

Expansion of Inefficient HIV-Specific CD8 T Cells during Acute Infection

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ABSTRACT

Attrition within the CD4⁺ T cell compartment, high viremia, and a cytokine storm characterize the early days after HIV infection. When the first emerging HIV-specific CD8⁺ T cell responses gain control over viral replication it is incomplete, and clearance of HIV infection is not achieved even in the rare cases of individuals who spontaneously control viral replication to nearly immeasurably low levels. Thus, despite their partial ability to control viremia, HIV-specific CD8⁺ T cell responses are insufficient to clear HIV infection. Studying individuals in the first few days of acute HIV infection, we detected the emergence of a unique population of CD38⁺ CD27⁻ CD8⁺ T cells characterized by the low expression of the CD8 receptor (CD8^{dim}). Interestingly, while high frequencies of HIV-specific CD8⁺ T cell responses occur within the CD38⁺ CD27⁻ CD8^{dim} T cell population, the minority populations of CD8^{bright} T cells are significantly more effective in inhibiting HIV replication. Furthermore, the frequency of CD8^{dim} T cells directly correlates with viral load and clinical predictors of more rapid disease progression. We found that a canonical burst of proliferative cytokines coincides with the emergence of CD8^{dim} T cells, and the size of this population inversely correlates with the acute loss of CD4⁺ T cells. These data indicate, for the first time, that early CD4⁺ T cell loss coincides with the expansion of a functionally impaired HIV-specific CD8^{dim} T cell population less efficient in controlling HIV viremia.

IMPORTANCE

A distinct population of activated CD8⁺ T cells appears during acute HIV infection with diminished capacity to inhibit HIV replication and is predictive of viral set point, offering the first immunologic evidence of CD8⁺ T cell dysfunction during acute infection.

Immense levels of human immunodeficiency virus (HIV) replication during the first days of infection are accompanied by dramatic changes in the immune system that may determine the quality of the subsequent immune response and ability to control HIV replication (1). The acute destruction of over half of the body's memory CD4⁺ T cells (2) is accompanied by changes in the immune system, including a drop in the B cell compartment and a major innate cytokine storm (3). Subsequent development of the adaptive HIV-specific CD8⁺ T cell response exerts selection pressure on the virus, forcing it to evolve to evade immune recognition and resulting in a lower level and semistable viral set point (4, 5). The level of the early viral set point is highly predictive for long-term disease outcome (6, 7), supporting the notion that the earliest events shaping the T cell responses are setting the stage for disease progression. Indeed, some individual HIV-specific CD8⁺ T cell responses during acute HIV infection have been identified to dictate long-term disease outcome (8, 9).

However, while the first emerging HIV-specific CD8⁺ T cell responses are able to gain initial control over viral replication, CD8⁺ T cell-mediated control is incomplete, immunological clearance of HIV infection is never observed, and viral replication

persists (10). This is partially due to HIV's ability to escape from CD8⁺ T cell targeted epitopes (11, 12), resulting in either the lack of recognition or generation of *de novo* CD8⁺ T cell responses against the variant epitope (13).

In the best (but rare) cases, HIV-specific CD8⁺ T cell responses are able to effectively control viral replication to nearly immeasur-

Received 30 October 2015 Accepted 28 January 2016

Accepted manuscript posted online 3 February 2016

Citation Eller MA, Goonetilleke N, Tassaneeritthep B, Eller LA, Costanzo MC, Johnson S, Betts MR, Krebs SJ, Slike BM, Nitayaphan S, Rono K, Tovanabutra S, Maganga L, Kibuuka H, Jagodzinski L, Peel S, Rolland M, Marovich MA, Kim JH, Michael NL, Robb ML, Streeck H. 2016. Expansion of inefficient HIV-specific CD8 T cells during acute infection. *J Virol* 90:4005–4016. doi:10.1128/JVI.02785-15.

Editor: G. Silvestri

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TABLE 1 RV217 patient demographics

Patient ID	Gender	Age (yr)	Country	CD4 T cell nadir (cells/ μ l)	Time to peak VL ^a (days)	Peak VL (log ₁₀ copies/ml)	Set point VL ^b (log ₁₀ copies/ml)
10220	F	33	Uganda	796	18	5.49	3.69
10428	F	27	Uganda	421	10	6.92	4.08
10435	F	18	Uganda	392	14	6.74	3.52
20225	F	24	Kenya	819	9	7.99	4.28
20263	F	20	Kenya	462	11	8.20	2.52
20337	F	24	Kenya	142	14	7.64	5.35
20355	F	24	Kenya	430	11	6.02	3.01
20368	F	18	Kenya	676	13	6.12	*
20509	F	24	Kenya	673	14	6.93	3.04
20511	F	24	Kenya	285	14	6.73	4.54
30112	F	25	Tanzania	884	18	7.50	4.38
30124	F	24	Tanzania	464	14	6.76	4.83
30190	F	25	Tanzania	511	11	7.34	5.96
40007	M	25	Thailand	406	15	7.31	5.35
40061	F	48	Thailand	312	14	5.79	*
40094	M	19	Thailand	866	16	6.40	4.64
40100	M	18	Thailand	467	10	8.46	5.22
40123	M	23	Thailand	616	14	6.24	4.47
40168	MtF ^c	25	Thailand	444	11	6.66	5.28
40250	M	35	Thailand	522	19	6.67	4.83
40257	MtF	18	Thailand	370	10	7.35	5.39
40265	M	23	Thailand	476	12	6.49	4.83
40353	M	21	Thailand	516	17	5.81	3.60
40363	MtF	29	Thailand	576	14	6.86	4.77
Average		25		522	13	6.85	4.44

^a Peak indicates the true peak; VL indicates HIV-1 load (copies/ml).

^b Set point VL is the average of all measured viral loads between day 80 and day 365 in the absence of treatment (requiring at least two measurements). An asterisk indicates that a set point cannot be defined because of treatment.

^c MtF, male-to-female transgender.

ably low levels. However, even then HIV cannot be cleared, and the ongoing fight between virus and T cells leads to a deterioration and exhaustion of the CD8⁺ T cell responses (14–16). This exhaustion is characterized by a hierarchical loss of functions and significant changes in the surface receptors, including the upregulation of inhibitory receptors such as programmed death 1 (PD1). Thus, besides the generation of the large breadth and magnitude of CD8⁺ T cell responses, the adaptive immune response appears to suffer from insufficient effector function after acute HIV infection that can be explained neither by exhaustion nor CD8⁺ T cell escape. Here, we assessed HIV-infected individuals at the earliest phase of acute infection to determine whether the failure to mount effective HIV-specific CD8⁺ T cell responses can be traced to early immunological changes and describe a population of CD8⁺ T cells that is associated with a lack of subsequent control.

MATERIALS AND METHODS

Study participants. Twenty-four HIV-1 acutely infected participants identified from the RV217 early-capture HIV cohort were selected based on preinfection sample availability and at least two time points sampled after infection and prior to peak viremia. RV217 is a multicenter, nonrandomized clinical observational study designed to describe the biological characteristics of acute HIV-1 infection in high-risk volunteers from Africa and Southeast Asia. Acute HIV-1 infection was determined from twice-weekly blood draws of at-risk populations using a nucleic acid test, the Aptima HIV-1 RNA qualitative assay (Hologic Gen-Probe Inc., San Diego, CA, USA), and confirmed by enzyme linked immunoassays and Western blotting after the advent of detectable antibodies. HIV-1 viral load was determined at every study visit with longitudinal samples using

the Abbott real-time HIV-1 assay with a detection limit of 40 HIV RNA copies/ml (Abbott Laboratories, Abbott Park, IL, USA). The HIV-1 viral set point was defined as the average of all viral load measurements between day 80 and day 365 in the absence of treatment, and it required at least two measurements. Two study participants were considered to have a missing set point viral load due to one individual initiating antiretroviral therapy (ART) at day 35 and the other having only one measurement available after day 80 prior to ART initiation; therefore, they were excluded from analysis with disease progression. Lymphocyte immunophenotyping was performed on fresh whole blood using the single-platform BD Multiset TruCount method to determine absolute counts and percentages (BD Biosciences, San Jose, CA, USA). Table 1 summarizes patient demographics. CD8⁺ T cell evaluation was completed through a median of 54 days for all participants with an average of 7 time points analyzed for the 13 East Africans and 11 Southeast Asians. One individual initiated antiretroviral therapy prior to the establishment of set point HIV-1 viral load.

Ethics statement. All individuals participating in this study provided written informed consent. Ethical approval was obtained from institutional review boards in each country as well as the Human Subjects Protection Branch at the Walter Reed Army Institute of Research, which approved the overall protocol.

Phenotypic analysis. One hundred sixty-seven cryopreserved peripheral blood mononuclear cell (PBMC) samples of 24 individuals were thawed and used for this study. Cells were washed in serum-free medium and stained with Live/Dead fixable aqua (Life Technologies, Grand Island, NY, USA) to exclude nonviable cells. PBMC then were washed with staining buffer containing 0.5% bovine serum albumin (BSA)–0.01% azide and stained with 2 panels of monoclonal antibodies for polychromatic flow-cytometric analysis. Antibodies used included CD4 Qdot605 (clone

S3.5), CD8a phycoerythrin (PE)-TR (clone 3B5), CD14 Tri-Color (clone Tük4), and CD19 Tri-Color (clone SJ25-C1), all from Life Technologies. CD3 allophycocyanin (APC)-eF780 (clone UCHT1), CD45RO eFluor 650NC (clone UCHL1), and HLA-DR eFluor 450 (clone L243) were from eBioscience, Inc., San Diego, CA, USA. CD27 peridinin chlorophyll protein (PerCP)-Cy5.5 and Alexa Fluor 700 (clone O323), CD28 biotinylated (clone CD28.2), CD45RA PerCP-Cy5.5 (clone HI100), CD57 fluorescein isothiocyanate (FITC) (clone HCD57), CD62L Alexa Fluor 700 (clone DREG-56), CD95 Alexa Fluor 647 (clone DX2), and CD127 BV421 (clone A019D5) were all from BioLegend (San Diego, CA, USA). CD38 APC (clone HB7), CD56 PE-Cy5 (clone B159), CD95 PE-Cy7 (clone DX2), CCR5 (CD195) FITC (clone 2D7/CCR5), CCR7 (CD197) PE-Cy7 (clone 3D12), CXCR3 (CD183) PE (clone 1C6/CXCR3), and PD-1 (CD279) PE (clone EH12.1) all were procured from BD Biosciences. α 4 β 7 (ACT-1 clone) (NIH AIDS Research and Reference Reagent Program) was conjugated in-house with an eFluor 650 nanocrystal conjugation kit–amine-reactive kit (eBioscience). After incubation, cells were washed with staining buffer and secondary Qdot 800 Streptavidin conjugate (Life Technologies) was applied for CD28. Cells were washed, fixed with 2% formaldehyde for 20 min, washed again, and resuspended in staining buffer. Samples then were acquired on an LSRII with a 4-laser configuration (BD Biosciences) and analyzed using Flow Jo, version 9.7.5 (TreeStar, Inc., Ashland, OR, USA).

Assessment of autologous HIV-specific CD8⁺ T cell responses.

Founder viruses of three study participants were deduced from plasma from the first time point preceding peak viremia with greater than 10,000 copies/ml following full-length single-genome amplification sequencing using Sanger technology as described elsewhere (17). Autologous overlapping peptides (18mer overlapping by 10 amino acids) were synthesized on the basis of the viral sequence for each individual. Five peptide pools then were generated for Gag, Env, Nef, Pol, and accessory proteins. PBMC were thawed, counted, and plated as described above. Cells were stimulated in the presence of 3 μ g/ml HIV peptide pools, costimulatory antibodies CD28/CD49d and CD107a⁻ FITC (clone H4A3) (BD Biosciences), and incubated at 5% CO₂ at 37°C for 6 h. Brefeldin A and monensin were added 2 h after stimulation. Cells were processed as described above. Antibodies include CD14 Tri-Color (clone Tük4) and CD19 Tri-Color (clone SJ25-C1), both from Life Technologies, CD38 APC (clone HB7), CD56 PE-Cy5 (clone B159), CD197 PE-Cy7 (clone 3D12), and HLA-DR APC-H7 (clone L243), all from BD Biosciences, CD45RO eFluor 650NC (clone UCHL1) from eBioscience, and CD27 AL700 (clone O323) from BioLegend. Cells then were washed, fixed in 2% formaldehyde, and permeabilized using Perm/Wash buffer (BD Biosciences). Cells then were stained intracellularly with CD3 PE-TR (clone S4.1) and CD4 Qdot605 (clone S3.5) from Life Technologies, CD8 PerCP-Cy5.5 (clone SK1) and interleukin-2 (IL-2) FITC (clone 5344.111) from BD Bioscience, and gamma interferon (IFN- γ) eFluor 450NC (clone 4S.B3) from eBioscience. Cells were washed, resuspended in staining buffer, and acquired on an LSRII flow cytometer (BD Biosciences).

Viral inhibition assay. PBMC from six patients identified in early acute infection were sorted using a FACSAria SORP (BD Biosciences) into CD4⁺, CD8^{bright}, and CD8^{dim} T cells. Sorted cells were rested overnight at 37°C in RPMI containing 20% fetal bovine serum (FBS), IL-2 (50 IU/ml), and IL-7 (5 ng/ml) in preparation for a modified viral inhibition assay as previously described (18). The target cells (autologous CD4⁺ T cells) were infected with a nevirapine-resistant virus at a multiplicity of infection (MOI) of 0.01 for 2 h. Excess virus was washed off and 100,000 cells were added to a 96-well plate in RPMI–10% fetal calf serum (FCS). Effector cells (CD8^{bright} and CD8^{dim} T cells) were added to the targets at a ratio of 1:1. Nevirapine (NIH AIDS Reagent Program) was added to cultures on day 0 at a concentration of 10 μ g/ml from a stock solution of 0.1 mg/ml. Supernatant was removed 3, 5, and 7 days postinfection and frozen at –80 before quantitative PCR (qPCR).

Soluble factor analysis. The cytokine levels of IL-2, IL-15, and IL-21 were assessed using a standard Luminesx multiplexed bead system (Mil-

liplex) according to the manufacturer's instructions and as previously described (19). Results obtained from the Bio-Plex system were analyzed automatically by the Bio-Plex Manager software program (Bio-Rad Laboratories Inc., Hercules, CA, USA) using a (5PL-fit) standard curve derived from recombinant cytokine and chemokine standards. IL-7 was quantified from longitudinal citrate plasma samples by electrochemiluminescent detection enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Meso Scale Discovery, Rockville, MD). Briefly, samples were diluted 1:2 in assay buffer and loaded onto the MSD plate in duplicate along with an 8-point, 4-fold standard curve, also in duplicate. After 2 h, plates were washed, and detection antibody bound to an electrochemiluminescent label was added for 2 h. Plates were washed again and read buffer added before the image was captured using a Meso QuickPlex SQ120 imager and analyzed with Discovery Workbench 4.0 software.

Statistical analysis. Statistical analysis was performed using Graph Pad Prism software version 6.0a for Mac OS X (GraphPad Software, La Jolla, CA, USA), JMP version 10.0.0, and SAS v9.3 (SAS, Cary, NC, USA). The standard score (Z score) for each analyte at each time point was calculated as sample measurement minus mean at baseline divided by standard deviations at baseline. Analysis of immunologic variables before and after infection was completed using the Friedman test for matched nonparametric data. Comparisons between groups were performed using the nonparametric Mann-Whitney U test or Wilcoxon method for continuous data. Correlations between continuous outcomes were determined by Spearman's rank correlation. For predictive associations of set point HIV viral load, linear regression models were generated. Assessing model fit for maximum CD8^{dim} CD38⁺ CD27⁻ T cell frequency revealed two observations with a high Cook's distance, and models were fit with and without these observations. Statistical significance was assessed at the 0.05 level.

RESULTS

Acute emergence of HIV-specific CD8⁺ T cells with a unique phenotype. To investigate the limited ability of HIV-specific CD8⁺ T cells to completely control viremia during acute HIV infection, we studied a prospective cohort of high-risk HIV-uninfected individuals from East Africa and Thailand (RV 217/ECHO) undergoing twice-weekly nucleic acid testing (Aptima HIV-1 RNA qualitative assay) to identify individuals in the earliest period after HIV infection (20). Twenty-four participants with extensive peripheral blood sampling before infection and also prior to peak HIV viral load were included in this study. Patient characteristics and demographics are shown in Table 1. Viral load peaked on average 13 days after the first reactive HIV RNA test and ranged from 5.49 log₁₀ to 8.46 log₁₀ HIV RNA copies/ml, with a median of 6.7 log₁₀ HIV RNA copies/ml. Viral set point occurred an average of 41 days after peak and ranged from 2.52 log₁₀ to 5.96 log₁₀ HIV RNA copies/ml. The initial CD4 count dropped from 855 to 472 cells/ μ l (range, 142 to 884 cells/ μ l) within 20 days from the first reactive HIV RNA test. In contrast, the CD8⁺ T cell count increased from 500 cells/ μ l (range, 209 to 1,150 cells/ μ l) to 1,413 cells/ μ l (range, 558 to 3,581 cells/ μ l; $P < 0.001$) (Fig. 1). The flow-cytometric assessment of CD8⁺ T cell phenotype before and during acute HIV infection demonstrated dramatic and synchronized changes to T cell surface receptor expression in parallel to HIV viral load kinetics (Fig. 2 and 3A). In particular, CD8⁺ T cell activation indices coincided with HIV expansion and were associated with the loss of IL-7 receptor (CD127) expression. However, we found most phenotypic markers did not occur singularly on CD8⁺ T cells; rather, we noted a shift or emergence of specific clusters of CD8⁺ T cell populations (Fig. 3B). In particular, we noted a profound contraction of the CD28⁺ CD127⁺ CD8⁺ T cell population and a corresponding increase of activated CD8⁺ T

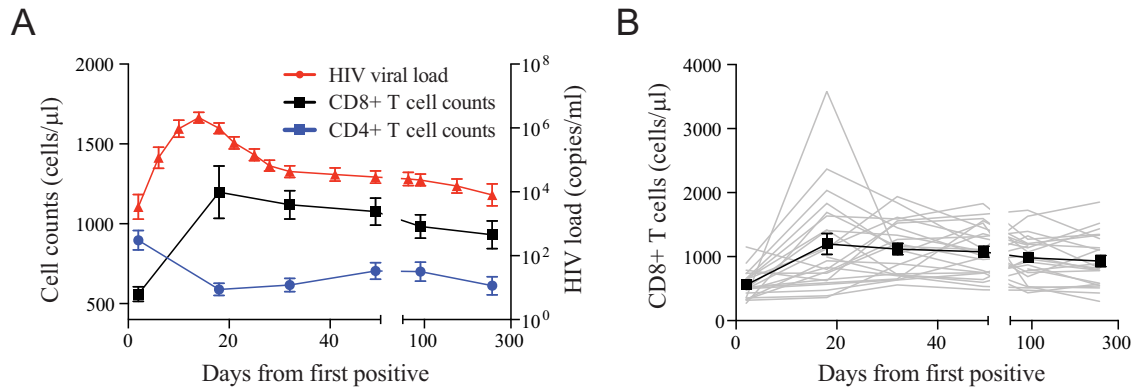


FIG 1 Longitudinal changes in HIV-1 load and CD4 and CD8 T cell absolute counts. (A) Average absolute CD4 T cell count (blue box), absolute CD8 T cell count (black box), and viral load (red circle) are presented for 24 individuals identified during acute HIV infection. (B) Aggregate CD8 T cell absolute counts are displayed for all 24 individuals during acute HIV infection.

cells with the CD38⁺ HLADR⁺ phenotype. The most dramatic changes were observed with the emergence of a CD8⁺ T cell population characterized by the CD38⁺ CD27⁻ phenotype (Fig. 3B and C), which appeared a median of 8 days after the detection of HIV-1 RNA in the periphery, closely followed viral load trajectory, but it continued to be significantly elevated for the remainder of the study ($P < 0.001$) (Fig. 3D). This population, clearly distinguishable by the lower expression of the CD8 receptor (CD8^{dim}) (Fig. 3E), was absent prior to HIV infection. Taken together, we found dramatic harmonized changes within the CD8⁺ T cell compartment characterized by the emergence of a unique CD38⁺ CD27⁻ CD8^{dim} T cell subset.

Early changes in the CD8⁺ T cell compartment is associated with loss of CD4⁺ T helper cells. Given the dramatic loss of peripheral CD4⁺ T cells during acute HIV infection, we wondered whether the emergence of this aberrant CD8⁺ T cell population is associated with the early changes in the CD4⁺ T cell compartment. While CD4⁺ and CD8⁺ T cell homeostasis are believed to be rather independent (21), we found that the CD4 nadir, defined as the minimum CD4⁺ T cell absolute count in acute HIV infection prior to day 80, was inversely correlated with HIV viral load ($\rho = -0.478$, $P = 0.024$) (Fig. 4A) and HIV levels of CD38⁺ CD27⁻ CD8^{dim} T cells ($\rho = -0.495$, $P = 0.014$) (Fig. 4B) at a time when viral decline is most profound, suggesting that at least in part the damage to the CD4⁺ T cell compartment is associated with the emergence of this unique CD8⁺ T cell phenotype. Indeed, we observed inverse trajectories of CD4 absolute counts and CD38⁺ CD27⁻ CD8^{dim} T cell frequency longitudinally during acute HIV-1 infection (Fig. 4C). To further understand the link between CD4 loss and CD8⁺ T cell proliferation, we next determined plasma levels of proliferative cytokines, including IL-2, IL-7, IL-15, and IL-21 (22). While we found no direct link between a single cytokine and the emergence of CD8^{dim} T cells, we nevertheless observed that a canonical burst of the cytokines preceded the expansion of the CD38⁺ CD27⁻ CD8^{dim} T cell subset (Fig. 4D). Thus, our data suggest that the extent of damage to the CD4⁺ T cell compartment during acute HIV infection may in part contribute to the development of unique phenotypes of activated CD8⁺ T cells.

High frequency of HIV-specific CD8⁺ T cells are CD38⁺ CD27⁻ CD8^{dim} T cells. We next investigated whether the expan-

sion of the CD8^{dim} population is virus specific or a nonspecific alteration within the CD8⁺ T cell compartment. Given the vast amount of HIV sequence diversity and recombinant forms in particular in the East-African population, we sequenced the founder virus from three study participants and generated autologous peptide sets spanning the entire HIV proteome to accurately determine the contribution of virus-specific CD8⁺ T cells to the increase of CD38⁺ CD27⁻ CD8^{dim} T cells (23, 24). In all three longitudinally studied individuals, we observed the emergence of HIV-specific CD8⁺ T cells, determined by either degranulation (CD107a) or cytokine production (IFN- γ or IL-2), early in acute HIV infection with various kinetics and specificities (Fig. 5A). While the majority of the responses increased over time, there were some individual responses targeting more variable segments of the viral proteome that showed an early expansion but then declined over the initial period of infection, as previously described (23, 24). Interestingly, both CD8^{bright} and CD8^{dim} T cells contained HIV-specific responses, yet a higher frequency of HIV-specific CD8⁺ T cell responses are within the CD8^{dim} population (Fig. 5B). It is important to note that no specific trends or patterns emerged with regard to the epitope targeting between the CD8^{bright} and CD8^{dim} T cells. We further characterized the HIV-specific CD8⁺ T cell population and found that they demonstrated lower expression of CD27, with an effector memory phenotype (CCR7⁻ CD45RO⁺), and were consistently HLADR⁺ (Fig. 5C). Coordinated expression patterns of CCR7, CD27, CD38, CD45RO, and HLA-DR were significantly different between HIV-specific CD8^{bright} and CD8^{dim} T cells ($P < 0.001$) (Fig. 5D). Interestingly, the only distinct subset we observed within this homogenous virus-specific CD8⁺ T cell population was the CD38⁺ CD27⁻ CD8^{dim} T cell population (Fig. 5E).

CD38⁺ CD27⁻ CD8^{dim} T cells are inefficient in control of viral replication. We next asked whether the two phenotypically distinct CD8⁺ T cell populations with HIV-specific functional activity were equally capable of controlling HIV viremia. Therefore, we purified CD8^{bright} and CD8^{dim} T cells from 6 patients immediately following peak viremia (average of 4 days after peak viremia [14 to 21 days following the first positive HIV determination]), where the most precipitous viral load decline occurs. We simultaneously activated and infected autologous CD4⁺ T cells as target cells with nevirapine-resistant virus in culture (Fig. 6A).

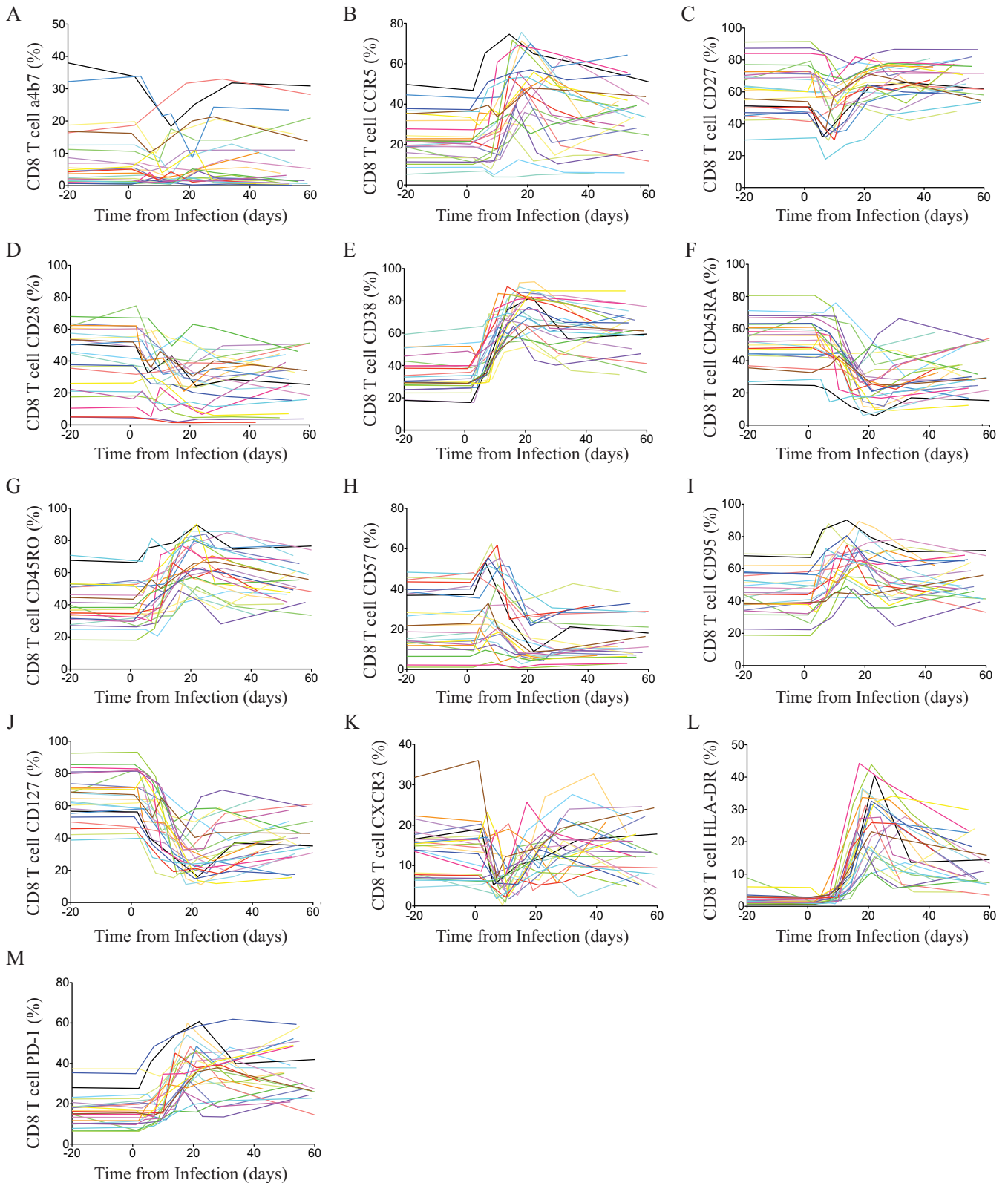


FIG 2 Bulk CD8 T cell receptor changes through acute HIV-1 infection. Changes of individual markers on CD8 T cells for all patients during acute HIV infection. The relative frequency of CD8 T cells expressing $\alpha 4\beta 7$ (A), CCR5 (B), CD27 (C), CD28 (D), CD38 (E), CD45RA (F), CD45RO (G), CD57 (H), CD95 (I), CD127 (J), CXCR3 (K), HLA-DR (L), and PD-1 (M) is shown. Color-coding across panels indicates each subject, and day 0 indicates the day of the first reactive HIV-1 Aptima nucleic acid test.

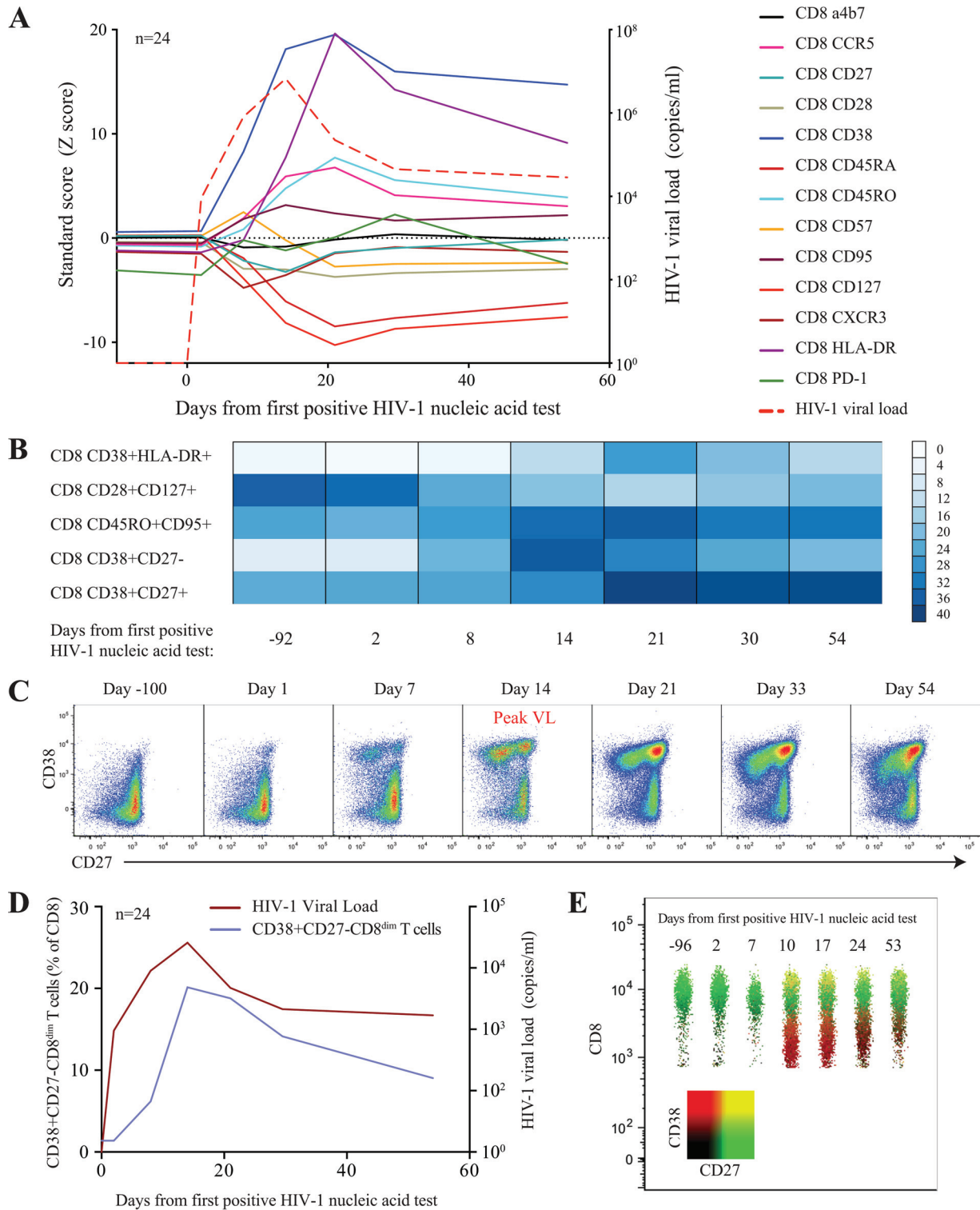


FIG 3 Longitudinal changes of surface marker expression on CD8⁺ T cells during acute HIV infection. (A) Relative changes (Z-score) of surface markers on CD8⁺ T cells during acute HIV infection. Average viral loads are depicted as red-segmented lines from the first RNA-positive HIV nucleic acid test. (B) Color intensity map of the most dramatic shifts within the CD8⁺ T cell populations during acute HIV infection. Populations are shown as a median frequency of CD8⁺ T cells based on specific combinations of receptors from green (no expression) to red (~40% expression). (C) Successive pseudocolor plots from CD8⁺ T cells, demonstrating CD38 against CD27 longitudinally, through acute HIV-1 infection and the emergence of a CD38⁺ CD27⁻ population in a representative donor. (D) Expansion of cells in association with viral loads. (E) Concatenated polychromatic plot of CD8 over time depicts the emergence of the CD38⁺ CD27⁻ population as a CD8^{dim} subset.

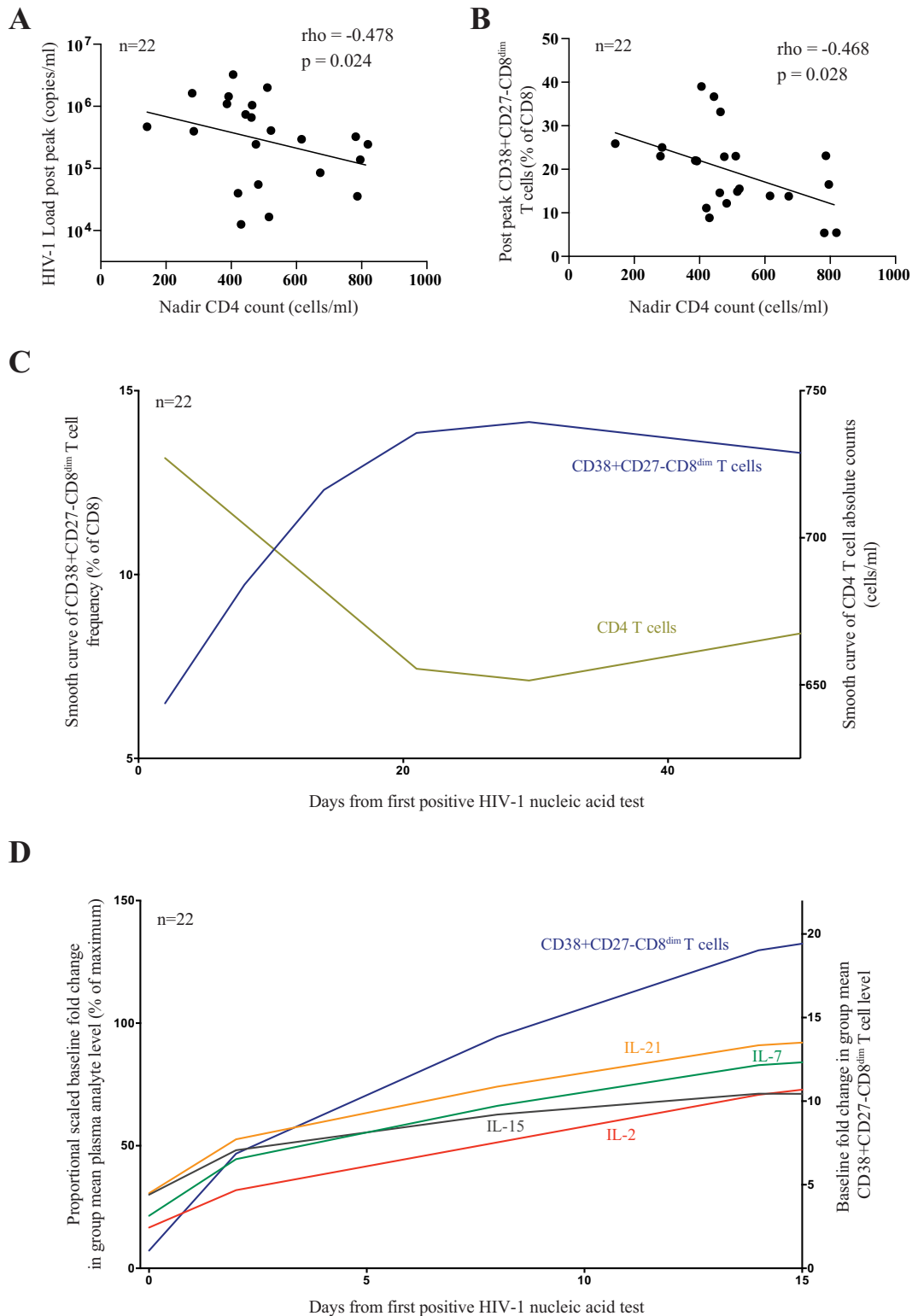


FIG 4 Temporal dynamics of CD4⁺ T cell count, HIV-1 viral load, CD38⁺ CD27⁻ CD8^{dim} T cell population, and plasma cytokine levels during acute HIV infection ($n = 22$). Two study participants were considered to have missing set point viral loads and were excluded. (A and B) Correlation of acute CD4 count nadir and HIV-1 viral load (A) and frequency of CD38⁺ CD27⁻ CD8^{dim} T cells an average 7 days after peak viral load (B). (C) Smoothed curve of means showing trends in 22 HIV-1-infected individuals of CD4 absolute counts (green) and CD38⁺ CD27⁻ CD8^{dim} T cell frequency (blue). (D) Scaled proportional group means of IL-2 (red), IL-7 (green), IL-15 (gray), and IL-21 (orange) relative to plasma baseline levels for each analyte. Mean fold difference from the background is presented for CD38⁺ CD27⁻ CD8^{dim} T cell frequency.

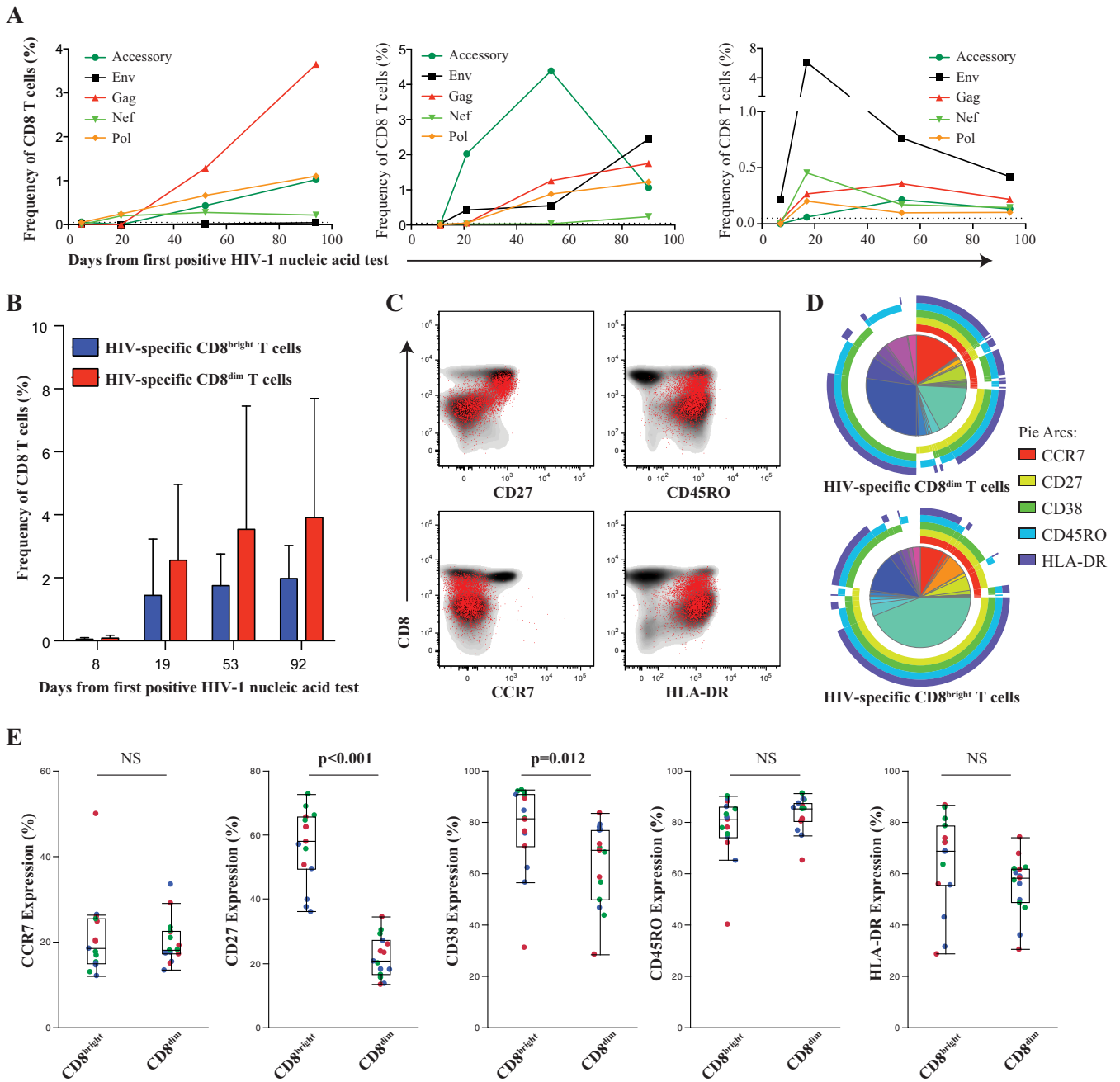


FIG 5 HIV-1-specific CD8⁺ T cells within the CD8^{bright} and CD8^{dim} population. PBMC from three donors were chosen an average 8, 19, 53, and 93 days after the 1st RNA-positive HIV nucleic acid test. (A) Shown are the kinetics of total HIV-specific CD8⁺ T cell responses (CD107a, IFN- γ , and/or IL-2) in each individual measured by flow cytometry. (B) The majority of HIV-specific CD8⁺ T cell responses emerge from the CD8^{dim} (red) T population. (C) Representative donor demonstrating the effector phenotype of the HIV-specific CD8⁺ T cell responses (in red) overlaying the total CD8 compartment (contour plot, in gray). (D) Pie charts showing average frequency of HIV-specific CD8^{bright} and CD8^{dim} T cells with combinatorial expression of 5 phenotypic markers assessed. Arcs show individual expression levels of each phenotypic marker. (E) Box-and-whisker plots show the relative expression of phenotypic markers between HIV-specific CD8^{bright} and CD8^{dim} T cells 39 days after peak viremia. Different donor responses are indicated by different colors for various peptide pools. Nonparametric comparisons were performed using the Wilcoxon method, and nonsignificant relationships are denoted as NS.

Immunofluorescently stained and sorted CD8^{bright} and CD8^{dim} T cells were added as effector cells at a ratio of 1:1. Strikingly, after 5 days of infection, we found that the CD8^{bright} T cell population was significantly more efficient at viral inhibition than the CD8^{dim} T cell population (65% versus 45%, respectively; $P = 0.016$) despite the higher frequency of HIV-specific CD8⁺ T cells observed

in the CD8^{dim} T cell population (Fig. 6B). We next assessed whether this population shows any association with levels of HIV viral load. Indeed, we found that 7 days after peak viremia the frequency of CD38⁺ CD27⁻ CD8^{dim} T cells was positively associated with viral load ($\rho = 0.591$, $P = 0.004$) (Fig. 6C). Examining absolute CD4⁺ T cell counts at contemporaneous time points

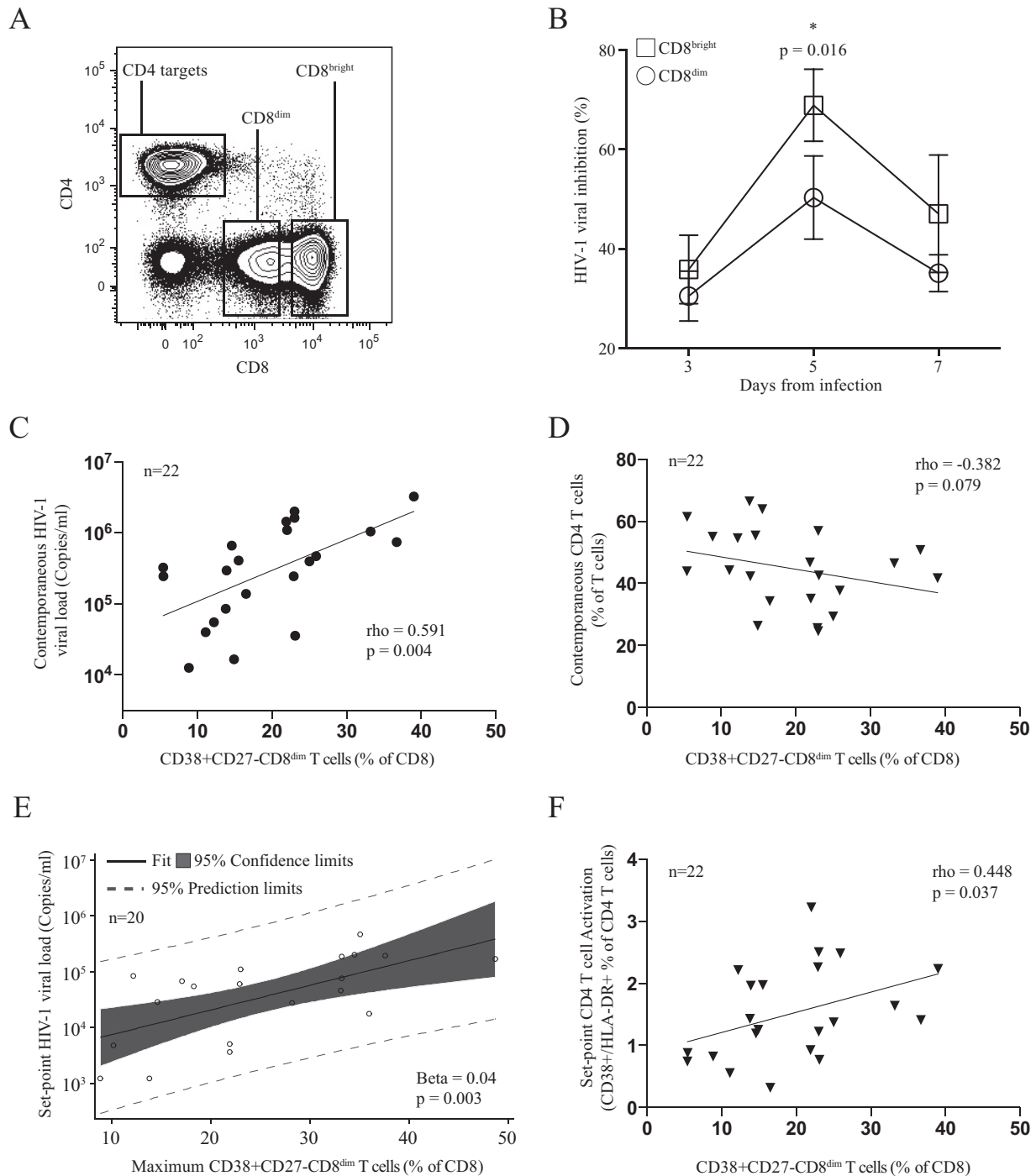


FIG 6 CD8^{dim} CD38⁺ CD27⁻ T cell population demonstrates a less efficient ability to inhibit viral replication *in vitro* and is associated with higher viral loads *in vivo*. (A) Sorting strategy for CD8^{bright} and CD8^{dim} cells. (B) Differences in viral inhibition between CD8^{bright} and CD8^{dim} T cells. (C) CD8^{dim} CD38⁺ CD27⁻ T cell frequency correlates positively with contemporaneous viral loads an average of 7 days after peak viral load and (D) negatively correlates with contemporaneous CD4⁺ T cell count. (E) Maximum CD8^{dim} CD38⁺ CD27⁻ T cell frequency was predictive of HIV set point using linear regression after the removal of two potential outliers. (F) CD8^{dim} CD38⁺ CD27⁻ T cell frequency an average of 7 days after peak viral load correlates directly with set point activation of CD4⁺ T cells (CD38⁺ HLA-DR⁺).

revealed a trend toward an inverse relationship with the frequency of CD38⁺ CD27⁻ CD8^{dim} T cells ($\rho = -0.382$, $P = 0.079$) (Fig. 6D). The maximum frequency of CD38⁺ CD27⁻ CD8^{dim} T cells, which occurred an average of 18 days after the first positive HIV-1

nucleic acid test, was significantly correlated with set point viral load (Spearman $\rho = 0.498$, $P = 0.018$). Fitting a linear regression model with the maximum frequency of CD38⁺ CD27⁻ CD8^{dim} T cells as a predictor yielded a positive relationship with a

marginally significant P value for set point viral load ($\beta = 0.03$, $P = 0.066$). Further examination of model fit revealed two data points with a large Cook's distance. Removing these potential outliers and refitting the model yielded a similar result with a significant P value ($\beta = 0.04$, $P = 0.003$) (Fig. 6E). In addition, the frequency of less functional $CD38^+ CD27^- CD8^{dim}$ T cells was positively associated with higher set-point $CD4^+$ T cell activation ($\rho = 0.448$, $P = 0.037$) (Fig. 6F), another marker of rapid HIV disease progression. Taken together, these data suggest that the loss of $CD4^+$ T cells in acute HIV infection drives the emergence of a suboptimal $CD8^+$ T cell population that is less capable of controlling viral replication and may reflect faster disease progression.

DISCUSSION

The efficacy of $CD8^+$ T cell-mediated control of HIV replication is one of the leading underlying mechanisms that determine the long-term control and disease progression in the absence of antiretroviral therapy. However, in most cases of HIV-infected individuals, $CD8^+$ T cells are incapable of achieving durable control of HIV replication, and there is no documented case of natural clearance or cure from HIV infection. Here, we found that the inefficiency of $CD8^+$ T cells to control viral replication is associated with the emergence of a unique $CD8^+$ T cell phenotype. These $CD8^+$ T cells are characterized by the expression of $CD38^+ CD27^-$ and low expression of the $CD8^+$ T cell receptor. While these cells harbor a high frequency of HIV-specific $CD8^+$ T cell responses, they are less efficient at suppressing viral replication. Moreover, we found that the frequency of this particular phenotype was associated with viral loads and markers for disease progression, suggesting that the generation of $CD8^+$ T cells with the aberrant phenotype greatly influences long-term disease outcome.

$CD8^+$ T cells with low expression of the $CD8^+$ receptor have been observed previously in persistent viral infections and described to have inefficient cytotoxic activity (25–27). Indeed, the presence of a $CD8^{dim}$ population also has been described in chronic HIV infection and associated with poor disease outcome (28). The physiologic role for $CD8^{dim}$ T cells currently is unknown. It has been speculated that either antigen persistence drives the development of an inefficient cellular T cell population or that these cells emerge as a regulatory population that controls the intensity of ongoing cytolytic and tissue-damaging activity (29). While both scenarios have been described in chronic infection and later stages of viral infections, the emergence of these cells during the acute phase of HIV infection is surprising and has been described previously only in simian immunodeficiency virus (SIV) infection (26). In particular, as a large fraction of HIV-specific $CD8^+$ T cell responses are contained in the $CD8^{dim}$ population, their overall contribution to the control of viral replication appears to be relatively minor. Indeed, the frequency of these $CD8^+$ T cells positively correlates with HIV viral load and portend a worse prognosis for disease progression. A potential explanation for the emergence of a dysfunctional $CD8^+$ T cell population is the lack of $CD4$ help during the acute phase of HIV infection due to the massive destruction in the $CD4^+$ T cell compartment. While studies have demonstrated that efficient $CD8^+$ T cell responses can be primed in the absence of $CD4$ help (reviewed in reference 30), $CD4$ -mediated helper signals nevertheless often are required for the generation of long-lived, functional memory $CD8^+$ T cell

responses. Indeed, it has been described that the lack of $CD4^+$ T cell help in the acute phase of infections can influence the frequency, phenotype, and function of $CD8^+$ T cell responses (31, 32). Here, we found that the size of the $CD8^{dim}$ T cell population was associated with the early $CD4^+$ T cell nadir, suggesting that the loss of early $CD4^+$ T cells and their helper function is involved in the emergence of the unique $CD8^+$ T cell populations. Previous studies have already suggested that a low acute $CD4$ nadir is associated with a poor long-term disease outcome (33), but a link to the $CD8^+$ T cell population has not been established. A further indicator that insufficient $CD4$ help is driving the $CD8^{dim}$ phenotype is the activated $CD8^+$ T cell phenotype ($CD38$) but lack of $CD27$ expression. Previous studies suggested that $CD8^+$ T cells primed in the absence of $CD4$ help lack the expression of $CD27$, which renders them inefficient to expand in the recall phase of the response (34, 35).

Thus, our data suggest an explanation for differences in prognosis as estimated here by viral load set point and $CD4$ activation in which $CD4$ count loss and canonical bursts of proliferative cytokines drive the expansion of a less functional $CD8^+$ T cell population that is not able to fully suppress viremia. Furthermore, our data support the notion that ART treatment during early acute HIV infection leads to an overall improvement of $CD8^+$ T cell function by preventing or limiting the emergence of the $CD8^{dim}$ population and ultimately permit durable control of HIV with future treatment interruptions due to a more dominant and functional $CD8^{bright}$ HIV-specific immune response (36–39).

ACKNOWLEDGMENTS

We thank the women and men who participated in the RV217 study. We also thank the RV217 study team: Jeffrey R. Currier, Peter Dawson, Fatim Jallow, Silvia Ratto-Kim, Eugene Kroon, Cornelia Lueer, Jennifer Malia, Mark Manak, Mark Milazzo, Robert O'Connell, Joseph Oundo, Donald Stablein, Erica Sanga, Somchai Sriplichien, and Rapee Trichavaroj.

Financial support for this study was provided by the U.S. Army under cooperative agreement W81XWH-11-2-0174 and the National Institute of Allergy and Infectious Diseases, U.S. National Institutes of Health. The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: $\alpha 4\text{-}\beta 7$ monoclonal antibody (catalog no. 11718) from A. A. Ansari and nevirapine.

The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.

FUNDING INFORMATION

This work was supported by a cooperative agreement (W81XWH-11-2-0174) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DoD).

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