Short Communication: Low Immune Activation Is Associated with Higher Frequencies of Central Memory T Cell Subset in a Cohort of Indian Long-Term Nonprogressors

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Abstract

Persistent immune activation in human immunodeficiency virus (HIV) infection is responsible for alterations in immune system such as activation, apoptosis, and reduced frequencies. Reduced immune activation is known to be associated with virus control. Limited information is available on the influence of pan-immune activation on memory responses. Hence, we compared the T cell activation and memory profile in HIV-infected individuals exhibiting disease control such as long-term nonprogressors (LTNPs) and progressors. The activated CD4⁺ and CD8⁺ T cells were significantly lower and the CD4⁺ and CD8⁺ central memory T cell phenotypes were significantly higher in the LTNPs compared to the progressors. In addition, we observed significant inverse association between the T cell activation and frequencies of central memory T cells. Our findings indicate that patients with absence of disease progression have preserved central memory T cell population associated with lesser immune activation.

Keywords: long-term nonprogressors, T cells, immune activation

HUMAN IMMUNODEFICIENCY VIRUS (HIV) induces persistent immune activation resulting in pan-activation of the immune system. This is characterized by activatory molecule expression on immune cells, including T and B cells, natural killer cells, and secretion of proinflammatory cytokines.^{1,2} The persistent immune activation in HIV-infected individuals is correlated with faster disease progression.^{3–5} However, limited information is available on its influence on the memory T cell compartment. One study has shown that immune activation alters the CD8 memory cell differentiation.⁶ Since long-term maintenance of memory is important for the generation of faster and efficient recall T cell responses and impaired CD4 central memory T cell response (T_{CM}) was found to be associated with HIV disease progression,^{7,8} it is important to understand the influence of immune activation on memory T cell compartment.

We used HIV-infected long-term nonprogressors (LTNPs) as a model for control over HIV disease progression. LTNPs represent a group of asymptomatic, HIV-infected individuals

with stable CD4 count of ≥ 500 cells/mm³ for at least seven or more years of documented HIV infection in the absence of antiretroviral treatment (ART).⁹ LTNPs are shown to have a variable degree of immune activation in different studies.^{10–12} It was reported that despite a high viral load, the lower immune activation and preserved immune responses result in long-term asymptomatic disease stage in patients of Amsterdam cohort,¹⁰ while chronic immune activation has been shown to be associated with progressive HIV.^{13,14} Furthermore, the persistent immune activation is shown to drive the T cell differentiation resulting in replicative senescence.¹⁵ Hence, we hypothesize that lower immune activation of T cells in LTNPs may ultimately result in better preservation of memory T cell population compared to the chronic progressors having a higher percentage of activated T cells. To test this hypothesis, we assessed the frequencies of activated T cells expressing HLA-DR and CD38 as a marker of immune activation, as well as the frequencies of central (T_{CM}) and effector memory (T_{EM}) T cells in LTNPs.

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Thirty-one (11M/20F) LTNP were recruited from the LTNP cohort who are under regular follow-up at National AIDS Research Institute along with 21 (11M/10F) chronic progressors (ART-naïve HIV-infected patients with CD4 count <500 cells/mm³) attending the outpatient clinics of the institute. The median age was 38 years in both LTNP (range 30–75 years) and chronic progressors (range 22–48 years). The median seropositivity period of LTNP was 11 years (range 8–19 years). At the time of enrolment in this study, the median CD4 count was 630 and 300 cells/mm³ in LTNP and progressors, respectively. The median plasma viral load (pVL) was significantly lower (5,321 copies/ml; range 40–770,523) in LTNP than that in progressors (28,345 copies/ml; range 1,065–1,456,931) ($p = .012$). The participants were not tested for *M. tuberculosis* infection; however, none of them presented with any signs or symptoms of tuberculosis disease. In addition, five HIV-seronegative healthy individuals (healthy controls; range 28–33 years; 3M/2F) with self-reported history of no fever in last 2 weeks and no major systemic disease were also enrolled for comparison. The study was approved by the Institutional Ethics Committee and prior written informed consent was obtained from all the participants.

The frozen peripheral blood mononuclear cells (PBMCs) from the study participants were revived as described previously¹⁶ and viability of the cells was assessed using trypan blue dye exclusion method. The samples showing $>90\%$ viability and $>3 \times 10^6$ cell count were considered for further analysis. 1×10^6 PBMCs each were used to characterize activation status of T cells and determine frequencies of memory CD4⁺ and CD8⁺ T cells using multicolor flow cytometry. The following antibodies were used: anti-CD3-PE-Texas-Red-A (Invitrogen), anti-CD4-APC-Cy7, anti-CD8-PE-Cy7, anti-CD38-FITC, anti-HLA-DR-PerCP-Cy5.5, anti-CD45RA-APC, CD67L-PE, and CCR7-PE-Cy7 (all from BD Pharmingen). PBMCs were incubated with specific antibodies for 30 min in dark at RT, followed by washing with a wash buffer (phosphate-buffered saline +0.5% fetal calf serum). The cells were fixed in 3% formaldehyde, acquired, and analyzed on FACSaria (Becton Dickinson) using FlowJo software. A total of 50,000 gated events of lymphocytes were acquired. Appropriate cell and isotype controls were used for determination of CD38/HLA-DR/HLA-DR and CD38 coexpressing CD4⁺ and CD8⁺ T cells and to determine the frequencies of CD4 and CD8 central memory (CCR7⁺/CD62L⁺ and CD45RA⁻) and effector memory (CCR7⁺/CD62L⁻ and CD45RA⁻) population. The gating strategy to identify the different populations is shown in Supplementary Figure S1 (Supplementary Data are available online at www.liebertpub.com/aid). The GraphPad Prism version 4.0 software was used for statistical analysis and presentation. Kruskal–Wallis test with *post hoc* analysis was used to compare the differences in the levels of T cell activation markers (Fig. 1A). Nonparametric Mann–Whitney *U* test was used to compare the differences in the frequencies of memory T cell phenotype between LTNP and progressors, (Fig. 1F). Spearman test was used for correlation analyses. A *p*-value of $<.05$ was considered significant.

The progressors had a significantly higher expression of activation markers, HLA-DR, CD38, and HLA-DR+CD38 on CD4 and CD8 T cells compared to the healthy controls ($p < .05$; Fig. 1A). Progressors also had a higher expression of

HLA-DR and HLA-DR+CD38 on both CD4⁺ and CD8⁺ T cells compared to the LTNP ($p < .001$; Fig. 1A), but not CD38-expressing T cells ($p > .05$; Fig. 1A). The LTNP had similar frequencies of HLA-DR as well as HLA-DR+CD38-coexpressing CD4 and CD8 T cells compared with healthy controls ($p > .05$; Fig. 1A), except the expression of CD38, which was significantly higher in LTNP than in healthy individuals ($p < .05$; Fig. 1A). We also determined whether the immune activation had any association with age within each study group and found that the frequency of only CD38-expressing CD4 cells was positively associated with age in LTNP ($p < .0001$; $r = 0.50$), while the T cell activation (HLA-DR- and/or CD38-expressing T cells) was not associated with age among the progressors. The HLA-DR⁺ and CD38⁺ CD4 T cell frequencies were negatively associated with CD4 cell counts ($p < .0001$ and $p < .003$, respectively) (Fig. 1B). HLA-DR-expressing CD4⁺ and CD8⁺ T cells were associated with higher pVL ($p < .001$ and $p < .01$, respectively) (Fig. 1C). Higher pVL was also found to be associated with CD38⁺CD8⁺ T cells, close to the significance levels ($p = .056$; $r = 0.268$). Furthermore, the frequencies of T cells coexpressing HLA-DR+CD38 were negatively associated with CD4 cell count (Fig. 1D) and positively with pVL (Fig. 1E).

The frequencies of both CD4⁺ and CD8⁺ T_{CM} cells were significantly higher in LTNP than progressors ($p < .0001$) (Fig. 1F) and were associated with lower pVL ($p = .001$ and $p = .002$, respectively) (Fig. 1G), but not with CD4 count. In contrast, the frequencies of CD4⁺ T_{EM} cells were significantly lower in LTNP than in the progressors ($p < .0001$) (Fig. 1F) and significantly associated with higher pVL ($p = .0009$) (Fig. 1H). The CD8⁺ T_{EM} frequencies were similar in progressors and LTNP (Fig. 1F). We further assessed the association between the immune activation (activated T cells) and memory T cell population. A strong inverse correlation was observed between the activated CD4⁺ and CD8⁺ T cells expressing HLA-DR+CD38⁺ with CD4⁺ and CD8⁺ T_{CM} (Fig. 1, J respectively), while the CD4⁺T_{EM} frequency was positively associated with HLA-DR+CD4⁺ ($r = 0.594$; $p < .0001$) and CD8⁺ T cells ($r = 0.435$; $p = .0001$).

Studies to identify correlates of immune mechanisms of HIV control are utmost essential for the development of new immune therapies as well as protection strategies. We, in this study, assessed the influence of immune activation on the memory T cell population that is important in mounting efficient recall responses. The study showed that the LTNP exhibit lower T cell activation, higher CD4⁺ and CD8⁺T_{CM}, and lower T_{EM} populations compared to the chronic progressors. We also showed that the immune activation correlated with lower frequencies of T_{CM} cells. A number of studies have shown that early immune activation causes damage to the immune system, which could result in chronic immune activation and further loss of immune function leading to disease progression.^{4,10,13,14,17} The CD38- and HLA-DR-expressing T cells are being used as a marker of immune hyperactivation and disease progression.⁴ These studies support our finding that LTNP show less activation of T cells, indicating low inflammatory environment and thus preservation of CD4 count, and control over disease progression.

Strong T_{CM} cell responses have shown to be associated with controlled disease progression.^{10,18–20} Our Indian LTNP

FIG. 1. Characterization of CD4⁺ and CD8⁺ T cells among Indian LTNP and chronic progressors. (A) Expression of activation markers HLA-DR and/or CD38 on CD4⁺ and CD8⁺ T cells of LTNP, progressors, and healthy controls (HC) using extracellular flow cytometry (Kruskal–Wallis test with a *post-hoc* analysis were used to determine statistical comparison between LTNP, progressors, and healthy controls). (B) Correlation of CD4 count with HLA-DR⁺CD4⁺ and CD38⁺CD4⁺ T cells (nonparametric Spearman's rank correlation test was used to obtain *p*-values and *r* represents Spearman's correlation coefficient). (C) Correlation of pVL with HLA-DR⁺CD4⁺ and HLA-DR⁺CD8⁺ T cells (nonparametric Spearman's rank correlation test was used to obtain *p*-values and *r* represents Spearman's correlation coefficient). (D) Correlation of CD4 count with CD4⁺ and CD8⁺ T cells with CD4 count (D) and pVL (E) (nonparametric Spearman's rank correlation test was used to obtain *p*-values and *r* represents Spearman's correlation coefficient). (F) Frequencies of central memory (T_{CM}) CD4⁺ and CD8⁺ T cells in LTNP and progressors by using multicolor flow cytometry (nonparametric Mann–Whitney test with a confidence level of 95% was used to obtain statistical comparison between LTNP and progressors). Correlation of pVL with central memory T cells (G) (nonparametric Spearman's rank correlation test was used to obtain *p*-values and *r* represents Spearman's correlation coefficient). Correlation between the frequency of HLA-DR⁺CD38⁺ T cells with central memory CD4⁺ T cells (I) and central memory CD8⁺ T cells (J) (nonparametric Spearman's rank correlation test was used to obtain *p*-values and *r* represents Spearman's correlation coefficient). **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001. LTNP, long-term nonprogressor; pVL, plasma viral load.

population also showed higher T_{CM} population, supporting these observations. We also confirmed the previous finding of association of T_{EM} with faster disease progression.⁶ The association of T_{EM} frequencies with higher immune activation is also supported by previous observation in a mouse model where the immune activation showed a progressive conversion of naive T cells into effector memory cells.²⁰

The increased immune activation is known to drive T cell differentiation leading to replicative senescence and immunodeficiencies.^{15,21} In HIV infection, this scenario in early course of HIV infection might result in loss of virus control leading to disease progression. During early HIV infection, higher T_{CM} and T_{EM} are associated with low and high viral load set point, respectively, indicating that early decrease in T_{CM} cells might be contributing to high viral load and thus faster disease progression.⁶ In support to these previous observations, our study has shown that preserved T_{CM} frequencies and lower immune activation are associated with controlled disease progression, although the causal relationship could not be established. It might be possible that lower immune activation in LTNP is responsible for reduced perturbation in T cell differentiation and further apoptosis of T cells, resulting in preserved central memory T cells, which can effectively respond to the antigenic stimulus. This scenario might be collectively and/or partially contributing to the sustained CD4 counts in these patients even in the presence of high viral load. Indeed, the natural host of SIV infection, the sooty mangabey monkeys, do not develop AIDS despite high levels of virus replication and exhibit very low levels of immune activation.²²

In summary, the low immune activation in HIV infection may contribute to preservation of memory cells and thus control over disease progression. The immune interventions to reduce the immune activation might be one of the useful anti-HIV strategies.

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Author Disclosure Statement

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