JOURNAL OF VIROLOGY, Aug. 2007, p. 8838–8842 Vol. 81, No. 16
0022-538X/07/$08.00+0 doi:10.1128/JVI.02663-06

Low Immune Activation despite High Levels of Pathogenic Human Immunodeficiency Virus Type 1 Results in Long-Term Asymptomatic Disease▽

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Received 1 December 2006/Accepted 20 May 2007

Long-term asymptomatic human immunodeficiency virus (HIV)-infected individuals (LTA) usually have low viral load and low immune activation. To discern whether viral load or immune activation is dominant in determining progression to AIDS, we studied three exceptional LTA with high viral loads. HIV type 1 isolates from these LTA were as pathogenic as viruses from progressors in organ culture. Despite high viral loads, these LTA had low levels of proliferating and activated T cells compared to progressors, like other LTA. In contrast to those in progressors, HIV-specific CD4$^+$ T-cell responses in these LTA were maintained. Thus, low immune activation despite a high viral load preserved HIV-specific T-cell responses and resulted in a long-term asymptomatic phenotype.

Both viral load and immune activation have been shown to be associated with progression to AIDS (2, 5–8, 11, 12). Since immune activation and viral load are correlated (2, 5, 6, 8), it is hard to determine which factor is dominant in determining disease progression. To further elucidate the importance of immune activation relative to viral load in human immunodeficiency virus (HIV) disease progression, we studied three rare long-term-asymptomatic HIV-infected individuals (LTA) from the Amsterdam Cohort Studies on HIV infection and AIDS who have high viral loads (LTA-HVL) equal to the viral loads seen in progressors (Fig. 1).

Although the high viral loads of these LTA-HVL were consistent with a high replicative capacity of isolated virus clones in vitro (1), slow disease progression despite high viral load may be caused by a decreased pathogenic phenotype of the virus. We tested the cytopathic effects (CPE) of these viruses in human fetal thymic organ culture (FTOC) as described previously (3). The time points of viral isolation used in these assays are indicated in Fig. 1. Pieces of thymic tissue (8 mm^3) were infected with 8×10^3 50% tissue culture infective doses of HIV type 1 (HIV-1) clones derived from progressors, LTA, or LTA-HVL. Viral replication and percentages of CD4$^+$ and CD8$^+$ thymocytes were assayed over a 3-week time period by p24 enzyme-linked immunosorbent assay and flow cytometry with CD4-phycoerythrin and CD8-peridinin chlorophyll protein monoclonal antibodies, respectively. In mock-infected FTOC, approximately, 78% of light scatter-gated thymocytes expressed CD4 at 14 and 21 days postinfection (Fig. 2A). Both progressor- and LTA-HVL-derived R5-HIV-1 clones caused depletion of more than 35% of CD4$^+$ thymocytes compared to that in mock infection at 21 days postinfection ($P < 0.001$) (statistical significance was determined by analysis of variance with Tukey's test for all pairwise comparisons). Likewise, both progressor- and LTA-HVL-derived R5-HIV-1 clones replicated to similar levels, except clones from LTA-HVL patient 68, in which replication peaked later in the course of infection (Fig. 2B). In contrast, R5-HIV-1 clones derived from LTA with low viral loads failed to exhibit significant CPE and replicated poorly.

Next we tested the ability of progressor- and LTA-HVL-derived R5-HIV-1 clones to deplete CD4$^+$ T cells in secondary lymphoid tissue histoculture. Lymphocytes were isolated from human spleen histoculture on days 16 and 21 postinfection with HIV-1 clones derived from progressors or LTA-HVL, stained with CD4-phycoerythrin and CD8-peridinin chlorophyll protein monoclonal antibodies, and analyzed by flow cytometry with a lymphocyte gate based on 90° and low-angle light scatter. Again, progressor- and LTA-HVL-derived R5-HIV-1 clones depleted CD4$^+$ T cells significantly ($P < 0.0001$) compared to mock-infected tissue on both days 16 and day 21, as measured by the ratio of CD4$^+$ to CD8$^+$ cells (Fig. 3A). No statistical difference in CPE was observed between progressor- and LTA-HVL-derived clones in FTOC or in spleen histoculture. As expected, the R5-HIV-1 clones preferentially depleted CD4$^+$ CCR5$^+$ T cells, while the X4-HIV-1 molecular clone NL4-3 depleted the CD4$^+$ CXCR4$^+$ subset of T cells more efficiently (Fig. 3B and C). These results show that R5-HIV-1 clones obtained from LTA-HVL were equally replication competent and cytopathic as progressor-derived clones. Therefore, the replication and CPE of these R5-HIV-1 clones in primary and secondary lymphoid tissue organ culture were not reflective of the clinical status of the patients from which they were derived.

We hypothesized that the LTA-HVL showed slow disease

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▽ Published ahead of print on 30 May 2007.

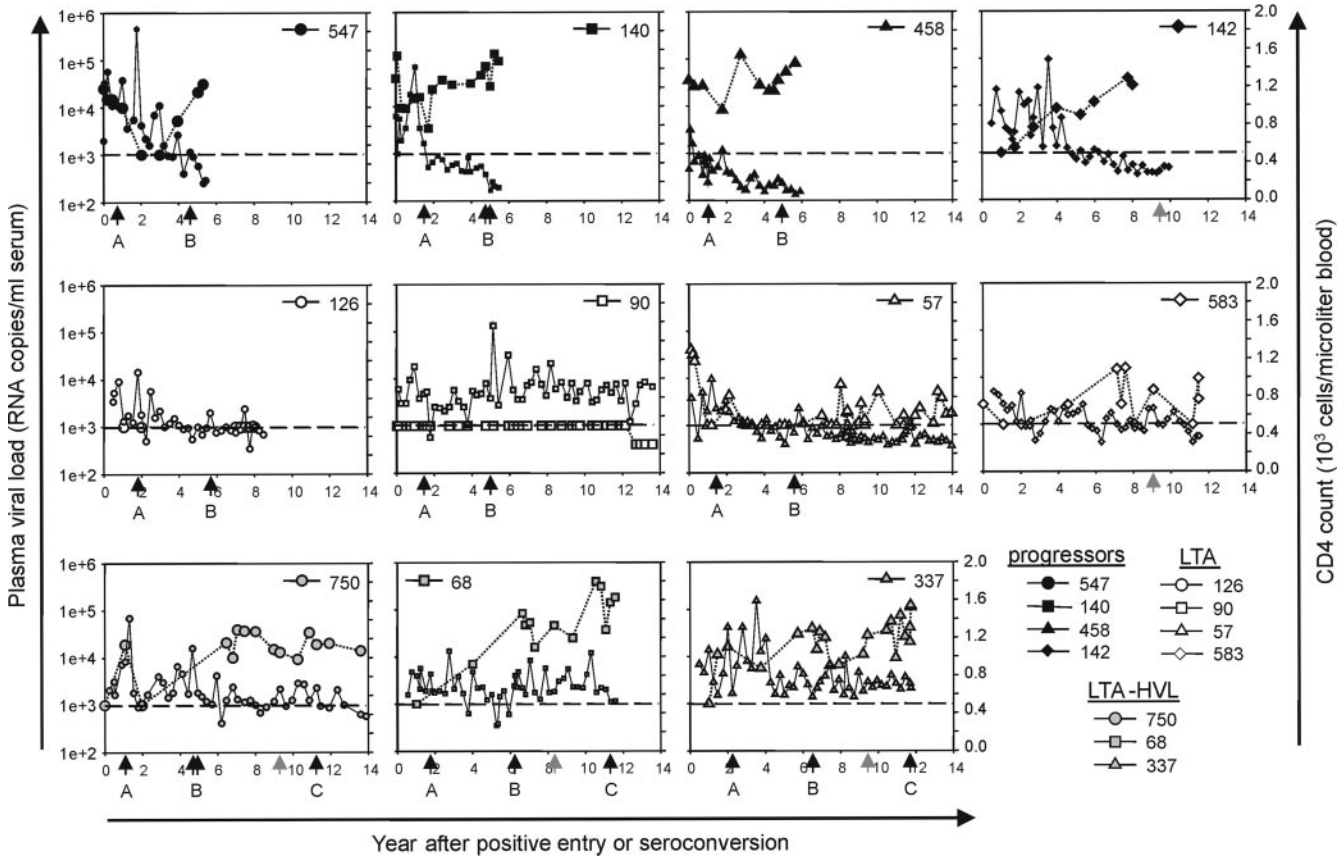

FIG. 1. Longitudinal analysis of $CD4^+$ T-cell numbers in blood and HIV-1 RNA copies in plasma of the patients used in this study. Plasma viral RNA copies per ml (left axes; large symbols and dashed lines) and $CD4^+$ T cells per μl blood (right axes; small symbols and solid lines) against time in years after seroconversion or after seropositive entry into the cohort are shown. The horizontal dashed lines represent the detection limit of 1,000 RNA copies/ml. Gray arrows along the *x* axes indicate the times of isolation of the HIV-1 clones used for Fig. 2 and 3, while black arrows with letters refer to time points of T-cell isolation for analyses shown in Fig. 4.

progression due to low-level immune activation despite a high viral load, since high levels of activated and proliferating ($Ki67^+$) $CD4^+$ and $CD8^+$ T cells during HIV infection are better prognostic factors than viral load (5, 6, 8) and preseroconversion immune activation has been shown to be predictive of disease progression (8, 15, 16). Moreover, patients who fail virologically on highly active antiretroviral therapy but have low levels of T-cell activation, proliferation, and apoptosis show a continuous increase in $CD4^+$ T cells (4, 9, 13). Similarly, during natural simian immunodeficiency virus infection, sooty mangabeys do not develop disease despite high viral loads, and their immune activation is as low as that in uninfected animals. In contrast, pathogenic simian immunodeficiency virus infection in macaques induces strong immune activation, $CD4^+$ T-cell loss, and disease progression (14). Collectively these data implicate immune activation as a driving force of HIV pathogenesis.

To test this hypothesis, we longitudinally followed the immune activation status (measured as previously described [8]) of the three LTA-HVL and compared these values with those for progressors matched for HIV viral load and for LTA with low viral loads (Fig. 1). The time points measured were approximately 1 year, 5 years, and for LTA-HVL additionally 11 years after seroconversion or seropositive entry into the cohort. The time-points used for analysis are indicated in Fig. 1.

Progressors showed high percentages of activated $HLA\text{-}DR^+$ $CD38^+$ $CD4^+$ and $CD8^+$ T cells within 5 years of HIV-1 infection. In contrast, the LTA-HVL had levels of activated T cells comparable to those of LTA with low viral loads even after more than 10 years of HIV-1 infection. A similar trend was found for proliferating, Ki67-expressing $CD4^+$ and $CD8^+$ T cells (Fig. 4A). The levels of proliferating T cells of both subsets was significantly lower in LTA and in LTA-HVL than in progressors at later time points by Tukey's test.

Jansen et al. (10) have shown that HIV-specific $CD4^+$ T-cell responses soon after seroconversion did not have predictive power for disease progression in a prospective cohort study in a multivariate analysis including immune activation. HIV-specific $CD4^+$ T-cell responses were lost over time in progressors, however, compared to LTA. To further elucidate whether loss of HIV-specific $CD4^+$ T cell responses was due to high viral load or to high-level immune activation, we determined absolute numbers of Gag-specific gamma interferon (IFN-γ)- and interleukin-2 (IL-2)-producing $CD4^+$ T cells (measured as previously described [10]). Gag-specific IFN-γ- and IL-2-producing $CD4^+$ T cells were readily detected early after infection in

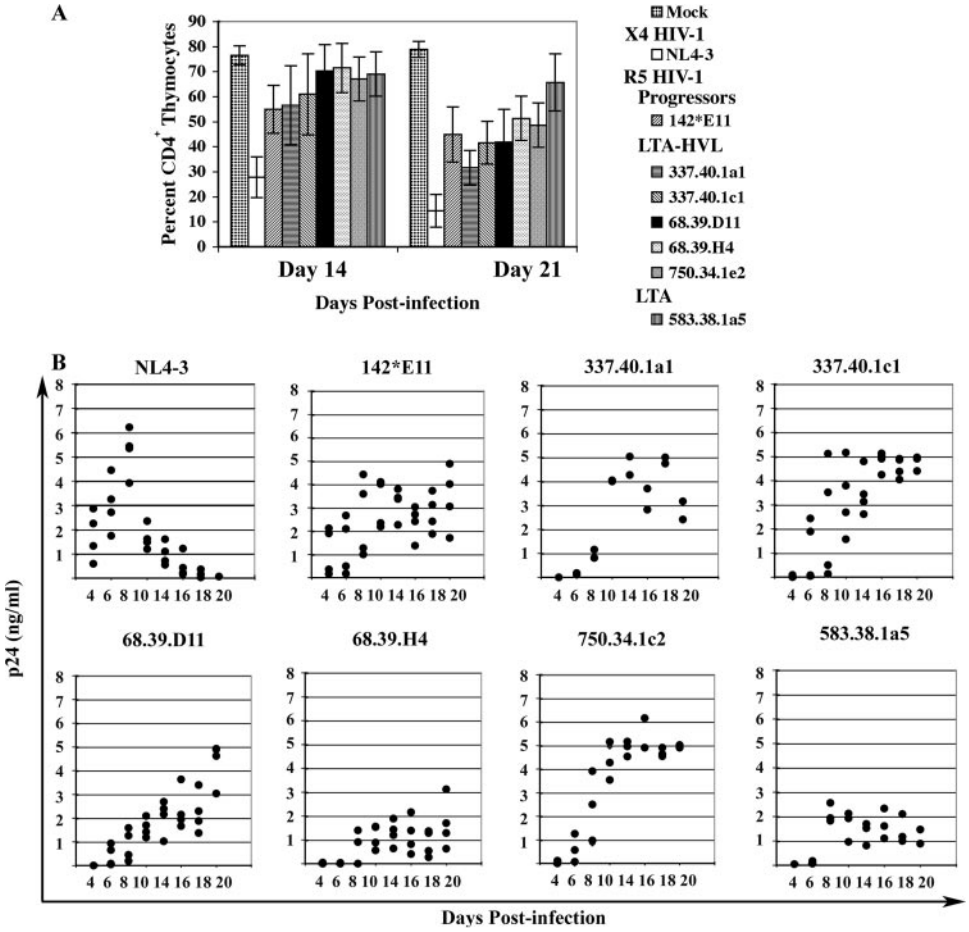

FIG. 2. R5-HIV-1 clones from LTA-HVL replicated to high levels and depleted $CD4^+$ thymocytes in FTOC. FTOCs were infected with the indicated HIV-1 clones. (A) The bars represent average percentages of $CD4^+$ thymocytes (CD4SP and -DP) in FTOC, with error bars indicating standard deviations. The numbers of samples used to obtain data on day 14 and day 21 postinfection, respectively, are as follows: $n = 9$ for mock in five experiments; $n = 4$ for NL4-3 in two experiments; $n = 8$ for ACH 142*E11 in five experiments; $n = 3$ for ACH 337.40.1a1 in two experiments; and $n = 5$ for ACH 337.40.1c1, ACH 68.39.D11, ACH 68.39.H4, ACH 750.34.1e2, and ACH 583.38.1a5 in three experiments. (B) Viral replication was quantified by measuring HIV-1 capsid antigen (p24) concentration in FTOC medium on days 4, 6, 8, 10, 14, 16, 18, and 20 postinfection with a commercial enzyme-linked immunosorbent assay kit (NEN Life Science Products). Each dot represents the medium from an individual FTOC.

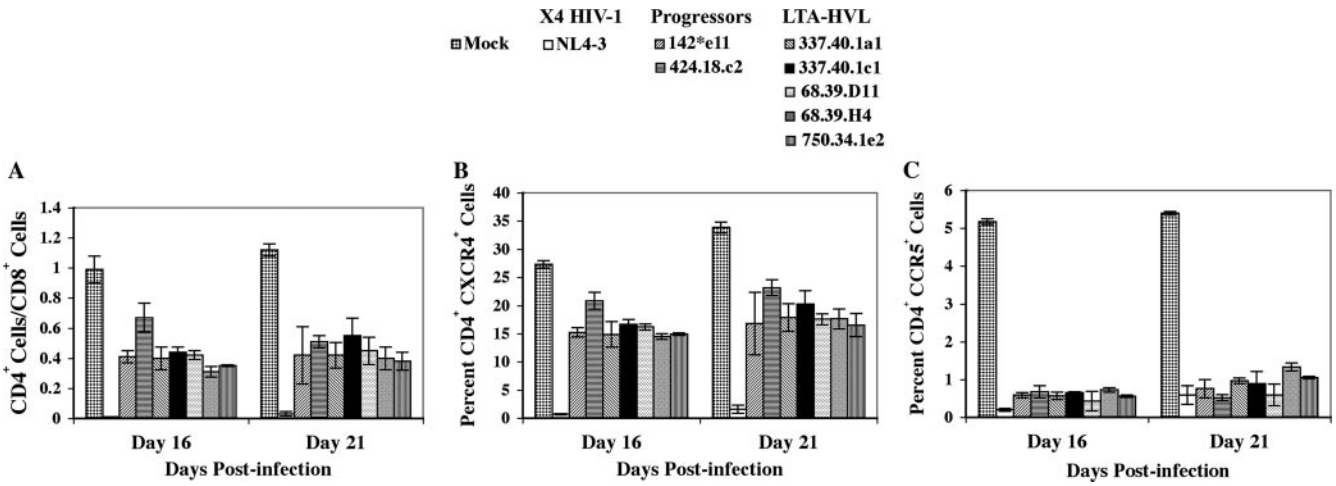

FIG. 3. Depletion of $CD4^+$ cells in human spleen histoculture infected ex vivo with HIV-1. Spleen histocultures were infected with the indicated HIV-1 clones. (A) Depletion of $CD4^+$ lymphocytes measured by change in the ratio of $CD4^+$ to $CD8^+$ cells. (B) Depletion of $CD4^+$ $CXCR4^+$ T lymphocytes. (C) Depletion of $CD4^+$ $CCR5^+$ T lymphocytes. Data shown are representative of two experiments done in duplicate, with error bars indicating standard deviations.

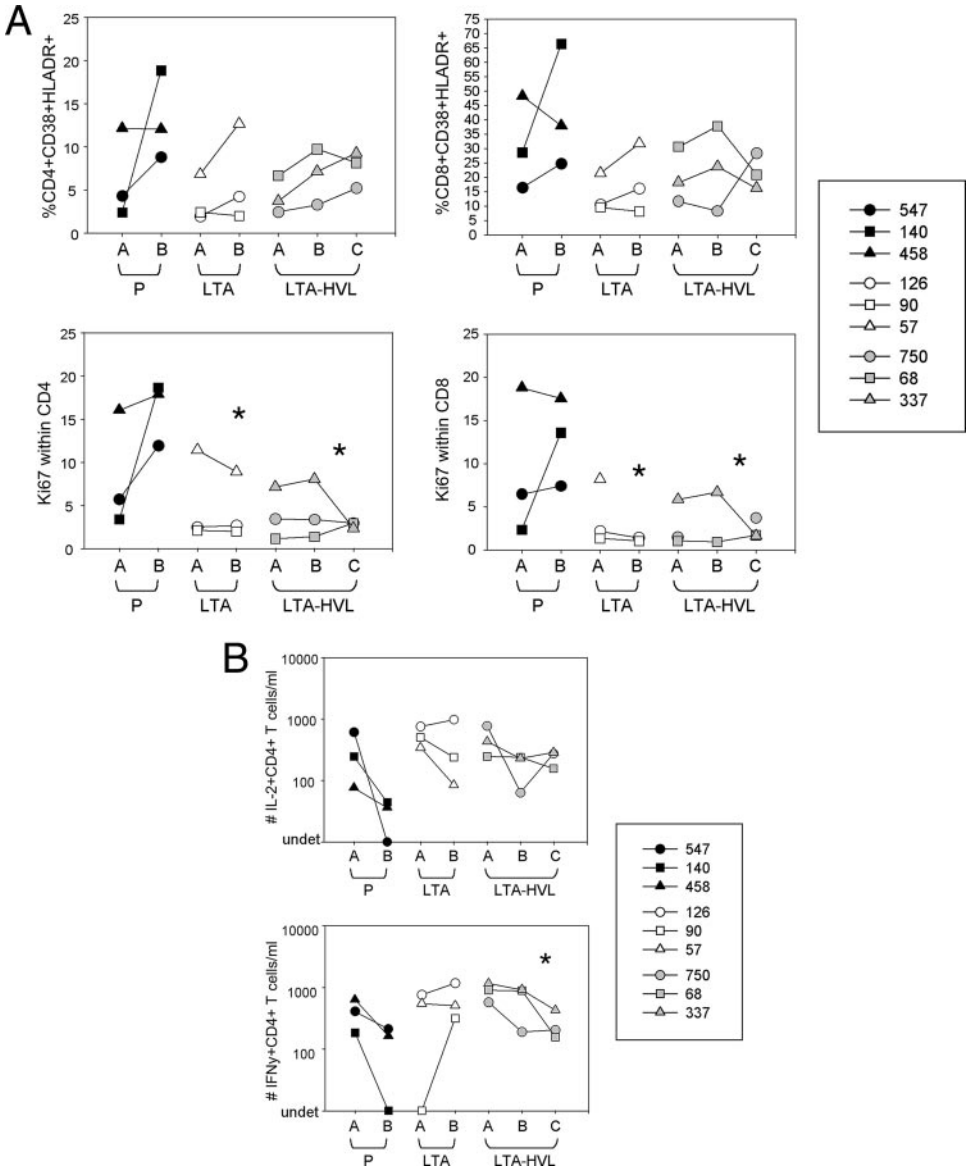

FIG. 4. LTA-HVL had low immune activation and preserved HIV-specific responses. (A) Percentages of activated ($CD38^+$ $HLA\text{-}DR^+$) and proliferating ($Ki67^+$) $CD4^+$ and $CD8^+$ T cells. (B) Absolute numbers of HIV-specific IL-2-producing (upper panel) and IFN-γ-producing (lower panel) $CD4^+$ T cells. Time points in panels A and B correspond to the black arrows in Fig. 1. Black symbols depict progressors, white symbols depict LTA with low viral loads, and gray symbols depict LTA with high viral loads. Asterisks indicate significant differences between LTA or LTA-HVL compared to progressors at time point B ($P < 0.01$ for LTA and LTA-HVL $Ki67^+$ $CD4^+$ cells, $P < 0.01$ for LTA $Ki67^+$ $CD8^+$ cells, $P < 0.05$ for LTA-HVL $CD8^+$ cells, and $P < 0.05$ for LTA-HVL IFN-γ^+ $CD4^+$ cells).

all subjects (Fig. 4B). These responses, while preserved in LTA with both high and low viral loads, however, tended to decrease over time in progressors. The effect was pronounced for absolute numbers of IL-2- and IFN-γ-producing $CD4^+$ T cells (Fig. 4B) but did not achieve statistical significance with only three patients in each group. When six more progressors studied previously were included in the comparison (10), however, the Gag-specific IFN-γ response of the LTA-HVL at time points B and C was significantly different from that of progressors at time point B, and the IL-2 response approached a significant difference ($P = 0.03$ and 0.07, respectively, by Student's t test). This suggests that it is not high viral load alone but instead chronic immune activation with high viral load that causes loss of functional Gag-specific $CD4^+$ T cells over time.

In summary, despite high viral loads, HIV-1 infection did not evoke high levels of immune activation or proliferation in these LTA-HVL. Although few patients could be studied, these data provide additional support for the idea that chronic immune activation is an important driving force for $CD4^+$ T-cell decline and loss of HIV-specific $CD4^+$ T-cell responses in HIV-1-infected individuals. Moreover, these data suggest that if there were no effective vaccine or treatment for AIDS, humans might eventually evolve resistance to HIV-1 by one of two mechanisms, exemplified by LTA, who suppress viral rep-

lication, and LTA-HVL, whose immune systems are not nonspecifically activated by HIV-1 infection.

We thank Argyrios Ziogas of the University of California, Irvine, for help with statistical analyses of the data.

We acknowledge support from NIH grant AI-55385 awarded to D.C. and the Amsterdam Cohort Studies on HIV Infection and AIDS, a collaboration between the Amsterdam Health Service, the Academic Medical Center of the University of Amsterdam, the Sanquin Blood Supply Foundation, and the University Medical Center Utrecht, part of The Netherlands HIV Monitoring Foundation, which is financially supported by The Netherlands National Institute for Public Health and the Environment.

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