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Central Memory CD4+ T Cell Responses in Chronic HIV Infection Are Not Restored by Antiretroviral Therapy

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A strong CD4+ T cell response has been correlated with better control of HIV infection. However, the effect of HIV on the maintenance of Ag-specific memory CD4+ T cells is not fully understood. We characterized the function and phenotype of memory CD4+ T cells generated by mumps and influenza A or B viruses in HIV-infected individuals receiving highly active antiretroviral therapy (n = 21), HIV-infected long-term nonprogressors (n = 10), and HIV-seronegative volunteers (n = 10). We observed significantly decreased proliferation of the Ag-specific central memory CD4+ T cell population (CD28+/CCR7+/CD45RA−) in the antiretroviral treated HIV-infected individuals compared with the seronegative controls. Restored CD4+ T cell count and decreased HIV viral load while on highly active antiretroviral therapy did not result in increased proliferation, whereas nadir CD4+ T cell count predicted the presence of Ag-specific proliferation. Our results indicate that HIV infection leads to impaired maintenance of virus-induced or vaccine-generated central memory CD4+ T cells that is not restored by HAART. The Journal of Immunology, 2004, 173: 2184–2189.

Protection following viral clearance or successful immunization requires the generation and maintenance of long-lived Ag-specific CD4+ Th cells, defined as central memory CD4+ T cells. Memory T cells have distinct phenotypes (reviewed in Ref. 1). Central memory CD4+ T cells express CCR7, a homing molecule to the lymph nodes, and CD28, a costimulatory molecule that provides signals for specific CD4+ T cell activation. Central memory CD4+ T cells produce IL-2, undergo rapid expansion in response to Ag restimulation (2, 3), and are critical for the development of CD8+ T cell memory (4–6).

HIV infection results in a progressive decline in CD4+ T cell number and function, resulting in an increased risk of opportunistic infections (7–10). The introduction of highly active antiretroviral therapy (HAART) has significantly reduced HIV-related morbidity and mortality (11–14). These clinical benefits have been attributed to decreased HIV replication and increased CD4+ T cell number (reviewed in Ref. 15). The full effect of HAART on T cell immunity is still debated. HAART initiation has been associated with the detection of proliferative and Ab responses following immunization with influenza vaccine (16), tetanus toxoid, and inactivated hepatitis A vaccine (17), although these responses are often incomplete (18–21). We report in this work the consequence of chronic HIV infection on the maintenance of central memory CD4+ T populations. We characterized the function and phenotype of memory CD4+ T cells specific for mumps and influenza A or B (A/B) viruses in HIV-infected individuals. We demonstrated evidence of impaired maintenance of virus-induced or vaccine-generated central memory CD4+ T cells in HIV-infected individuals that was not restored by HAART.

Materials and Methods

Study subjects and samples

HIV-positive volunteers (n = 31) were recruited from the Study of the Consequences of the Protease Inhibitor Era (SCOPE) cohort at San Francisco General Hospital (San Francisco, CA) (22). SCOPE is an ongoing cohort of 500 HIV-1 chronically infected adults. Subjects are evaluated every 4 mo. Prestudy treatment history and treatment responses (plasma HIV RNA, CD4+ T cell count) were obtained via standardized patient interviews and medical chart review. A summary of the patient clinical data is shown in Table I. HIV RNA in the plasma (viral load) was quantified by the branched chain-DNA amplification test (Chiron, Emeryville, CA), and the lower limit of detection was 75 copies/ml. The study protocol was approved by the University of California Committee on Human Research, and all participants provided informed written consent. HIV-positive volunteers were divided into two groups: 1) 10 long-term nonprogressors (LTNP) having stable CD4+ T cell counts for >10 years and no history of any antiretroviral treatment; 2) 21 HIV-positive volunteers currently receiving HAART who had achieved either complete (HIV RNA <75 copies/ml) or partial virologic response. Ten age-matched, HIV-seronegative control volunteers were also included in the study. None of the study participants had received a mumps vaccine booster for at least 10 years, and all had Abs against mumps, influenza A, and influenza B in the plasma, as determined by the standard diagnostic test, enzyme immunoassay (California Department of Health Services, Richmond, CA) (23, 24). PBMC were separated and cryopreserved in liquid nitrogen until assay time.

Antigens

Mumps virus Ag was prepared from the Enders strain (Microbix Biosystems, Toronto, Canada). Influenza A/B virus vaccine (Fluzone; Aventis Pasteur, Swiftwater, PA) was produced for the 2002–2003 influenza season, and was formulated to contain the prototype strains of influenza A and B viruses. The vaccine was dialyzed before use to remove all traces of the preservative thimerosal.

Proliferation of memory CD4+ T cells

PBMC (2 × 10^6) were labeled with 4 µM CFSE (Molecular Probes, Eugene, OR) in PBS, then quenched with 100% FCS (Sigma-Aldrich, St. Louis, MO). Cells were resuspended in RPMI 1640 (Sigma-Aldrich) with

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5% CO₂ in the presence of costimulatory anti-CD49d (1/H11021) was used as a control. Percentage of cytokine-producing CD3⁺ T cells without Ag stimulation was measured as: net percentage of IL-2-positive CD4⁺ T cells (net percentage = percentage of Ag-specific – percentage of negative control).

**Statistical analysis**

Statistical analysis and comparisons were performed with PRISM software version 2.0 (GraphPad, San Diego, CA). Analysis was determined by Mann-Whitney U test and linear regression test. Statistical significance was defined as p < 0.05.

**Results**

**Characteristics of study participants**

The clinical characteristics of all HIV-positive volunteers are shown in Table I. There was no significant difference in age between the HIV-positive LTNP (aged 32–55; median = 46.5) and those receiving HAART (aged 33–66; median = 50) volunteers, and the HIV-seronegative (aged 26–61; median = 45.5) volunteers (p = 0.27; p = 0.05, respectively). All HIV-positive and -seronegative study participants had Abs against mumps, influenza A, and influenza B virus Ags (as determined by enzyme immunoassay). HAART resulted in a significant increase in the mean CD4⁺ T cell count (nadir = 100; current = 382; p < 0.0001) with nine volunteers having a restored CD4⁺ T cell count >350 cells/μl (ID 1092, 2070, 2074, 2001, 2009, 2013, 3085, 3135, 3167, 3158), while seven volunteers achieved a complete virologic response (HIV RNA <75 copies/ml; ID 1092, 2015, 2070, 2074, 2001, 2009, 2013).

**Phenotypic analysis of central memory CD4⁺ T cell subset**

We first examined the phenotypes and proliferative ability of CD4⁺ T cells following stimulation with mumps or influenza A/B virus Ags. Representative plots of the phenotype of the proliferating mumps- and influenza A/B-specific CD4⁺ T cells are shown in Fig. 1, A and B, respectively. Most of the proliferating mumps-specific CD4⁺ T cells expressed the CD28⁺/CCR7⁺/CD45RA⁻/CD49d⁻ phenotype.
phenotype in both HIV-negative \((n = 10)\) and HIV-positive volunteers \((n = 8; \text{Fig. 1C})\). The proliferating influenza A/B-specific CD4\(^+\) T cells in both HIV-seronegative \((n = 10)\) and HIV-positive \((n = 9)\) volunteers also expressed similar phenotype (Fig. 1D). All proliferating mumps- and influenza A/B-specific CD4\(^+\) T cells expressed the memory marker CD45RO (data not shown). This is consistent with the phenotypic criteria defining CD28\(^+\)/CCR7\(^+\)/CD45RA\(^-\)/CD45RO\(^+\) as central memory CD4\(^+\) T cells (2, 3). In addition, proliferation was associated with IL-2 production by the CD4\(^+\) T cells in both HIV-seronegative and HIV-positive volunteers in response to mumps and influenza A/B virus Ags (Fig. 2), and differences between the two groups were not statistically significant \((p = 0.96\) and \(p = 0.24\) for mumps and influenza A/B, respectively).

**Effect of HIV infection on maintenance of mumps- and influenza-specific central memory CD4\(^+\) T cell responses**

We next examined the effect of chronic HIV infection on the maintenance of memory CD4\(^+\) T cells specific for mumps and influenza A/B virus Ags. All HIV-seronegative volunteers \((n = 10)\) demonstrated significant proliferative responses to both mumps (median = 2.05%; Fig. 3A) and influenza A/B (median = 4.75%; Fig. 3B) virus Ags. Similarly, 8 of 10 and 9 of 10 HIV-positive LTNP volunteers displayed significant proliferative responses to mumps
response demonstrated signifi-

cant differences were

responses to either mumps (Fig. 4A) or influenza A/B (Fig. 4B) virus Ags. Surprisingly, the two HIV-positive LTNP, who had a nadir CD4+ T cell count ≤350 cells/mm³, also demonstrated no significant central memory CD4+ T cell responses to mumps (Fig. 4A), and only one of two was able to maintain significant central memory CD4+ T cell responses to influenza A/B (Fig. 4B). In contrast, all of the HIV-positive LTNP, who had a nadir CD4+ T cell count >350 cells/mm³ (n = 8), were able to maintain significant central memory CD4+ T cell responses to mumps (Fig. 4A) and influenza A/B (Fig. 4B) virus Ags.

Previous studies have demonstrated that HIV viremia correlated with diminished HIV-specific CD4+ T cell proliferation in chronic HIV infection (25, 26). We determined the relationship between HIV viral load and the magnitude of central memory CD4+ T cell responses and found no correlation between the viral load and responses to mumps (Fig. 5A) or influenza A/B (Fig. 5B) virus Ags.
The hallmark of immune protection is the long-term maintenance of memory T cell responses. CD4⁺ T cell help is required for the control of infection and for the generation of functional memory CD8⁺ T cells (27, 28). A strong CD4⁺ T cell response has been correlated with better control of HIV infection (29). However, the mechanism by which HIV affects the homeostasis and the proliferative ability of memory CD4⁺ T cells remains unresolved (reviewed in Ref. 30).

We first characterized the central memory CD4⁺ T cells specific for mumps and influenza A/B virus Ags in a cohort of chronic HIV-infected volunteers. HIV-positive LTNP maintained significant proliferative responses to mumps and influenza A/B virus Ags comparable to that observed in HIV-seronegative volunteers. Detection of mumps-specific responses 10 years after natural infection or immunization suggests that central memory CD4⁺ T cells are long-lived in the absence of Ag re-exposure. These results also suggest that the ability to generate or maintain effective central memory CD4⁺ T cell responses is preserved in nonprogressive HIV disease with low-level HIV replication.

In contrast, none of the antiretroviral treated individuals had detectable proliferative responses to either mumps or influenza A/B virus Ags. This lack of responses persisted even in individuals with clear evidence of a treatment-mediated increase in CD4⁺ T cell count.

FIGURE 4. Relation of mumps- and influenza A/B-specific central memory CD4⁺ T cell proliferation to the nadir CD4⁺ T cell count in HIV-positive individuals. CFSE-labeled PBMC were stimulated with mumps (A) or influenza A/B virus (B) Ag for 5 days, then assessed for proliferation by flow cytometry. Results are percentage of proliferating CD4⁺ T cells as measured by the extent of CFSE dilution. Dashed line represents the cutoff for significant proliferation. Differences in A between 4 vs 1, 2, or 3 were statistically significant (p = 0.0003, p < 0.0001, and p = 0.04, respectively). Differences in B between 4 vs 1 or 2 were statistically significant (p = 0.0003 and p < 0.0001, respectively). Differences between 4 vs 3 in B, between 1 vs 2 or 3 and between 2 vs 3 in A and B were not statistically significant (p > 0.05).

1 = On HAART, nadir CD4 ≤ 350/current CD4 ≤ 350
2 = On HAART, nadir CD4 ≤ 350/current CD4 > 350
3 = LTNP, nadir CD4 ≤ 350/current CD4 > 350
4 = LTNP, nadir CD4 > 350/current CD4 > 350

FIGURE 5. Relation of mumps- and influenza A/B-specific central memory CD4⁺ T cell proliferation to HIV RNA titer. The CD4⁺ T cell proliferative response to mumps (A) and influenza A/B virus (B) Ag, as measured by flow cytometry, is plotted against the HIV RNA titers (copies x 10⁷/ml). No correlation between the two parameters was observed in A, R² = 0.008, p = 0.62 or B, R² = 0.03, p = 0.35.
and low or undetectable viremia. These results suggest that progressive HIV disease may cause global impaired maintenance of central memory CD4+ T cell responses, and that reconstitution of pre-existing immunity remains defective despite viral suppression and normalization of CD4+ T cell count in response to HAART.

Data from several cohort studies support initiating treatment of HIV-positive individuals when CD4+ T cell count reaches 200 cells/mm3 (31–33). Introduction of HAART has significantly reduced HIV-related morbidity and mortality (11–14). However, the optimal time to initiate HAART in asymptomatic HIV-positive individuals with CD4+ T cell count of <350 cells/mm3 is still debated (reviewed in Ref. 34). In our study, indicators of clinical responses to HAART including restored current CD4+ T cell count or suppression of HIV replication had no impact on maintaining effective virus-induced or vaccine-generated central memory CD4+ T cell responses. In contrast, low nadir CD4+ T cell count (≤350 cells/mm3) predicted failure to preserve these responses. The ability to maintain central memory CD4+ T cell responses in our HIV-positive study participants may reflect on immunologic characteristics specific to HIV-positive LTNP. However, the two LTNP that had a low nadir CD4+ T cell count (<350 cells/mm3) were also unable to maintain mumps-specific central memory CD4+ T cell responses. This interesting finding leads us to postulate that a low nadir CD4+ T cell count results in a permanent loss of the long-lived Ag-specific central memory CD4+ T cells. Our findings should contribute to decisions toward initiation of antiretroviral therapy and strategies of vaccination targeted to the HIV-infected population.

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