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Differential Gag-Specific Polyfunctional T Cell Maturation Patterns in HIV-1 Elite Controllers

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A small fraction of HIV-infected individuals (<1%), referred to as elite controllers (EC), are able to maintain undetectable viral loads indefinitely without treatment. The role of the maturational phenotype of T cells in the control of HIV infection in these individuals is not well described. We compared the maturational and functional phenotypes of Gag-specific CD4 and CD8 T cells from EC, who maintain undetectable viral loads without treatment; relative controllers (RC), who maintain viral loads of <1,000 copies/ml without treatment; and noncontrollers (NC), who fail to control viral replication. EC maintained higher frequencies of HIV-specific CD4 T cells, less mature polyfunctional Gag-specific CD4 T cells ($CD27^+$ $CD57^-$ $CD45RO^+$), and Gag-specific polyfunctional CD4 T cells than those observed in NC. In EC, the frequency of polyfunctional Gag-specific CD8 T cells was higher than that observed in RC and NC. RC had a similar functional phenotype to that observed in NC, despite consistently lower viral loads. Finally, we found a direct correlation between the frequency of Gag-specific $CD27^+$ $CD57^-$ $CD45RO^+$ $CD4^+$ T cells and the frequency of mature HIV-specific CD8 T cells. Altogether, our data suggest that immature Gag-specific interleukin-2 (IL-2)-producing $CD4^+$ T cells may play an important role in spontaneous control of HIV viremia by effectively supporting HIV-specific CD8 T lymphocytes. This difference appears to differentiate EC from RC.

Most untreated HIV-infected patients have high levels of HIV replication that lead to a rapid deterioration of the immune system. HIV controllers are able to maintain low viral loads (VLs) in the absence of antiretroviral therapy, delaying the progression to AIDS by years (5). A rare subset (<1% of all HIV-infected individuals), known as elite controllers (EC), are capable of spontaneously maintaining undetectable viral loads (15).

Determining the host immune mechanisms responsible for the control of HIV viremia would facilitate the future design of immunotherapeutic studies. It is generally accepted that CD8 cell cytotoxic responses are at least partially responsible for the control of viral replication (12). Preserved CD8 T cell polyfunctionality (2, 16, 19) and degranulation (18), interleukin-2 (IL-2) secretion to support CD4-independent proliferation (8, 13), and overrepresentation of protective HLA alleles (9, 17) have all been suggested to contribute to the control of viral replication in HIV controllers. In addition, preservation of IL-2-producing HIV-specific CD4 T cells has long been thought to contribute to the control of viremia (reviewed in reference 20).

Differences in maturation phenotype also appear to contribute to improved viral control. In progressors, HIV-specific CD8 T cells display a more immature profile than that observed for other antigen-specific CD8 T cells (3, 7), and terminally differentiated HIV-specific CD8 T cells are observed more frequently in relative controllers (RC) than in progressors (1). HIV-specific CD4 T cells, responsible for supporting long-term memory, acquire a more mature phenotype and lose their self-renewing capacity (8, 24). In contrast to progressors, controllers preserve IL-2 production even in effector CD4 T cells (21). Even though altered T cell maturation patterns are consistently modified in HIV progressors (7, 24), their role in the control of viremia is not well described. In this study, we characterized the maturational and functional phenotypes of RC and EC and compared them to those observed for noncontrollers (NC).

MATERIALS AND METHODS

Subjects. Peripheral blood mononuclear cells (PBMCs) were obtained from 40 untreated asymptomatic chronically HIV-infected subjects recruited from the Infectious Diseases Service at Virgen del Rocío University Hospital in Seville, Spain, and were stored at the HIV Biological Bank of the Spanish AIDS Research Network (RIS). Twenty samples were from individuals with persistent viral control (median, 215.7 months; interquartile range [IQR], 132.8 to 241.16 months). Nine of the 20 controllers had undetectable viral loads (<50 HIV RNA copies/ml) and were defined as EC. The remaining 11 controllers showed viral loads between 50 and 1,000 HIV RNA copies/ml and were defined as RC. In addition, 20 study subjects with persistent viral loads of >2,000 HIV RNA copies/ml (median, 21.7 months; IQR, 17.9 to 155.4 months) were defined as NC. Informed consent was obtained and was reviewed and approved by the ethical committee of the hospital for all subjects prior to enrollment in this study.

Cell stimulation. Frozen PBMCs were thawed and washed twice with R-10 medium (RPMI 1640 supplemented with 10% heat-inactivated calf serum, 100 U/ml penicillin G, 100 μl/ml streptomycin sulfate, and 1.7 mM sodium glutamine). Thawed cells were resuspended in R-10 containing 10 U/ml DNase I (Roche Diagnostics) and rested for 2 h before being used. Cells were stimulated at 2×10^6 PBMCs/ml in the presence of 1 μg/ml of anti-CD28, 1 μg/ml of anti-CD49d (BD Biosciences), 10 μg/ml of brefeldin A (BFA) (Sigma Chemical Company), and 0.7 μg/ml of

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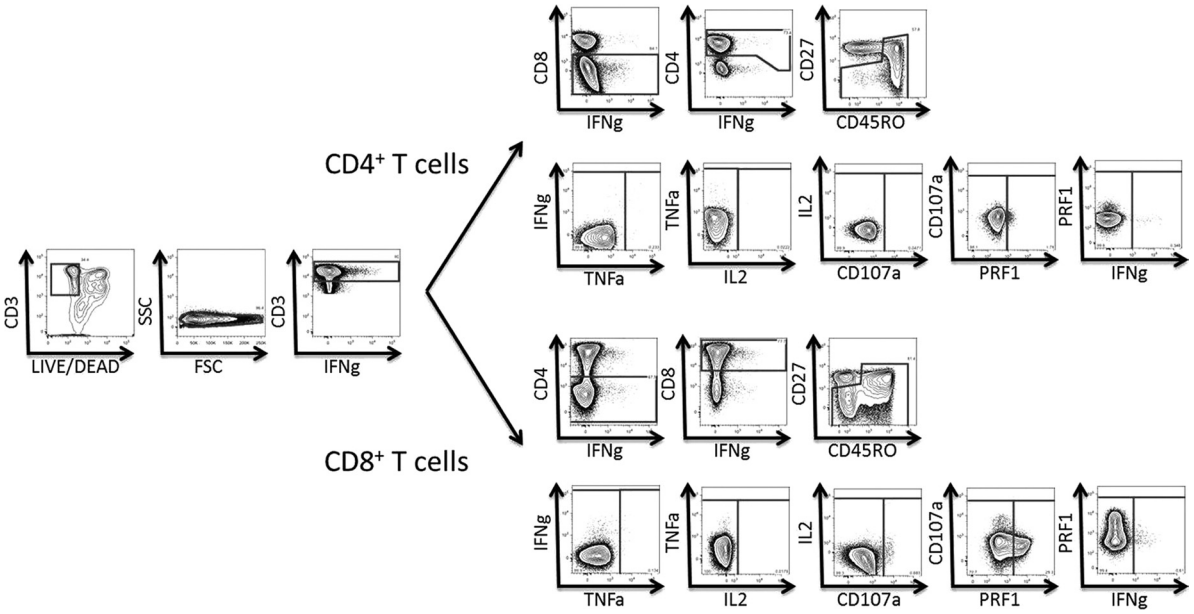

FIG 1 Representative plots showing the gating strategy and functional responses to Gag 15-mers overlapping by 11 amino acids.

monensin (BD Biosciences), in the absence or presence of peptide antigens. Directly conjugated monoclonal anti-CD107a was added at the beginning of incubation. Six-hour stimulations were performed for both cytomegalovirus (CMV) (pp65)- and HIV (Gag)-specific peptide pool stimulation.

Antibodies. Directly conjugated anti-IL-2–Cy55–peridinin chlorophyll protein (PerCP), anti-CD3–Cy7–allophycocyanin (APC), anti-gamma interferon (anti-IFN-γ)–phycoerythrin (PE), anti-tumor necrosis factor (anti-TNF)–Cy7–PE, anti-CD14–Pacific Blue (PB), and anti-CD19–PB monoclonal antibodies were obtained from BD Biosciences; anti-CD45RO–Texas Red PE (TRPE) and anti-CD27–Cy5–PE were from Beckman Coulter; anti-CD4–Cy55–PE was from Caltag; and anti-PRF1–fluorescein isothiocyanate (FITC) clone B-D48 was from Cell Sciences. Anti-CD107a–Alexa 680, anti-CD8–Q-Dot 655 (QD655), and anti-CD57–QD565 were conjugated in our laboratory according to standard protocols (http://drmr.com/abcon/index.html).

Immunofluorescence staining and flow cytometric analysis. Previously stimulated PBMCs were washed and stained for 10 min with a pretitrated quantity of Live/Dead fixable violet dead cell stain (Invitrogen). Cells were then surface stained at room temperature for 30 min with 100 μl of Dulbecco's phosphate-buffered saline (PBS) containing pretitrated amounts of anti-CD14, anti-CD19, anti-CD8, anti-CD45RO, anti-CD27, and anti-CD57. Cells were then washed and permeabilized using a Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions. Cells were stained intracellularly with anti-CD3, anti-CD4, anti-IFN-γ, anti-TNF-α, anti-IL-2, and anti-PRF1 and then washed and fixed in PBS containing 1% paraformaldehyde (PFA). Flow cytometry was performed for all subjects on the same day that the experiment was performed. Twelve-color, 14-parameter flow cytometry was performed on an LSRII flow cytometer (BD Immunocytometry Systems) equipped for the detection of 18 fluorescence parameters. A minimum of 300,000 events were collected for each sample. Electronic compensation was conducted with antibody capture beads (BD Biosciences) stained separately with individual monoclonal antibodies. Analysis was performed using FlowJo, version 9.2 (Tree Star), as previously described (6). Briefly, singlet cells were identified based on forward scatter height (FSC-H) and forward scatter area (FSC-A). Dead cells, B cells, and monocytes were excluded from the live $CD19^-$ $CD14^-$ gate. $CD3^+$ $CD8^-$ $CD4^+$ T cells or $CD3^+$ $CD4^-$ $CD8^+$ T cells were selected sequentially. Different CMV- or HIV-specific T cells were identified by IFN-γ, TNF-α, IL-2, CD107a, and PRF1 production. Maturation patterns were then analyzed using CD45RO, CD27, and CD57 expression. According to these markers, naive T cells (T cells that have never encountered their specific antigen) are defined as $CD45RO^-$ $CD27^+$ cells, memory T cells (antigen-experienced T cells) are defined as $CD45RO^+$ $CD27^+$ cells, and mature effector T cells are defined by the absence of CD27 expression ($CD45RO^+$ for effector memory T cells and $CD45RO^-$ for terminally differentiated effector memory $CD45RA^+$ T cells). The accuracy of these phenotypes has been reported previously (10). A representative example of the gating strategy is shown in Fig. 1. A standard virus-specific response was defined by intracellular cytokine production of IFN-γ, TNF-α, and/or IL-2 in response to virus-specific peptide pools. Subjects were categorized as responders or nonresponders according to their standard response. Graphs were made using Pestle, version 1.6.2 (provided by M. Roederer, NIH, Bethesda, MD), and Spice, version 5.2 (provided by M. Roederer, NIH, Bethesda, MD) (22).

Peptide pools. Peptide pools consisting of 15-mer peptides overlapping by 11 residues covering the entire Gag and pp65 proteins were constructed (NIH AIDS Research and Reference Reagent Program [https://www.aidsreagent.org/index.cfm]) (6). Each pool contained 400 μg of each peptide, and peptides in incubation mixtures were each present at a concentration of 2 μg/ml. All peptides were >70% pure.

Statistical analysis. Continuous variables are expressed as medians (IQR), and categorical variables are expressed as percentages. Nonparametric linear regression analysis was done using the Spearman rank test. The Mann-Whitney U test was used to analyze differences between unpaired groups. Differences between paired samples were determined by the Wilcoxon signed rank test. All P values of <0.05 were considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS 17.0; SPSS, Chicago, IL). Prism, version 5.0 (GraphPad Software, Inc.), was used for the generation of graphs.

RESULTS

Spontaneous control of HIV viremia is associated with Gag-specific T cell responses. Gag and pp65 peptide pools were used to determine the frequencies of pp65- and Gag-specific CD4 and CD8 T cells (defined by detectable IFN-γ, TNF-α, and/or IL-2 cytokine expression) in our cohort. The gating strategy is shown in

TABLE 1 Patient characteristics and percentages of HIV- and CMV-specific T cell responses[c]

Patient	Age (yr) (sex[a])	HLA-B type	VL (HIV RNA copies/ml)	CD4 count (cells/ml)	% HIV response[b]		% CMV response[b]	
					CD4 cells	CD8 cells	CD4 cells	CD8 cells
EC01	46 (M)	NA	<50	619	0.327	0.668	0.303	1.143
EC02	52 (M)	B35/B44	<50	552	1.214	1.803	NR	0.651
EC03	48 (M)	B08/B13	<50	597	0.690	0.972	6.770	1.312
EC04	40 (M)	B14/B51	<50	950	0.876	1.142	0.652	0.467
EC05	45 (M)	B27/B49	<50	676	1.645	3.993	1.855	NR
EC06	47 (F)	B07/B55	<50	429	0.515	1.103	0.422	3.003
EC07	48 (F)	B51/B57	<50	963	NR	1.387	NR	NR
EC08	54 (F)	B07/B55	<50	414	1.144	NR	2.534	NR
EC09	42 (F)	B35/B53	<50	562	1.535	1.136	2.285	1.486
RC01	43 (F)	B08/B27	60	714	NR	4.296	1.533	0.459
RC02	34 (M)	NA	173	555	NR	2.952	2.260	NR
RC03	44 (F)	B14/B44	269	470	2.585	0.921	0.302	1.131
RC04	45 (M)	B38/B44	356	428	8.049	3.726	NR	0.332
RC05	36 (F)	NA	392	580	NR	0.423	NR	0.750
RC06	26 (F)	B13/B18	537	783	0.663	0.301	1.943	0.312
RC07	40 (M)	NA	540	719	0.336	0.831	0.362	NR
RC08	41 (M)	B40/B44	623	624	NR	1.903	NR	NR
RC09	44 (F)	B14/B35	675	412	1.398	1.519	0.768	1.719
RC10	25 (F)	NA	851	497	0.306	NR	0.559	NR
RC11	46 (F)	B07/B45	979	289	0.946	3.547	2.146	3.147
NC01	46 (M)	B14/B51	2,150	319	0.360	2.069	0.463	NR
NC02	49 (F)	B35/B50	2,930	291	0.933	2.017	6.273	0.628
NC03	26 (F)	B51/B52	5,100	726	NR	1.524	0.601	0.315
NC04	48 (M)	B40/B44	6,350	151	0.301	0.342	NR	1.025
NC05	44 (F)	B07/B15	10,000	347	NR	5.514	0.309	5.114
NC06	33 (M)	B50/B57	10,400	152	NR	NR	0.334	0.375
NC07	45 (F)	B45/B51	18,399	572	0.351	0.332	0.499	0.378
NC08	52 (M)	B08/B50	20,000	450	0.502	1.455	0.539	NR
NC09	26 (F)	B18/B58	26,700	478	0.954	2.181	0.734	NR
NC10	40 (M)	B18/B37	35,800	575	NR	NR	0.306	0.433
NC11	43 (F)	B07/B18	40,500	414	0.622	0.596	2.522	1.784
NC12	40 (M)	B07/B14	40,500	984	NR	1.254	NR	NR
NC13	43 (M)	NA	43,400	457	NR	NR	0.300	0.565
NC14	45 (M)	B15/B44	49,400	567	NR	NR	3.024	0.356
NC15	47 (M)	NA	51,800	94	0.346	0.659	NR	1.356
NC16	39 (F)	B08/B27	67,205	355	1.272	NR	1.222	NR
NC17	45 (M)	NA	88,700	650	NR	NR	0.970	NR
NC18	41 (M)	NA	107,000	355	NR	1.526	0.775	0.730
NC19	32 (M)	B18/B35	109,000	479	NR	NR	NR	NR
NC20	42 (M)	B35/B45	152,000	259	NR	2.441	1.186	0.961

[a] M, male; F, female.
[b] Percentage of cytokine-based T cell response after Gag (HIV)- or pp65 (CMV)-specific stimulation. NR, nonresponder.
[c] NA, not available.

Fig. 1. As shown in Table 1, a larger number of EC (8/9 patients [89%]) than RC (7/11 patients [64%]) showed HIV-specific CD4 T cell responses. Moreover, only 45% (9/20 patients) of the NC group showed Gag-specific CD4 cell responses. These differences were statistically significant ($P = 0.027$). HIV-specific CD8 T cells were found in approximately 90% of controllers (8/9 EC and 10/11 RC) and only 65% of NC (13/20 patients [65%]). Differences between groups did not show any statistical significance for CD8 T cell responses, although there was a trend toward higher responses among the controllers ($P = 0.102$). Six of 20 NC (30%), but none of the controllers—either EC or RC—completely lacked Gag-specific responses. In contrast, similar response rates were found among all groups for both CD4 (EC, 78%; RC, 73%; NC, 80% [$P = 0.826$])- and CD8 (EC, 67%; RC, 64%; NC, 65% [$P = 0.951$])-specific pp65 responses.

Immature HIV-specific CD4 T cells are increased in elite controllers and are associated with spontaneous control of HIV viremia. To analyze the level and characteristics of the HIV-specific response better, only subjects categorized as responders (Table 1) were considered. Absolute numbers of HIV-specific CD4 T cells were increased in the EC group (for EC versus NC, $P = 0.0041$). Maturation patterns of the HIV-specific CD4 cell responses were then analyzed. As shown in Fig. 2A, all three groups showed statistically significantly different maturation profiles as defined by surface expression of CD45RO, CD27, and CD57. The EC group had a significantly increased frequency of the most immature ($CD4^+$ $CD45RO^+$ $CD27^+$ $CD57^-$; $Mem57^-$) T cell subset (Fig. 2B). Nevertheless, when maturation patterns of CMV-specific T cell responses were analyzed, no differences were found between groups. Absolute numbers of the $Mem57^-$ CD4

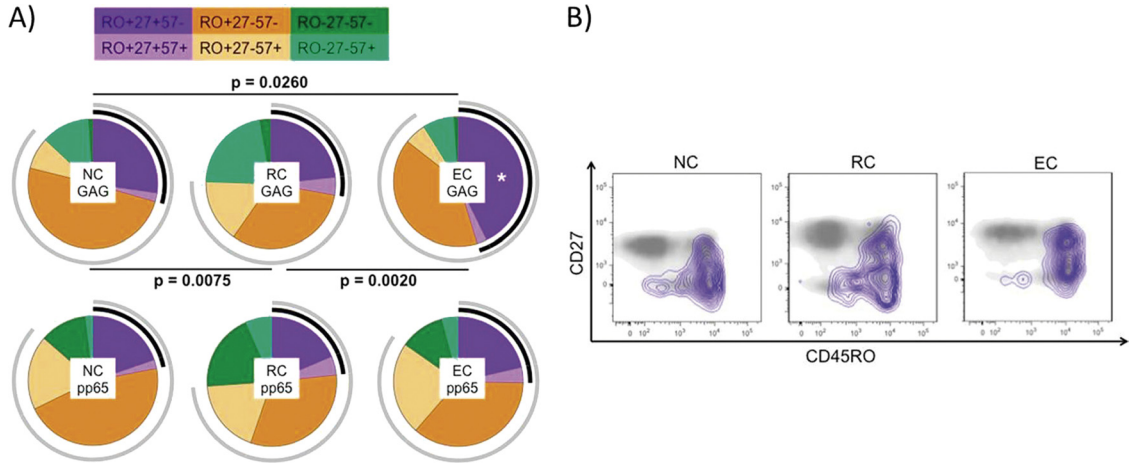

FIG 2 Gag-specific CD4 T cell response. (A) Maturation patterns (analyzed by CD45RO, CD27, and CD57 expression) of pp65- and Gag-specific T cells. Black arcs correspond to CD27 expression, and gray arcs correspond to CD45RO expression. Statistically significant differences among the maturation patterns are shown in the figure. Gag-specific CD4 T cells with the CD45RO$^+$ CD27$^+$ CD57$-$ (Mem57$^-$) maturation profile were increased significantly (*) in the EC group. (B) Gag-specific CD4 T cells (defined by intracellular cytokine production of IFN-γ, TNF-α, and/or IL-2) are shown with purple isobars and overlaid onto two-dimensional plots for total CD4$^+$ T cells plotted against CD27 and CD45RO. EC, elite controllers; RC, relative controllers; NC, noncontrollers.

cell subset were significantly increased in EC (for EC versus NC, $P = 0.038$; for EC versus RC, $P = 0.0311$), but not in RC, compared to NC (for RC versus NC, $P = 0.7577$).

To determine whether HIV-specific Mem57$^-$ CD4 T cells were more polyfunctional than their more mature counterparts, cytokine expression patterns were determined for immature (Mem57$^-$) and mature effector (CD4$^+$ CD45RO$^+$ CD27$^-$ CD57$^-$; $T_{EM}57^-$) HIV-specific CD4 T cells. When the average polyfunctionality was analyzed, a higher frequency of IFN-γ$^+$ TNF-α$^+$ IL-2$^+$ (3-function) polyfunctional T cells was observed in the Mem57$^-$ immature subset than in the $T_{EM}57^-$ subset (Fig. 3A). In addition, all IL-2-expressing phenotypes were also more frequent in the immature subset (Fig. 3A and B). Only the less polyfunctional phenotypes, i.e., IFN-γ$^+$ TNF-α$^+$ IL-2$^-$ and IFN-γ$^+$ TNF-α$^-$ IL-2$^-$, were overrepresented in the HIV-specific mature CD4 T cell subset (Fig. 3B). Since HIV-specific Mem57$^-$ CD4 T cells were increased in the EC group, while RC and NC HIV-specific responses showed a more mature profile, and since both subsets showed different polyfunctionality profiles, we tested whether the HIV-specific Mem57$^-$/$T_{EM}57^-$ CD4 T cell ratio could be associated with the patient's CD4 T cell count and viral load. Interestingly, higher ratios were associated with both lower viral loads (Fig. 3C) and higher absolute CD4 T cell counts (Fig. 3D). A higher Mem57$^-$/$T_{EM}57^-$ ratio could be achieved by two different situations: (i) accumulation of Mem57$^-$ immature T cells that do not progress to T_{EM} maturational status and (ii) a higher loss of T_{EM} T cells because of cells undergoing terminal differentiation. However, as shown in Fig. 2A, terminally differentiated CD4 T cells (CD27$^-$ CD45RO$^-$) were not increased in the EC group, suggesting that EC somehow preserve the immature phenotype of HIV-specific CD4 T cells.

Mature HIV-specific CD8 T cells with differential functional profiles are overrepresented in elite controllers. When the standard virus-specific CD8 cell response was analyzed, no differences were found among the different HIV groups (data not shown), as previously reported for HIV-specific CD8 T cells (1). However, EC showed different maturation patterns from those of RC or NC (Fig. 4A). Both RC and NC showed similar HIV response profiles, while EC HIV-specific CD8 T cells preferentially showed a mature phenotype ($T_{EM}57^-$) (Fig. 4A), to the detriment of more immature (Mem57$^-$) subsets. Polyfunctionality levels of these subsets were then analyzed. EC showed higher frequencies of polyfunctional HIV-specific T cells in both the Mem57$^-$ and $T_{EM}57^-$ subsets (Fig. 4B). However, when all functions were analyzed separately, $T_{EM}57^-$ mature HIV-specific CD8 T cells showed a statistically significant increase in IFN-γ$^+$ TNF-α$^+$ IL-2$^-$ double-positive T cells in the EC group (Fig. 4C). Mem57$^-$ immature CD8 T cells, however, showed similar cytokine expression in every HIV group (data not shown).

Since degranulation and cellular killing play an important role in viral control, we determined the frequency of cytotoxic T lymphocytes (CTL) which surface mobilized CD107a and produced perforin (PRF1) in response to antigenic stimuli. No difference in the frequency of PRF1-producing cells was observed between HIV- and CMV-specific T cells (data not shown). However, although not statistically significant, there was a trend toward a higher-frequency IFN-γ$^+$ TNF-α$^+$ CD107a$^+$ triple-positive subset in the EC group (Fig. 5A). Expression of this polyfunctional subset in the CMV-specific CD8 T cell response was unchanged among the different groups analyzed. To determine whether the relationship between mature and immature HIV-specific CD8 T cells might influence the efficacy of the HIV-specific CD8 T cell response, we calculated the T_{EM}/Mem ratio of these CD107a-expressing polyfunctional T cells. Again, although the difference was just short of statistical significance, the EC group showed higher ratios (namely, a more mature, CD27$^-$ response) than the other groups (Fig. 5B).

Maturation profiles overrepresented in elite controllers are directly correlated in all HIV-infected patients. Finally, to test whether the presence of the more immature, more polyfunctional Mem57$^-$ CD4 T cells might help to support the more polyfunctional, more mature $T_{EM}57^-$ CD8 T cells, we compared the levels of these CD4 and CD8 T cell subsets in all HIV-infected individuals in our cohort. As shown in Fig. 6, individuals with a larger

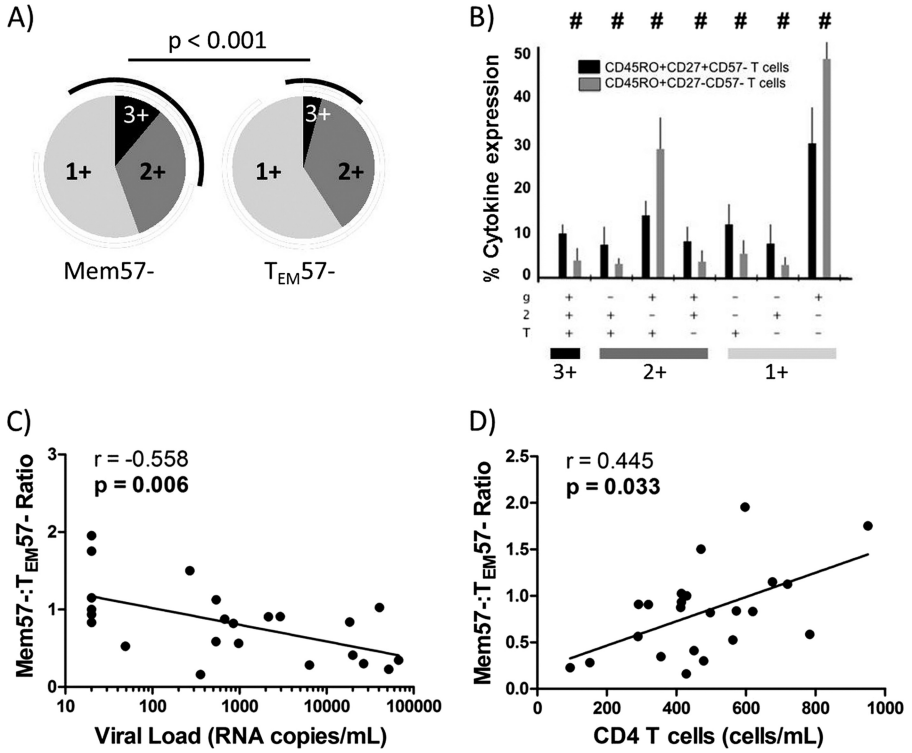

FIG 3 Gag-specific CD4 T cell maturation patterns and immunovirological status. (A) Pie charts showing Mem57$^-$ (CD45RO$^+$ CD27$^+$ CD57$^-$) and $T_{EM}57^-$ (CD45RO$^+$ CD27$^-$ CD57$^-$) HIV-specific T cells which produce 1, 2, or 3 functional responses to Gag stimulation. Black arcs show IL-2-containing functional responses. (B) Comparison of functionality of Gag-specific memory CD4 T cells (black bars) and Gag-specific effector CD4 T cells (gray bars). T, TNF-α; 2, IL-2; g, IFN-γ. (C) Correlation between the $T_{EM}57^-$/Mem57$^-$ ratio and HIV load. (D) Correlation between the $T_{EM}57^-$/Mem57$^-$ ratio and total peripheral CD4 T cell counts.

immature HIV-specific CD4 cell response also had higher frequencies of $T_{EM}57^-$ HIV-specific CD8 cell response for both absolute numbers and percentages (Fig. 6A and B, respectively). This result strongly suggests that the maintenance of a less mature memory CD4$^+$ T cell population provides the necessary T cell help for optimal maturation of effective CD8$^+$ T cell responses.

DISCUSSION

Our results show that higher frequencies of HIV-specific $T_{EM}57^-$ CD8 T cells are associated with spontaneous control of HIV replication in EC and suggest that the maintenance of these cells is important in controlling HIV infection. In addition, these data argue that it is important to maintain HIV-specific Mem57$^-$ CD4 cells during HIV infection; these cells most likely play an important role in support of the $T_{EM}57^-$ CD8 T cell population.

The lack of HIV-specific proliferative CD4 cell responses is a hallmark of progressive HIV infection (14, 23). Our data confirm and extend the observations of others which have shown that individuals who are able to spontaneously control HIV replication are more likely to have a high frequency of HIV-specific CD4 T cells that produce IL-2 when stimulated. Production of IL-2 by CD4 T cells has consistently been associated with increased replicative capacity. In contrast, we found no difference in CMV-specific CD4$^+$ T cell responses between controllers and NC, with CMV infection being controlled by both groups of individuals in our cohort. The EC and RC groups had the same frequency of HIV-specific CD8$^+$ T cells (90%). However, EC had more HIV-specific CD4 T cells (89%) than RC (64%), thus revealing differences in HIV-specific responses between the EC and RC groups. It should be noted that while EC and RC always had an HIV-specific CD4 and/or CD8 cell response, 30% of the NC individuals completely lacked measurable HIV-specific T cells. This straightforward analysis shows the importance of T cell responses in HIV replication control.

Not only did EC have a higher magnitude of HIV-specific CD4 T cell response than RC, but they also had more polyfunctional T cells with a particular maturation phenotype. In agreement with this result, Emu et al. (8) previously reported that lower VLs (VLs of <10,000 RNA copies/ml in untreated or highly active antiretroviral therapy [HAART]-treated, partially suppressed individuals) were associated with higher percentages of memory CD4 T cells. For the HIV controller scenario, Potter et al. (21) compared RC subjects (defined as those having HIV VLs of <400 RNA copies/ml) to NC and found similar rates of IFN-γ-producing HIV-specific CD4 T cells in both groups. They also showed that p24-specific central memory (CD45RA$^-$ CCR7$^+$) and effector memory (CD45RA$^-$ CCR7$^-$) CD4 T cells from RC showed higher frequencies of IL-2 production than similar cells from NC. We extended these results by showing that HIV-specific CD4 T cells are increased in EC compared to RC. Our study extends this interesting work by demonstrating a greater effect in EC, which strongly suggests the importance of retaining a relatively immature phenotype in the CD4 T cell subset for the spontaneous control of HIV replication.

In contrast to our results, Addo et al. (1) reported that termi-

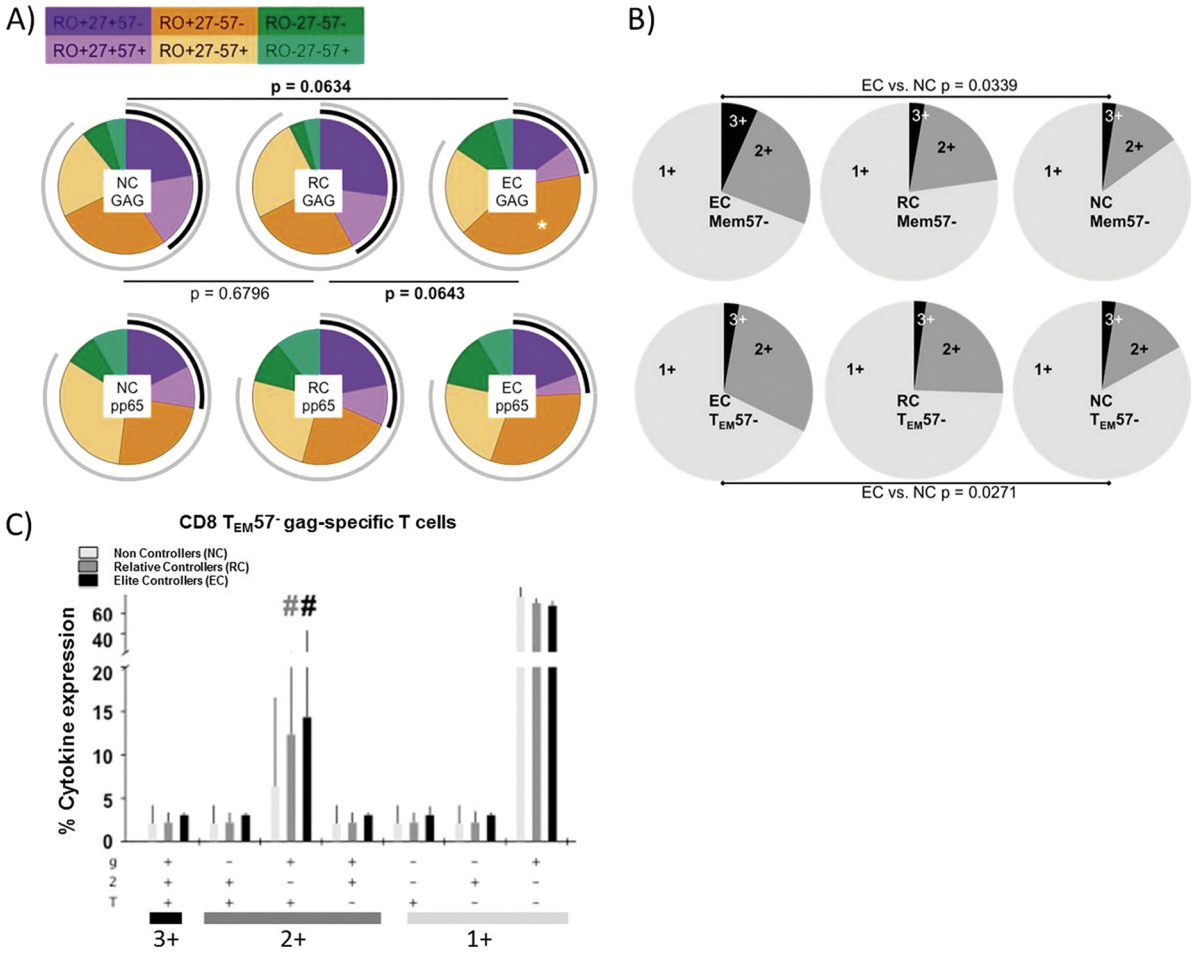

FIG 4 Gag-specific CD8 T cell response. (A) Maturation patterns of pp65- and Gag-specific CD8 T cells. Black arcs correspond to CD27 expression, while gray arcs correspond to CD45RO expression. Statistically significant differences among the pies are shown. Gag-specific CD8 T cells with the $T_{EM}57^-$ maturation profile were increased significantly (*) in the elite controller group. (B) Individual pie charts show Mem57$^-$ and $T_{EM}57^-$ HIV-specific T cells which produce 1, 2, or 3 functional responses to Gag stimulation for every HIV group. (C) Comparison of functionality of Gag-specific $T_{EM}57^-$ effector T cells among the different HIV groups. T, TNF; 2, IL-2; g, IFN-γ; EC, elite controllers; RC, relative controllers; NC, noncontrollers. #, $P < 0.05$.

nally differentiated (CD45RA$^+$ CCR7$^-$) HIV-specific cells are increased preferentially in RC compared to NC. Our data show that it is the CD27$^-$ CD45RO$^+$ CD57$^-$ HIV-specific response that is increased significantly in controllers. This increase results in a more effective polyfunctional response (4). We saw no difference between the functional responses of NC, RC, and EC for the CD27$^-$ CD45RO$^-$ CD57$^-$ CD8$^+$ population. In addition, we saw no significant difference in the frequency of the CD27$^-$

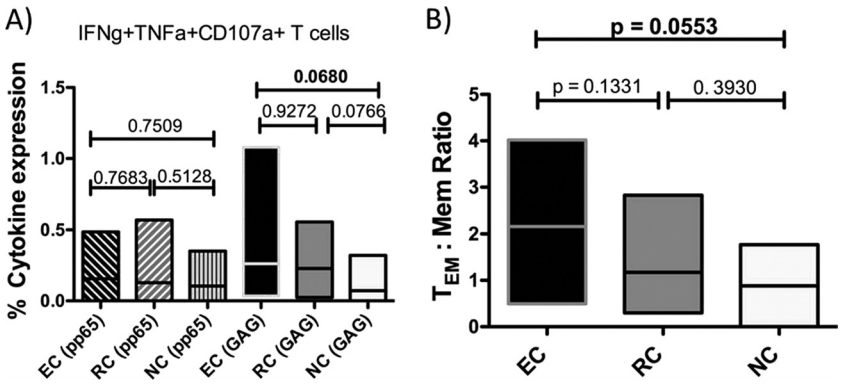

FIG 5 Polyfunctional Gag-specific CD8 T cells. (A) Elite controllers have increased percentages of Gag-specific IFN-γ$^+$ TNF-α$^+$ CD107a$^+$ T cells, while levels of pp65-specific IFN-γ$^+$ TNF-α$^+$ CD107a$^+$ T cells are similar in every group. (B) The T_{EM}/Mem ratio for Gag-specific IFN-γ$^+$ TNF-α$^+$ CD107a$^+$ T cells is increased in elite controllers. T_{EM} cells, CD45RO$^+$ CD27$^-$ cells; Mem cells, CD45RO$^+$ CD27$^+$ cells; EC, elite controllers; RC, relative controllers; NC, noncontrollers.

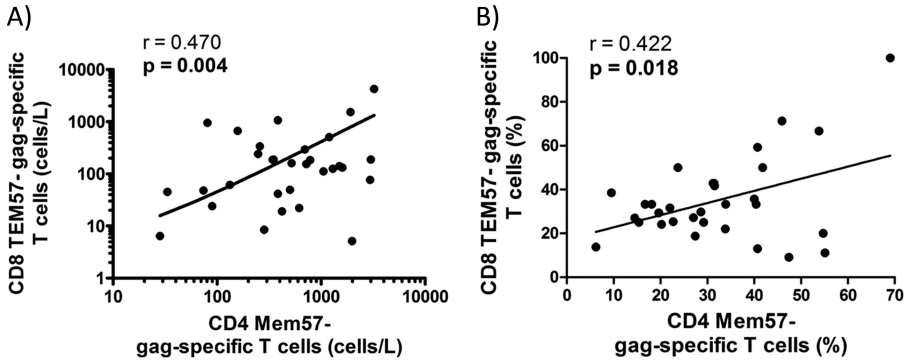

FIG 6 Relationship between Gag-specific CD4 and CD8 T cell responses. A higher CD8 $T_{EM}57^-$ Gag-specific T cell response is directly correlated with a higher CD4 Mem57$^-$ Gag-specific T cell response by both absolute numbers (A) and percentages (B).

CD45RO$^-$ CD57$^-$ CD8$^+$ T cell response in these groups. Several differences in experimental design could contribute to this difference. Addo et al. did not use asymptomatic viremic progressors as we did, and they also used different maturation markers. In addition, Addo et al. used single peptides, whereas we used pooled consensus Gag 15-mers overlapping by 11 amino acids.

In a recent study, a new functional feature of EC, i.e., rapid secretion of perforin (PRF1), was related to viral load control. Hersperger et al. (11) reported PRF1 level differences between EC and chronic progressors (CP), while EC and RC had similar Gag-specific PRF1 expression. Like Hersperger et al. (11), we included both surface mobilization of CD107a and increased PRF1 production as markers of cytolytic activity to determine whether subsets with different maturation patterns also showed enhanced cytotoxicity. Mature HIV-specific CD8 T cells from EC, but not CMV-specific T cells, showed an increase in the IFN-γ^+ TNF-α^+ CD107a$^+$ triple-positive subset, suggesting that more mature HIV-specific CD8 T cells have higher cytotoxic abilities. However, we observed no differences in isolated PRF1 expression among our HIV groups. Hersperger et al. set their cutoff for CP as a load of >10,000 RNA copies/ml (9), while we set our cutoff at >2,000 RNA copies/ml. A limitation of our study is the modest number of EC analyzed ($n = 9$), since these patients, representing less than 1% of the overall total of HIV-infected patients, are difficult to recruit. Therefore, the lack of differences observed in the current study could be related to small sample numbers, and increased PRF1 levels may be detected in a larger cohort.

Despite the need for further longitudinal studies to establish whether these maturation phenotypes are the cause or consequence of the spontaneous control of viremia, our results strongly suggest that less mature HIV-specific "central memory-like" CD4$^+$ T cells, by providing the required T cell help for optimal maturation of the "effector memory-like" HIV-specific T cell population, play an important role in the control of HIV replication. Immunotherapeutic trials should attempt to foster conditions which result in the production and maintenance of these cell types.

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