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CD4 T cell regeneration in HIV-1 elite controllers

Yue YANG^{1,2,*}, Maha AL-MOZAINI^{1,2,3,*}, Maria J. BUZON^{1,2}, Jill BEAMON¹, Sara FERRANDO-MARTINEZ^{4,5}, Ezequiel RUIZ-MATEOS⁵, Eric S. ROSENBERG¹, Florencia PEREYRA², Xu G. YU², and Mathias LICHTERFELD¹

¹Infectious Disease Division, Massachusetts General Hospital, Boston, MA

²Ragon Institute of MGH, MIT and Harvard, Boston, MA

³King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

⁴Laboratory of Molecular Immuno-Biology, Gregorio Marañón Hospital, Madrid, Spain

⁵Laboratory of Immunovirology, Virgen del Rocío University Hospital, Institute of Biomedicine of Seville (IBiS) Seville, Spain

Abstract

Background—Elite controllers spontaneously control HIV-1 replication, which in many cases is associated with preservation of normal CD4 T cell counts. However, a subset of elite controllers has progressive CD4 T cell losses despite undetectable viral loads, for reasons that remain undefined. Here, we assessed mechanisms of CD4 T cell homeostasis in elite controllers with progressive vs. non-progressive HIV-1 disease courses.

Methods—Flow cytometry assays were used to determine the proliferation, activation and apoptosis levels of naïve T cells in elite controllers with high or low CD4 T cell counts, and reference cohorts of HIV-1 negative and HAART-treated persons. Thymic output was measured by sjTREC/βTREC ratios, and the frequency of circulating recent thymic emigrants was flow-cytometrically determined by surface expression of protein tyrosine kinase 7 (PTK7).

Results—Proportions of naïve T cells in elite controllers were severely reduced and closely resemble those of HIV-1 patients with progressive disease. Despite reductions of naïve T cells, most elite controllers were able to maintain normal total CD4 T-cell counts by preservation of uncompromised thymic function in conjunction with extrathymic processes that led to elevated levels of circulating recent thymic emigrants. In contrast, elite controllers with low CD4 T-cell counts had reduced thymic output that mirrored thymic dysfunction during untreated progressive HIV-1 infection.

Conclusion—These results indicate that both thymic and extrathymic mechanisms contribute to CD4 T cell maintenance in elite controllers and support the idea that CD4 T-cell homeostasis and control of viral replication are distinct, but frequently coinciding processes

Corresponding author Mathias Lichterfeld, M. D., Ph. D., Infectious Disease Division, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, Phone: 617-726-7516, Fax: 617-726-3351, mlichterfeld@partners.org.

*These authors contributed equally.

Author contribution

Study concept, data analysis and writing of manuscript: XGY, ML

Data generation and data analysis: YY, MAM, MB

Patient recruitment, critical review of manuscript: ESR, FP

Protocol development for TREC level analysis: SFM, ERM

Technical assistance with experiments: JB

Keywords

HIV-1; elite controllers; thymic function; naïve T cells

Introduction

A hallmark of HIV-1 infection is the progressive loss of CD4 T cells. This disruption of CD4 T cell homeostasis most prominently affects naïve T cells [1, 2], a group of long-lived, antigen-inexperienced T cells that have major roles for maintaining T cell diversity, replenishing effector/memory T cell populations and protecting the integrity of the total T cell pool [3]. Under physiologic conditions, a stable pool of naïve T cells is maintained by the thymus, which releases immature lymphocytes termed “recent thymic emigrants” (RTE) that serve as peripheral precursor cells for regenerating mature naïve T cells [4, 5]. Once thymic function is deteriorating, naïve T cell homeostasis can be mediated by peripheral homeostatic proliferation during which naïve T cells retain their phenotype and functional characteristics. During progressive HIV-1 infection, these mechanisms of naïve T cell homeostasis seem to be markedly disturbed. Indeed, thymic output of new naïve T cells, determined by measurements of sjTREC levels [6] or ratios of sjTREC/βTREC levels [7, 8] in the peripheral blood, is significantly inhibited during progressive HIV-1 infection.

Elite controllers (EC) represent a small proportion of HIV-1 infected persons who are able to maintain undetectable levels of HIV-1 replication without antiretroviral therapy [9–11]. In the majority of these patients, this spontaneous control of HIV-1 is associated with normal CD4 T cells, however, a small proportion of elite controllers develops declining number of CD4 T cells despite undetectable levels of HIV-1 viremia [12–15]. Here, we comprehensively assessed naïve T cell subsets, thymic output and frequencies of recent thymic emigrants in a cohort of EC with normal or low CD4 T cell counts.

Materials and Methods

Patients

Study participants gave written informed consent to participate in accordance with the Declaration of Helsinki. Blood samples were drawn in ACD tubes, and PBMC were isolated by using Ficoll density gradient centrifugation within six hours of the blood draw.

Flow cytometric studies

PBMCs were stained with blue viability dye, followed by surface staining with antibodies directed against CD3, CD4, CD8, CD45RA, CCR7, CD38, HLA-DR or PTK7 (Becton Dickinson) according to standard procedures [16]. Subsequently, cells were fixed and permeabilized using the FACSLysing/Permeabilizing buffers, followed by intracellular stainings with Ki-67 antibodies. Data were acquired on a LSR-II system and analyzed using Flowjo software.

TREC level analysis

For TREC quantification, semiquantitative real-time PCRs were carried out from cell lysates as previously described [8, 17]. Briefly, first-round PCRs were performed for sjTREC or the six DβJβ-TRECs using outer primer pairs (DTF6/DTR61 for sjTREC and T3A-T3F/A05AS for DβJβ-TRECs) [17]. Second round PCRs were performed using the Light-Cycler instrument and described primers/probes. All quantifications of sjTRECs and DβJβ-TRECs were run in duplicates. sjTREC and DβJβ-TREC levels were normalized to DNA levels of the house-keeping gene β-globin. A reference sample from an HIV-1 negative study subject

was run in each experiment to compensate for plate-to-plate variation; TREC levels of study samples were expressed as fold-changes to the reference sample.

Statistics

Data are summarized as median and range or using box-and-whisker plots, reflecting the minimum, maximum and the 25th, 50th and 75th percentile. Pearson's correlation coefficient was calculated to analyze correlations. Differences between study cohorts were tested for statistical significance by one-way ANOVA, followed by post-hoc analysis using the Tukey multiple comparison test. A p-value <0.05 was considered significant.

Results and discussion

To investigate mechanisms of T cell homeostasis in EC, we initially determined the relative and absolute numbers of naïve T cells in a cohort of EC with normal or declining CD4 T cell counts, and in reference populations of HAART-treated subjects, untreated HIV-1 progressors and HIV-1 negative persons. Demographic and clinical characteristics of the study groups are summarized in supplemental Table 1. Flow cytometric analysis demonstrated that the relative proportion of naïve CD4 and CD8 T cells, as defined by co-expression of CD45RA and CCR7, was severely reduced in both groups of EC and closely resembled the proportion of naïve T cells in patients with untreated progressive HIV-1 infection, while being significantly lower than in HIV-1 negative persons and HAART-treated individuals (Figure 1A). In contrast, relative proportions of CCR7-CD45RA-effector memory T cells, and to a lesser extent CCR7+ CD45RA-central memory T cells and CCR7-CD45RA+ terminally differentiated T cells, were higher in EC and progressors in comparison to HIV-1 negative persons (supplemental Figure 1). This relative redistribution of naïve and effector/memory T cells in EC and progressors was associated with reduced absolute naïve CD4 and CD8 T cell counts and corresponding increases in absolute counts of effector and memory T cells (supplemental Figure 1). Overall, this indicates that the characteristic disruption of T cell subset composition that is typically observed during chronic progressive HIV-1 infection occurs in a similar fashion in EC, despite their ability to spontaneously control viral replication.

The size of the naïve T cell pool is determined by the influx of new naïve T cells from the thymus, by rates of homeostatic naïve T cell proliferation and by losses of naïve cells to activation-induced death or conversion into the memory or effector cell pool. Assessments of immune activation markers demonstrated that surface expression of HLA-DR and CD38 on naïve CD4 and CD8 T cells were slightly higher in EC than in HIV-1 negative persons, while no substantial differences were found between EC with normal or low CD4 T cell counts (Figure 1B). Proportions of naïve CD8 T cells, but not CD4 T cells, were inversely correlated to corresponding levels of HLA-DR and CD38 surface expression, suggesting that naïve CD8 T cell losses are at least in part related to immune activation (Figure 1C). We next assessed proliferative activities of naïve CD4 and CD8 T cells in our study cohorts by analyzing expression of the intracellular proliferation-associated antigen Ki67. We observed that proportions of Ki67+ naïve CD4 T cells were elevated in all HIV-1 infected patients compared to HIV-1 negative persons; this increase in naïve CD4 T cell proliferation was highest in EC (Figure 1D). Proliferation of naïve CD8 T cells in EC was also elevated, although not to the same extent as in progressors. Notably, no significant differences were observed between the proliferative activities of T cell subsets from EC with normal or low CD4 T cell counts. Interestingly, we observed that levels of absolute and relative naïve CD4 and CD8 T cells were inversely correlated with proportions of naïve Ki67+ CD4 and CD8 T cells (Figure 1E), suggesting that increased proliferation of naïve T cells in controllers and progressors is associated with naïve T cell losses and therefore likely to reflect accelerated transitional proliferation of naïve T cells into more differentiated lymphocytes. This bias

towards transitional proliferation is likely to deprive naïve T cells of their ability to maintain adequate naïve T cell counts through homeostatic proliferation, and may leave naïve T cell replenishment entirely up to thymic function.

To investigate thymic output in our cohorts, we performed combined quantifications of sjTREC and β TREC levels. The ratio of sjTREC/ β TREC reflects proliferation of intrathymic precursor T cells, which is directly proportional to thymus output [18] and unaffected by peripheral naïve T cell proliferation [8]. As shown in Figure 2A, sjTREC/ β TREC ratios were similar between EC with normal CD4 T cells counts and age-matched HIV-1 negative persons, while being significantly smaller in age-matched HIV-1 progressors or EC with low CD4 T cell counts. sjTREC/ β TREC ratios were positively associated with absolute levels of naïve and total CD4 and CD8 T cells, consistent with the important role of thymic output for T cell regeneration (Figure 2B–C). Overall, this suggests that uncompromised thymic output represents the critical feature that distinguishes both groups of EC, and points towards intrinsic thymic dysfunction as a predominant factor contributing to HIV-1 disease progression, irrespectively of the degree of viral replication.

Despite similar ratios of sjTREC/ β TREC levels, both β TREC and sjTREC levels were elevated in non-progressive EC as opposed to HIV-1 negative persons (Figure 2A); this is consistent with specific peripheral, thymus-independent mechanisms that can increase the frequency of recent thymic emigrants (RTE) and have previously been described in persons with primary HIV-1 infection [8]. To investigate the frequency of RTEs in EC, we assessed the numbers of CD4 T cells with surface expression of protein tyrosine kinase 7 (PTK7), an optimized biomarker for peripheral RTEs [4]. As shown in Figure 2D, we observed that relative proportions of PTK7+ CD4 T cells in non-progressive EC exceeded corresponding levels in all other patient populations. Notably, absolute counts of PTK7+ RTEs were also increased in non-progressive EC, indicating an enrichment of the peripheral blood with RTEs in these patients, independently of the contraction of the naïve T cell pool. Relative proportions of PTK7+ naïve CD4 T cells in EC with progressive CD4 cell losses were slightly higher than in reference cell populations, while absolute numbers of PTK7+ naïve CD4 T cells were not different between these patients and reference cohorts (Figure 2D). Relative and absolute levels of PTK7+ naïve CD4 T cells were positively correlated to total and naïve CD4 T cell counts (Figure 2E); these relationships were most pronounced in EC with normal CD4 T cell counts. Overall, these data suggest that in contrast to progressors and EC with low CD4 T cell counts, loss of naïve CD4 T cell in non-progressive EC is compensated by uncompromised thymic function and by extrathymic mechanisms that contribute to an enrichment of PTK7+ RTEs in the peripheral circulation. The precise mechanisms underlying the higher frequency of PTK7+ naïve CD4 T cells in EC remain unclear at present.

An important question is whether thymic dysfunction in EC with low CD4 T cell counts is related to the degree of residual viral replication, which is typically detectable in almost all EC using ultra-sensitive assays and tends to inversely correlate with total CD4 T cells [19]. Future studies will be necessary to analyze the role of residual HIV-1 viremia for naïve CD4 T cell losses and thymic dysfunction in EC, and it will be important to determine in randomized clinical trials whether reduced thymic output and progressive CD4 T cell losses in EC can be reversed by antiretroviral therapy.

This study has several limitations: First, elite controllers with low CD4 T cell counts are extremely rare, and the number of subjects with such characteristics in our study was therefore relatively low. Moreover, we cannot exclude that unavoidable differences in clinical or demographical characteristics between the study cohorts may have confounded our data to some degree. Finally, due to reduced sample availabilities, TREC levels were

assessed in total PBMC instead of sorted CD4 and CD8 T cells; however, since TREC levels are formed before the initiation of CD4/CD8 T cell differentiation steps, this is unlikely to cause experimental artifacts [8]

In summary, this work demonstrates that loss of naïve CD4 T cell counts is a universal feature of elite controllers and occurs despite their ability to maintain undetectable viral loads. In the majority of EC, this loss of naïve T cells is balanced by thymus-dependent and peripheral compensatory mechanisms, which seem to be impaired in the small number of EC with progressive CD4 T cell losses. Whether and how impaired CD4 T cell regeneration in EC with progressive disease can be improved by antiretroviral therapy needs to be investigated in future work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Disruption of naïve T cell homeostasis in elite controllers

(A): Box and Whisker plots reflecting the relative proportions of naïve CD4 and CD8 T cell within the indicated study cohort. (B) Surface expression of CD38 and HLA-DR on naïve CD4 and CD8 T cells from indicated study cohort. (C): Correlations between proportions of naïve CD4 and CD8 T cells and corresponding expression of T cell activation markers. (D): Proportions of Ki67+ naïve CD4 and CD8 T cells from indicated study subject. (E): Correlations between proportion of naïve CD4 and CD8 T cells and corresponding expression levels of Ki67. R and p values indicated in bold print refer to the entire study population, R and p in regular font reflect data from elite controllers only.

Figure 2. Thymus-dependent and thymus-independent T cell regeneration in elite controllers (A) Box and Whisker plots indicating relative levels of β TREC, sjTREC and sjTREC/ β TREC ratios in indicated study groups. (B-C): Correlations between sjTREC/ β TREC ratios and absolute and naïve CD4 (B) and CD8 (C) T cell counts. Pearson's correlation coefficients are indicated. (D): Box and Whisker plots summarizing the relative and absolute counts of PTK7+ CD4 T cells. Significance was tested using Mann Whitney U tests. (E): Correlations between relative and absolute naïve PTK7+ CD4 T cells and corresponding total and naïve CD4 T cell counts. R and p values indicated in bold print refer to the entire study population; R and p in regular font reflect data from elite controllers only.