible with treatment; this is a response that was not predicated on reducing pVL.

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**CD14+ Monocytes**

Monocytes were gated on light scatter properties (Figure 5A) and were less frequent in HAM (P = .043; Figure 5B). Single monocytes were less frequent and monocytes were more likely to be excluded through doublet discrimination in both uHAM and tHAM compared with AClo (P < .001; Figure 5C). This was not due to a greater frequency of cells excluded through live–dead discrimination, which were slightly more frequent in AClo (P = .027; Figure 5D). Compared with AClo, CD14+CD56- monocytes were less frequent among live, single monocytes in tHAM compared with AClo (P = .033), and there was a trend for higher frequency of expression of ICOS-L (Figure 5E and F). No significant differences in absolute numbers of NK cells were observed across all 4 groups (Supplementary Figure 4).

**Figure 3.** Frequency of CD4+ and CD8+ PD-1+ and CD28null T cells in individuals with asymptomatic and symptomatic HTLV-1 infection. A. The gating strategy and FMO control used to identify and select live CD3+CD4+ and CD3+CD8+ T cells expressing PD-1 (upper left panel). B. The frequency of CD4+ PD-1+ (upper middle panel). The comparison between the proportion of individuals with >20% of CD4+ T cells expressing PD-1, depicted by the dotted line, in the AClo vs the uHAM group is indicated above the graph, P value calculated using Fisher exact test; * P<.05, ** P<.01, *** P<.001. C. The comparison of frequency of CD8+ T-cells that expressed PD-1 (upper right panel). D. The gating strategy to identify CD28null live CD3+CD4+ and CD3+CD8+ T cells (lower left panel). E. The comparison of frequency of CD4+CD28null T cells (lower middle panel). F. The comparison of frequency of CD8+CD28null T cells (lower right panel). All graphs show, from left to right, AClo, AChi, uHAM, and tHAM. The multiple group comparison with the AClo control group was calculated using the Kruskal-Wallis test with Dunn’s multiple test comparison. The P value is indicated below the graph; * P<.05, ** P<.01, *** P<.001, **** P<.0001. Abbreviations: AClo, asymptomatic HTLV-1 infection with low pVL; AChi, asymptomatic HTLV-1 infection with high pVL; FMO, fluorescence minus one; HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; pVL, proviral load; SSC-A, side scatter-area; tHAM, treated HAM; uHAM, untreated HAM.

**Figure 5.** Frequency of CD4+ and CD8+ PD-1+ and CD28null T cells in individuals with asymptomatic and symptomatic HTLV-1 infection. A. The gating strategy and FMO control used to identify and select live CD3+CD4+ and CD3+CD8+ T cells expressing PD-1 (upper left panel). B. The frequency of CD4+ PD-1+ (upper middle panel). The comparison between the proportion of individuals with >20% of CD4+ T cells expressing PD-1, depicted by the dotted line, in the AClo vs the uHAM group is indicated above the graph, P value calculated using Fisher exact test; * P<.05, ** P<.01, *** P<.001. C. The comparison of frequency of CD8+ T-cells that expressed PD-1 (upper right panel). D. The gating strategy to identify CD28null live CD3+CD4+ and CD3+CD8+ T cells (lower left panel). E. The comparison of frequency of CD4+CD28null T cells (lower middle panel). F. The comparison of frequency of CD8+CD28null T cells (lower right panel). All graphs show, from left to right, AClo, AChi, uHAM, and tHAM. The multiple group comparison with the AClo control group was calculated using the Kruskal-Wallis test with Dunn’s multiple test comparison. The P value is indicated below the graph; * P<.05, ** P<.01, *** P<.001, **** P<.0001. Abbreviations: AClo, asymptomatic HTLV-1 infection with low pVL; AChi, asymptomatic HTLV-1 infection with high pVL; FMO, fluorescence minus one; HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; pVL, proviral load; SSC-A, side scatter-area; tHAM, treated HAM; uHAM, untreated HAM.

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our observations to single monocytes, CD14+CD56- in patients with HAM were less frequent compared with asymptomatic carriers, despite treatment. Our observations suggest a role for monocytes in the inflammatory state in HAM that needs further clarification.

CD4:CD8 ratio inflation occurred in all HTLV-1–infected individuals, including asymptomatic carriers, indicating T-cell dysregulation despite control of pVL. Inversion (<1) of the CD4:CD8 ratio and accumulation of CD28null cells can be driven by CMV or HIV-1 infection and by age-related changes in T-cell homeostasis [23, 24]. In HTLV-1 infection, integration of the provirus is associated with oligoclonal proliferation of CD4+ T cells [25]. Inversion of the CD4:CD8 ratio has been posited as a clinical biomarker of chronic immune activation in HIV-1 infection [26]. Given the high prevalence of HTLV-1 in specific global communities, interpretation of such a biomarker should be in the context of local viral epidemiology.

Unlike our findings related to the CD4:CD8 ratio, the altered maturational profile of T-cell subsets in HAM was associated with stage of disease and presence of symptoms. HTLV-1–specific cytokine-secreting T cells occur at a median frequency of 0.9% of CD4+ T cells in HAM [27]. Consistent with previous findings in CD8+ T cells, expansion of mature CD4+ and CD8+ T cells was not attributable solely to expansion of antigen-specific cells [28]. Generalized expansion of this subset would increase the niche for HTLV-1, which preferentially infects effector memory CD4+ T cells [29]. Reduced frequency of naive T cells correlated inversely with pVL, and CD4+TEM were less frequent and TEMRA expanded in uHAM compared with asymptomatic carriers. More specifically, the ratio of circulating CD4+TEM to CD4+TEM was slightly higher in asymptomatic carriers than in those with high pVL or treated HAM and was lowest in untreated HAM. TEMRA frequency increases with age and as a consequence of antigen experience [30–32]. Many of those with high pVL had frequencies of CD8+ TEMRA greater than 30% and CD4+ TEMRA greater than 0.9%.

Figure 4. Frequency of cTFH cells and association with HTLV pVL. A, The gating strategy to identify cTFH T cells, CD3+CD4+CXCR5+CD45RA-, of these a CCR7+PD-1- subset was identified and selected (upper panel). To measure activation of this subset, cells expressing ICOS were selected. B, The inverse correlation of the log10 of HTLV-1 pVL DNA copies/100 PBMCs with the frequency of cTFH cells across all individuals studied (lower left panel). The Spearman Rho correlation coefficient and associated P value are depicted. C, The comparison of frequency of these cells that expressed ICOS in individuals with, from left to right, AClo, AChi, uHAM, and tHAM (lower right panel). In all panels, the multiple group comparison with the AClo control group was calculated using the Kruskal-Wallis test with Dunn’s multiple test comparison; the P value is indicated below the graph. Abbreviations: AClo, asymptomatic HTLV-1 infection with low pVL; AChi, asymptomatic HTLV-1 infection with high pVL; HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; ICOS, human inducible costimulator; PBMC, peripheral blood mononuclear cell; cTFH, T-follicular helper-like cells; FMO, fluorescence minus one; pVL, proviral load; tHAM, treated HAM; uHAM, untreated HAM.
Figure 5. Aggregation and activation of monocytes. A, The gating strategy and FMO control used to identify and select monocyte subsets (upper left panel). Live singlet monocytes were gated using a dead cell discriminator, and CD14+CD56- monocytes were selected. ICOS-L expression was measured using an FMO control. All panels show comparison, from left to right AClo with AClo, uHAM, and tHAM. B, The comparison of the frequency of monocytes gated on light scatter properties (upper right panel). C, The frequency of single monocytes gated by doublet exclusion (middle left panel). D, The frequency of live monocytes (middle right panel). E, The frequency of CD14+CD56- in live monocytes (lower left panel). F, The frequency of these cells expressing ICOS-L (lower right panel). The multiple group comparison with the AClo control group was calculated using the Kruskal-Wallis test with Dunn's multiple comparisons test; the P value is indicated below the graph. Significant differences between AClo and the other groups studied are indicated above the graph; *P < .05, **P < .01, ***P < .001. Abbreviations: AClo, asymptomatic HTLV-1 infection with low pVL; AClo, asymptomatic HTLV-1 infection with high pVL; FMO, fluorescence minus one; FSC, forward scatter; HAM, human T-lymphotropic virus type 1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; ICOS-L, human inducible costimulator-ligand; PBMC, peripheral blood mononuclear cell; pVL, proviral load; SSC-A, side scatter-area; uHAM, untreated HAM; tHAM, treated HAM.
5%, and there was a correlation with disease state, with this most commonly observed in HAM, then in AC\textsuperscript{th}, and then AC\textsuperscript{th}.

T-cell phenotypes that indicate exhaustion and senescence reflected HTLV pathogen burden. Frequencies of CD28\textsuperscript{null} T cells and PD-1\textsuperscript{+} T cells were higher in those with high pVL and highest in those with HAM. Loss of CD28 expression is associated with cellular senescence due to antigen experience and with maladaptation in aging [33]. PD-1 is part of a checkpoint pathway associated with T-cell exhaustion in infectious disease and is expressed on HTLV-1–specific CD8\textsuperscript{+} T cells in ATLL [34–36]. Taken together, our observations indicate a widespread proinflammatory cellular phenotype in untreated infection where there has been poor viral control that is not restricted to antigen-specific cells. Given that this phenotype was observed, albeit less markedly, in some asymptomatic individuals with high pVL, immunophenotyping could assist with clinical risk stratification prior to the onset of symptoms, with the aim of guiding and monitoring treatment. Our data indicate the ratio of circulating CD4\textsuperscript{+} T\textsubscript{CM}T\textsubscript{EM} to be the potential biomarker most suitable for this approach. This biomarker could be utilized in treatment decisions of HAM, a disease that is currently managed syndromically. Furthermore, our findings could underpin the development of more detailed investigations to predictively manage presymptomatic HTLV-1 infection.

Our study was limited by its cross-sectional design. Prospective studies are needed to tease out the temporal relationship between poor viral control and symptomatic inflammatory disease and the impact of anti-inflammatory medication. For example, patients with tHAM may represent a group with more advanced disease than those with uHAM, which might account for why a treatment effect was not always observed. The possible inverse association between the frequency of cTFH and HTLV-1 pVL is an observation worthy of further study to determine whether higher frequencies might contribute to immune control of HTLV-1 infection.

In summary, we report widespread immunopathology in the circulation of individuals with HTLV-1 infection, with poor viral control affecting both innate and adaptive immunity and with a cell profile indicative of activation, maturation, and senescence in patients with untreated HAM. These changes are evident prior to the onset of symptoms, particularly in T-cell maturational subsets, and may be amenable to anti-inflammatory medication.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Acknowledgment. The authors thank the staff and patients of the National Centre for Human Retrovirology.

Disclaimer. Adults who attended the National Centre for Human Retrovirology (Imperial College Healthcare National Health Service Trust, London, United Kingdom [UK]) gave written informed consent to donate blood for viable cryopreservation of peripheral blood mononuclear cells. All participants were aged 218 years. All research was conducted under the auspices of the Communicable Diseases Group Tissue Bank, approved by the UK Research Ethics Service (09/H0606/106, 15/SC/0089).

Financial support. This work was supported by the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre (BRC).

Potential conflicts of interest. A. R. reports salary support from grants from the Wellcome Trust (Senior Investigator grant to Prof Charles Bangham) and from the NIHR BRC during the conduct of the study and nonfinancial support from Kyowa Kirin outside the submitted work. K. P. M. reports grants and salary from NIHR during the conduct of the study and grants from Gilead Sciences Europe Ltd outside the submitted work. K. P. is an NIHR clinical lecturer. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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