Kinetics of Expansion of Epitope-Specific T Cell Responses during Primary HIV-1 Infection[1]

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Multiple lines of evidence support a role for CD8$^+$ T cells in control of acute/early HIV replication; however, features of the primary HIV-specific CD8$^+$ T cell response that may impact on the efficiency of containment of early viral replication remain poorly defined. In this study, we performed a novel, comprehensive analysis of the kinetics of expansion of components of the HIV-specific CD8$^+$ T cell response in 21 acutely infected individuals. Epitope-specific T cell responses expanded asynchronously during primary infection in all subjects. The most rapidly expanded responses peaked as early as 5 days following symptomatic presentation and were typically of very limited epitope breadth. Responses of additional specificities expanded and contracted in subsequent waves, resulting in successive shifts in the epitope immunodominance hierarchy over time. Sequence variation and escape were temporally associated with the decline in magnitude of only a subset of T cell responses, suggesting that other factors such as Ag load and T cell exhaustion may play a role in driving the contraction of HIV-specific T cell responses. These observations document the preferential expansion of CD8$^+$ T cells recognizing a subset of epitopes during the viral burst in acute HIV-1 infection and suggest that the nature of the initial, very rapidly expanded T cell response may influence the efficiency with which viral replication is contained in acute/early HIV infection. *The Journal of Immunology,* **2009, 182: 7131–7145.**

To inform rational design of prophylactic vaccines and therapeutic strategies to combat HIV-1 infection, a better understanding of features of the acute-phase immune response that may be beneficial or detrimental to restriction of virus replication is paramount. Multiple arms of the immune system may contribute to control of acute/early HIV-1 replication, but CD8$^+$ T cells are known to be of particular importance. Virus-specific CD8$^+$ T cell activity has been detected in primary HIV-1 and SIV infection at the time of resolution of the acute-phase viral burst (1–3) and has been shown to have a major impact on containment of early virus replication (4, 5). Quantitative, qualitative, and/or kinetic aspects of the CD8$^+$ T cell response mounted in primary HIV-1 infection may therefore have a significant impact on the efficiency of control of virus replication and the prognostically important persisting viral load established in early infection (6). Despite this, there have been few detailed studies of the HIV-specific CD8$^+$ T cell response during the acute and early stages of infection. Notably, the kinetics with which epitope-specific response(s) expand and decline in acute and early HIV-1 infection and how they relate to the kinetics of the acute burst of viral replication are not well defined.

Studies in SIV-infected rhesus macaques and HIV-infected humans have reported detection of virus-specific CTL within the first 3 wk following infection or symptomatic presentation (1–3, 7–10), and in some cases as early as 6 or 7 days following symptomatic presentation (2, 11). T cell responses in acute/early HIV-1 infection have been shown to be of more restricted epitope breadth than those observed in chronic infection (8, 12–16), implying that expansion of responses to different epitopes occurs with different kinetics during the course of infection. However, we are not aware of any study that has addressed this directly, and many important questions remain unanswered about the early-phase T cell response, for example: how many epitopes are typically targeted by the first T cell responses to expand in acute HIV infection; how quickly do responses of additional specificities expand and broaden the overall response; does the magnitude of the most rapidly expanded responses remain high as additional responses are expanded, or does it decline such that waves of T cell expansion and contraction occur; why do T cell responses of different specificities expand at different times after infection; and does the speed at which the first T cell responses emerge (or features of these responses) correlate with the efficiency of early control of virus replication?

In an attempt to answer some of these questions, we studied 21 subjects recruited during acute HIV-1 infection from whom blood samples were drawn at multiple, sequential time points over at least the first 6 mo following symptomatic presentation. The epitopic regions targeted by each patient's HIV-specific T cell response in early infection were mapped, and the kinetics of the response to each of these were analyzed using peptide-stimulated IFN-γ ELISPOT assays or tetramer immunolabeling. We demonstrate that epitope-specific T cell responses expand asynchronously in acute and early HIV-1 infection, with the first wave of responses reaching their peak frequencies as early as (and potentially before)

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5 days following the onset of symptoms (DFOSx).[3] Notably, the most rapidly expanded responses were typically focused on a small number of highly immunodominant epitopes and frequently underwent a rapid decline in magnitude after their peak. In many subjects, there was evidence of a second wave of expansion of responses, peaking ~6–12 wk after symptom onset, followed by slower evolution of the T cell immunodominance hierarchy, with delayed expansion of responses of additional specificities. We show that several mechanisms may contribute to the decline of T cell responses during acute/early HIV-1 infection, including fluctuations in Ag load, T cell exhaustion, and T cell-driven escape.

The first epitope-specific $CD8^+$ T cell responses to be expanded in acute infection may have a critical impact on the efficiency of containment of primary viremia, and should be the focus of future studies aiming to identify correlates of good control of HIV-1 replication.

Materials and Methods

Patient samples

Individuals acutely infected with HIV-1 were recruited at the Mortimer Market Centre for Sexual Health and HIV Research. Ethical approval for these studies was obtained from the local ethical review committee, and written informed consent for participation in the study was obtained from all patients. Subjects were recruited into the study after presenting with acute retroviral symptoms, typically following a known/suspected recent high-risk HIV exposure incident. Blood samples were drawn into EDTA (Sigma-Aldrich), and PBMC were isolated over a Histopaque 1.077 density gradient and cryopreserved until use. Duration of infection is measured as time following the onset of symptoms (FOSx).

Patient HLA class I typing

Genomic DNA was purified from patient PBMC using a QIAamp DNA blood mini kit (Qiagen). High resolution class I typing was conducted on purified DNA by the Oxford Transplant Centre (Churchill Hospital) using a PCR method with sequence-specific primer mixes.

Autologous virus sequencing

In five patients, sequence analysis of the plasma virus population was performed at selected time points throughout the course of acute and early infection, as previously described (17). Briefly, HIV-1 RNA was isolated from plasma using a QIAmp viral RNA mini kit (Qiagen), and cDNA was synthesized from replicate plasma virus RNA preparations using SuperScript III reverse transcriptase (Invitrogen Life Technologies). Replicate cDNA samples (200–1200 RNA molecules/reaction) were subjected to nested PCR amplification. PCR was performed using Elongase enzyme kit (Invitrogen). All PCR products were sequenced directly.

Peptides

All peptides were produced in a peptide-amino acid format. Clade B consensus sequence (2001) and autologous virus sequence 20-mer peptides were purchased as PepSet peptide libraries from Mimotopes. Autologous virus sequence 18-mer peptides were purchased as PEPscreen peptide libraries from Sigma-Aldrich.

IFN-γ ELISPOT assay

Multiscreen plates (MAHAS45 or MAIPS45; Millipore) were coated overnight at 4°C with 10 μg/ml anti-human IFN-γ capture mAb 1-D1K (Mabtech) in sterile PBS. The following morning, plates were washed three times with sterile PBS and then blocked for 2–3 h at 37°C with R10 (RPMI 1640 medium with Glutamax and 25 mM HEPES (Invitrogen) supplemented with 10% FCS (Invitrogen), 100 U/ml penicillin, and 100 μg/ml streptomycin). Patient PBMC were thawed and washed in R10, and viable cell counts were determined by trypan blue exclusion. Cells were plated out at $1.5–2 \times 10^5$/well and stimulated with peptide pools/individual peptides (20-mer peptides were used at a final concentration of 10^{-5} M, and 18-mer peptides at 2 μg/ml) for 24 h at 37°C. Cells stimulated with R10 only or 10 μg/ml PHA served as negative and positive controls, respectively. Plates were subsequently washed six times with PBS/0.05% Tween 20 and incubated overnight at 4°C with 1 μg/ml biotinylated anti-human IFN-γ detection mAb 7-B6-1 (Mabtech) in PBS/10% FCS. Plates were washed again six times with PBS-Tween 20, and then biotin was detected using one of two different methods. In the first, plates were incubated with 1/1000 goat anti-biotin alkaline phosphatase mAb (Vector Laboratories) for 2 h at room temperature before washing three times with PBS-Tween and three times with PBS. Alkaline phosphatase was then developed for 30 min at room temperature using a NBT/5-bromo-4-chloro-3-indolyl phosphate substrate (Bio-Rad) before the reaction was terminated by submerging the plates in water. In the second, plates were incubated with an avidin-HRP conjugate (Vector Laboratories) for 1 h at room temperature and washed three times with PBS-Tween 20 and three times with PBS, and then peroxidase was developed for 2–3 min using 0.33 mg/ml 3-amino-9-ethylcarbazole in *N*,*N*-dimethylformamide and 0.015% hydrogen peroxide (all Sigma-Aldrich) in 0.1 M acetate buffer. The reaction was terminated by submerging the plates in water. Plates were dried thoroughly, and spots were enumerated using an AID image analysis system with AID ELISPOT software version 2.5 (Autoimmun Diagnostika). Spot-forming cells were always readily quantifiable and never exceeded the enumeration limits of detection of the ELISPOT reader.

Mapping of HIV-specific T cell responses using clade B consensus sequence peptides

HIV-specific T cell responses in 16 patients (MM12, MM13, MM19, MM23, MM25, MM28, MM38, MM40, MM46, MM47, MM48, MM49, MM50, MM51, MM55, and MM56) were mapped using 20-mer peptides (overlapping by 10 aa) corresponding to the entire HIV-1 2001 clade B consensus sequence. Peptides were arranged into pools according to protein (Gag, Pol, Env, Nef, and accessory/regulatory protein pools, with each peptide occurring in two separate pools), following peptide matrix systems, as described by Addo et al. (16). Cryopreserved patient PBMC (typically pooled cells from two sample time points between 5 wk and 4 mo FOSx) were screened for responses to all peptide pools by IFN-γ ELISPOT assay. From the matrices, a ranked list of potential epitope-containing peptides was deduced (in accordance with which peptide pools stimulated the largest responses), and the top 30 peptides most likely to contain an epitope were retested individually in a second assay. Peptide stimulations measuring at least 3× background counts and ≥50 IFN-γ^+ spot-forming cells per million PBMC were classed as positive responses.

Mapping of HIV-specific T cell responses using autologous virus sequence peptides

HIV-specific T cell responses in five patients (MM33, MM39, MM42, MM43, and MM45) were mapped using peptides based on the sequence of their individual autologous virus determined at the earliest sample time point available ($t = 0$; mean = 16 DFOSx; median = 21 DFOSx). Peptides (18 mer; overlapping by 10 aa) were assembled into 126 pools of 10 (with each peptide occurring in 3 separate pools), and pools were tested for reactivity in an IFN-γ ELISPOT assay. For each patient, full mapping was performed at ~1 and ~6 mo FOSx. From the matrices, a ranked list of potential epitope-containing peptides was deduced (in accordance with which peptide pools stimulated the largest responses), and putative epitope-containing peptides were retested individually in a second round assay. Peptides stimulating responses measuring ≥3× background counts and ≥50 IFN-γ^+ spot-forming cells per million PBMC were classed as positive responses.

Analysis of effect of intraepitopic sequence variation on T cell recognition (escape analysis)

Peptide-titrated IFN-γ ELISPOT assays were used to address whether sequence evolution occurring during the course of acute and early infection had direct effects on peptide recognition by T cells. Log-fold concentrations of either wild-type or mutant epitope peptide (corresponding to known optimal epitope sequences 9 or 10 aa in length) were used to stimulate a constant number of patient PBMC (typically $1.5–2 \times 10^5$/well), and the number of IFN-γ spot-forming cells was enumerated. A mutant peptide was deemed as an escape variant if at least 1 log more peptide was required to elicit the same magnitude of response as the wild-type peptide.

Preparation of short-term cell lines for cultured IFN-γ ELISPOT assay

Ag-specific cells within total PBMC were expanded in frequency during a 10-day culture period with specific peptide and IL-2. Briefly, PBMC were resuspended in RAB-10 (RPMI 1640 plus 10% human AB serum plus penicillin/streptomycin) at 2×10^6 cells/ml in a 24-well plate with 25

[3] Abbreviations used in this paper: DFOSx, days following the onset of symptoms; FOSx, following the onset of symptoms.

Table I. *Time (measured in DFOSx) at which the first PBMC sample was collected from each patient, and timing of HIV seroconversion*

Patient	DFOSx of First PBMC Sampling Time Point	DFOSx of Last HIV Ab^- Test[a]	DFOSx of First HIV Ab^+ Test
MM12	16	ND	7
MM13	16	ND	15
MM19	13	Evolving at 8	13
MM23	9	5	9
MM25	10	ND	7
MM28	6	6	9
MM33	12	Evolving at 9	12
MM38	29	25	93
MM39	5	Evolving at 3	23
MM40	11	Evolving at 11	18
MM42	22	Evolving at 8	22
MM43	21	Evolving at 6	13
MM45	22	Evolving at 1	22
MM46	5	1	5
MM47	28	Evolving at −1	8
MM48	22	1	Evolving at 16
MM49	16	9	35
MM50	32	Evolving at 15	27
MM51	18	5	18
MM55	31	6	31
MM56	15	4	13

[a] ND, Not determined.

ng/ml IL-7 (R&D Systems) and 10 μM peptide. A total of 10 U/ml human rIL-2 (Roche) was added on days 3 and 7, and cells were fed with fresh RAB-10 on day 7. After 10 days in culture, cells were washed three times with sterile PBS, then resuspended in fresh RAB-10, and cultured for 30 h at 37°C before use in IFN-γ ELISPOT assay, as above.

Phenotyping of HIV-specific $CD8^+$ T cells

Ag-specific $CD8^+$ T cells were identified by surface immunolabeling with PE-conjugated tetramers or pentamers purchased from Beckman Coulter and ProImmune, respectively. The specificity of all multimeric reagents was tested by comparing staining of PBMC from HIV^+ individuals known to respond to the epitope concerned with staining of PBMC from HLA class I-matched, HIV-seronegative individuals. PBMC were labeled for 20 min at 37°C with a preoptimized concentration of the relevant tetramer. For analysis of PD-1 expression, cells were labeled for 30 min at 4°C with CD8 FITC (eBioscience), CD3 PerCP (BD Biosciences), and PD1 allophycocyanin (eBioscience). Data were acquired on a CyAn ADP flow cytometer (DakoCytomation) and analyzed with Flow Jo 8.8.3 software. Fluorescence minus one controls were used to set negative gates.

Statistics

Differences in the mean values between two independent groups were tested for statistical significance using a two-sample, unpaired Student's *t* test. Differences between group means for two sets of data derived from the same set of individuals were tested for statistical significance using a paired Student's *t* test. To test for differences between mean values for multiple groups, ANOVA (using Tukey's correction for multiple pairwise comparisons) was used. The relationship between continuous variables was tested by Pearson's correlation analysis. Statistical analyses were performed using Prism (version 4) or Minitab (version 15) software and independently reviewed by S. Leidi of the Statistical Services Centre, Section of Applied Statistics (University of Reading, Reading, U.K.).

Results

Determination of the epitope specificity of the primary HIV-specific T cell response to enable subsequent analysis of response kinetics

To analyze the kinetics of expansion of HIV-specific T cell responses in primary HIV-1 infection, we studied 21 individuals from whom blood samples were available at sequential time points over at least the first 6 mo FOSx. Table I shows the time (DFOSx) at which the first PBMC sample was collected from each subject,

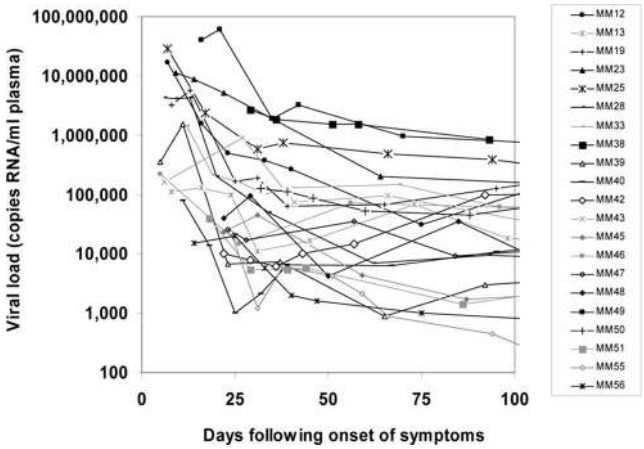

FIGURE 1. Alignment of viral load data from 21 subjects acutely infected with HIV over the first 100 DFOSx. For each patient, the viral load (RNA copies/ml plasma) determined at sequential time points throughout the course of acute and early infection is plotted against time, measured as DFOSx.

together with the timing of HIV seroconversion, and patients' viral loads over the first 100 DFOSx are shown in Fig. 1. The highest recorded acute-phase viral load ranged from 10,100 to 60,000,000 RNA copies/ml plasma (mean = 6,399,371; median = 224,100), and the first available PBMC sampling time point ranged from 5 to 32 DFOSx (mean = 17 DFOSx; median = 16 DFOSx), by which time most individuals had recently seroconverted. Together, these data show that our patients were sampled sufficiently early to enable study of the kinetics of expansion of T cell responses during acute and early HIV-1 infection.

In each subject, we initially mapped the epitopes to which T cell responses could be detected, so that we could then determine the kinetics with which the response to each epitope evolved over the course of acute and early infection. In 16 patients, HIV-specific T cell responses were mapped using PBMC pooled from time points typically between 2 and 3 mo FOSx by IFN-γ ELISPOT assay, and overlapping peptides corresponding to the HIV-1 2001 clade B consensus sequence. CD8 depletion experiments confirmed that the vast majority of T cell responses detected using this methodology were mediated by $CD8^+$ T cells (data not shown). In the individuals whose responses were mapped in this way, the number of epitope-containing regions to which responses were detected at 2–3 mo FOSx varied markedly, ranging from 2 to 21 (mean = 9.4; median = 8 epitopic regions). The most frequently targeted protein was Nef; 13 of 16 (81%) subjects recognized one or more epitopic regions within this protein. None of the subjects targeted Protease or Vpu at the 2- to 3-mo mapping time point.

Disadvantages of this approach are as follows: 1) that responses may have been missed due to failure of T cell responses induced to epitopes in the autologous virus sequence to cross-recognize the corresponding consensus B sequence peptide as a result of sequence difference(s); and 2) that some epitope-specific components of the primary T cell response may have declined in magnitude by 2–3 mo FOSx, and hence, may not have been detected in the mapping assays performed at this time. In five additional patients, T cell responses were thus mapped at ~1 and ~6 mo FOSx using autologous virus sequence-based peptides. At the 6-mo time point, responses were observed to between 4 and 15 epitopic regions (mean = 8.2; median = 7 epitopic regions). Of the total of 16 epitopic regions against which responses were detected in three of five individuals studied, there were differences between the autologous virus and clade B consensus virus sequence in 9. T cells

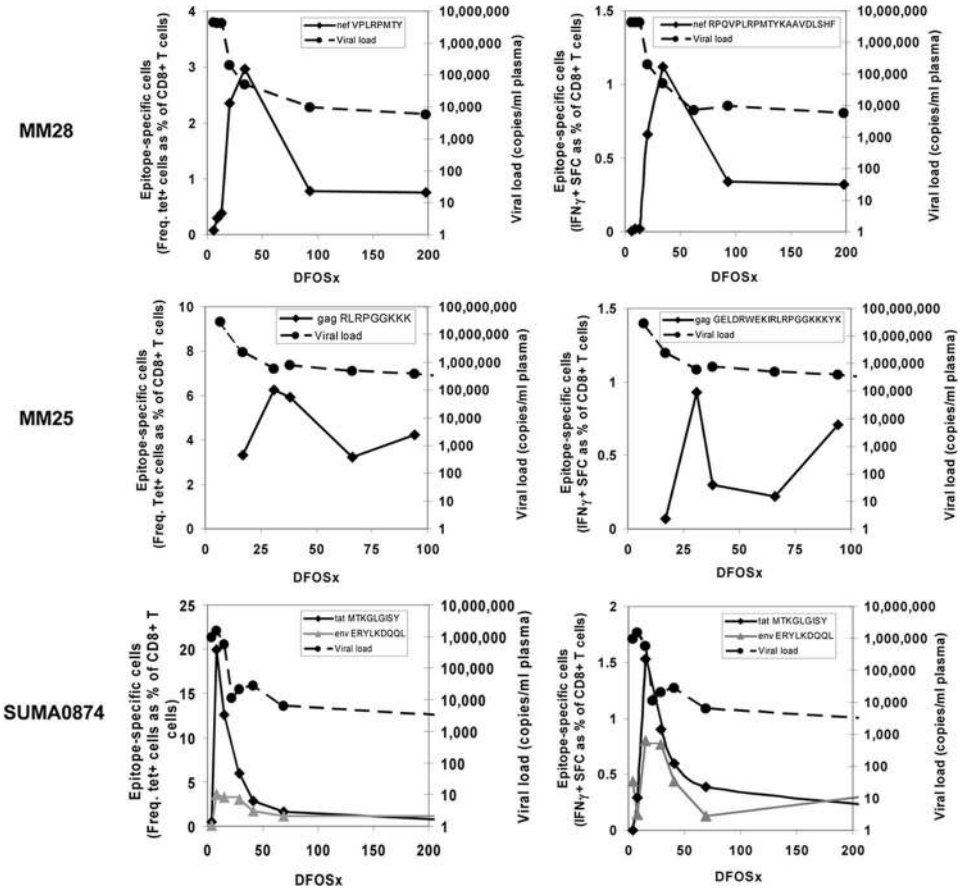

FIGURE 2. Comparison of the use of tetramer immunolabeling and IFN-γ ELISPOT assay to assess the kinetics of expansion of HIV-specific $CD8^+$ T cell responses in primary infection. PBMC cryopreserved at sequential time points over the course of primary infection from patients MM28, MM25, and SUMA0874 were labeled with Abs to CD3 and CD8 and MHC class I tetramers corresponding to epitopes found to be part of the patient's early HIV-specific $CD8^+$ T cell response (*left panels*), or stimulated in an IFN-γ ELISPOT assay with peptides containing the epitope sequences to enumerate the number of epitope-specific $CD8^+$ T cells (*right panels*). For each patient, the frequency of epitope-specific cells detected (solid lines) and the plasma viral load (dashed lines) over time (DFOSx) are plotted.

responsive to 6 of 9 of these were able to cross-recognize the clade B consensus sequence peptide, indicating that at least in these subjects, 19% of the responses would have been missed had mapping been conducted using the clade B consensus rather than autologous virus sequence-based peptides (data not shown). At the 1-mo mapping time point, responses were observed to only between 1 and 8 epitope-containing regions (mean = 3.6; median = 1 epitopic regions). In three of five subjects, the responses detected at 1 mo FOSx were a subset of those detected at 6 mo FOSx, but in the other two patients, a response was detected at 1 mo FOSx that was not observed at the 6-mo time point (data not shown). Together, these results suggest that where responses were mapped at 2–3 mo FOSx using clade B consensus sequence peptides, some epitope-specific components of the initial response were likely to have been missed due to lack of cross-recognition of the test peptides or rapid decline in frequency in the acute phase of infection. Nonetheless, the majority of the acute-phase responses would have been detected.

Effect of methodology used for following response kinetics on the observed pattern of kinetics of expansion of HIV-specific T cell responses

In all patients, HIV-specific T cell responses were initially mapped using overlapping long peptides. In some cases, optimal epitopes were deduced and epitope-specific responses were followed over time using the relevant tetramer/pentamer. However, because it was not feasible to generate multimers for analysis of all epitope-specific responses, in other cases response kinetics were followed by peptide-stimulated IFN-γ ELISPOT assay (using the long peptides corresponding to the epitopic region identified as being targeted by the HIV-specific T cell response in the earlier mapping studies).

Because T cell response kinetics were followed using different methods in different patients, we first thought it important to address whether the methodology adopted to detect Ag-specific T cell populations might impact on the observed results. Fig. 2 shows data from three patients (two subjects from this study, and an additional patient, SUMA0874, whose primary HIV-specific T cell response was mapped using autologous virus sequence-based reagents as part of a previous study (17)), in whom the magnitude of the T cell response to one or more epitopes was measured at sequential time points during acute/early HIV-1 infection by IFN-γ ELISPOT assay and by tetramer immunolabeling. In patient MM28, tetramer immunolabeling of HLA-A11 VPLRPMTY-specific $CD8^+$ T cells revealed that the response to this epitope increased in magnitude over acute-phase time points up to a peak of ~3% of $CD8^+$ T cells at 34 DFOSx, and contracted at time points thereafter (Fig. 2, *upper panels*). When the response to the same epitope-containing region was followed by peptide-stimulated IFN-γ ELISPOT assay and the data adjusted to account for the percentage of $CD8^+$ T cells in the PBMC sample, the magnitude of the response detected using the functional assay was lower than that detected by direct labeling of epitope-specific cells using the tetramer. Nevertheless, a very similar pattern of kinetics of expansion of the response was observed using both methods. Similarly, in patient MM25 (Fig. 2, *middle panels*), the T cell response to the HLA-A3 RLRPGGKKK epitope peaked at 31 DFOSx when kinetics were followed by peptide-stimulated IFN-γ ELISPOT assay or tetramer immunolabeling, although the ELISPOT assay again underestimated the true magnitude of the response. In patient SUMA0874, however, the situation was not so clear cut. Peak frequencies of T cells specific for the HLA-B15 MTKGLGISY and HLA-B44 ERYLKDQQL epitopes were recorded at 8 DFOSx when responses were tracked using tetramer immunolabeling (Fig. 2, *lower panels*), but responses of only low magnitude

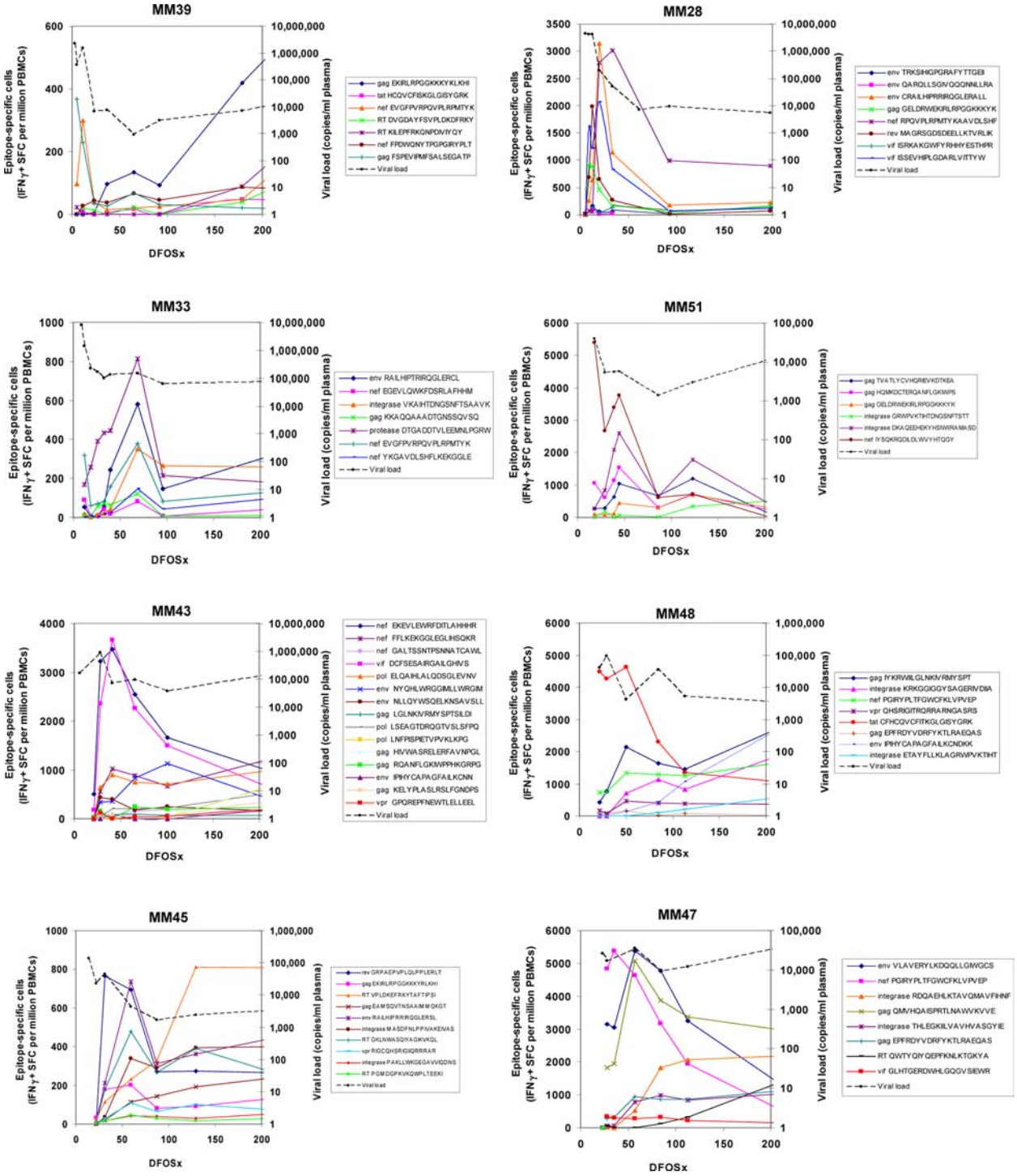

FIGURE 3. HIV-specific CD8$^+$ T cell responses expand asynchronously in acute/early HIV infection. The kinetics of expansion and decline of T cell responses to the entire repertoire of HIV epitopes mapped in each patient were followed by peptide-stimulated IFN-γ ELISPOT assay. Representative results from eight patients are shown as follows: MM39, MM28, MM33, MM51, MM43, MM48, MM45, and MM47. The frequency of epitope-specific cells detected over time (DFOSx) is shown for different epitope-containing peptides. Patient viral loads are indicated by the dashed lines.

were detected at this same time point by IFN-γ ELISPOT assay, and peak frequencies of epitope-specific T cells were instead detected at 15 DFOSx using this approach.

Together these results indicated that, as found in previous studies (18, 19), the IFN-γ ELISPOT assay underestimated the true magnitude of individual T cell responses, and importantly, showed that this effect was especially marked at the earliest sampling time points, highlighting the importance of using multimeric reagents (tetramers/pentamers) to detect Ag-specific T cell populations whenever possible. Nevertheless, when directly compared with tetramer immunolabeling, the IFN-γ ELISPOT assay gave a reasonable representation of the pattern of kinetics of T cell responses at all but the most acute time points.

HIV-specific T cell responses expand asynchronously during acute and early HIV-1 infection

Kinetic studies revealed that in each individual patient, T cell responses to the different epitopic regions defined by our initial

Table II. *Timing and specificity of the earliest studied T cell response(s) in 21 individuals acutely infected with HIV*

Patient	HLA Class I Type	Time of First Available PBMC Sample (DFOSx)	Time of Highest Recorded Magnitude of Earliest T Cell Response(s) Observed (DFOSx)	Number of Responses Peaking at This Time	Epitopes or Epitopic Regions Targeted by the First Analyzed T Cell Responses in Acute Infection (HLA Restriction of Optimally Defined Epitope Peptide Is Indicated)	HIV-1 Protein
MM12	A*03, A*68 B*07, B*44 Cw*07, Cw*07	16	40	2	(HLA-A*03) QIYAGIKVK (HLA-A*03) QVPLRPMTYK	RT Nef
MM13	A*01, A*01 B*08, B*57 Cw*06, Cw*07	16	≤16	3	(HLA-B*08) FLKEKGGL (HLA-B*57) KAFSPEVIPMF (HLA-B*08) GEIYKRWII	Nef Gag p24 Gag p24
MM19	A*01, A*68 B*44, B*57 Cw*06, Cw*07	13	31	2	PRGSDIAGTTSTLQEQIGWM IYKRWIILGLNKIVRMYSPT	Gag p24 Gag p24
MM23	A*02, A*23 B*44, B*49 Cw*05, Cw*07	9	≤9	1	QEEEEVGFPVRPQVPLRPMT	Nef
MM25	A*11, A*03 B*07, B*07 Cw*07, Cw*07	10	≤10	1	GELDRWEKIRLRPGGKKKYK	Gag p17
MM28	A*11, A*30 B*13, B*35 Cw*04, Cw*06	6	9	2	GELDRWEKIRLRPGGKKKYK ISSEVHIPLGDARLVITTYW	Gag p17 Vif
MM33	A*02, A*68 B*07, B*44 Cw*05, Cw*07	12	≤12	3	EGEVLQWKFDSRLAFHHM RAILHIPTRIRQGLERCL EVGFPVRPQVPLRPMTY	Nef Env Nef
MM38	A*01, A*02 B*08, B*07 Cw*07, Cw*07	29	51	1	TNNPPIPVGEIYKRWIILGL	Gag p24
MM39	A*02, A*03 B*15, B*35 Cw*09, Cw*04	5	≤5	1	FSPEVIPMFSALSEGATP	Gag p24
MM40	A*24, A*24 B*07, B*27 Cw*05, Cw*07	11	25	1	PGIRYPLTFGWCFKLVPVEP	Nef
MM42	A*02, A*32 B*07, B*39 Cw*07, Cw*15	22	≤22	2	FDSRLAFHHIARELHPEY VLSIVNRVRQGYSPLSLQ	Nef Env
MM43	A*02, A*02 B*55, B*40 Cw*10, Cw*09	21	27	1	NLLQYWSQELKNSAVSLL	Env
MM45	A*03, A*31 B*07, B*51 Cw*07, Cw*15	22	31	1	GRPAEPVPLQLPPLERLT	Rev
MM46	A*02, A*11 B*08, B*52 Cw*12, Cw*07	5	≤5	10	SRLAFHHMARELHPEYYKDC QEEEEVGFPVRPQVPLRPMT CRAILHIPRRIRQGLERALL VGSPQILVESPAVLESGTKE LKHIVWASRELERFAVNPGL YKAAVDLSHFLKEKGGLEGL TNNPPIPVGEIYKRWIILGL MAGRSGDSDEELLKTVRLIK HGMDDPEREVLVWKFDSRLA PEGTRQARRNRRRRWRERQR	Nef Nef Env Rev Gag p17 Nef Gag p24 Rev Nef Rev
MM47	A*24, A*24 B*39, B*14 Cw*02, Cw*07	28	≤28	2	GLHTGERDWHLGQGVSIEWR VLAVERYLKDQQLLGIWGCS	Vif Env
MM48	A*24, A*26 B*15, B*27 Cw*01, Cw*09	22	≤22	1	CFHCQVCFITKGLGISYGRK	Tat
MM49	A*02, A*02 B*35, B*51 Cw*04, Cw*07	16	35	1	IQAQPDKSESELVSQIIEQL	RT
MM50	A*02, A*03 B*13, B*40 Cw*10, Cw*06	32	39	5	EQLWVTVYYGVPVWKEATTT RSELYKYKVVKIEPLGVAPT CRAILHIPRRIRQGLERALL LRPGGKKKYKLKHIVWASRE KKRYSTQVDPDLADQLIHLY	Env Env Env Gag p17 Vif
MM51	A*02, A*30 B*13, B*44 Cw*06, Cw*05	18	≤18	2	HQMKDCTERQANFLGKIWPS IYSQKRQDILDLWVYHTQGY	Gag p2p7p1p6 Nef
MM55	A*01, A*33 B*14, B*15 Cw*08, Cw*07	31	≤31	4	VLAVERYLKDQQLLGIWGCS QIRSISEWILSTYLGRPAEP EGAVVIQDNSDIKVVPRRKA LVWKFDSRLAFHHMARELHP	Env Rev Integrase Nef
MM56	A*24, A*02 B*35, B*57 Cw*04, Cw*06	15	≤15	1	RDYGKQMAGDDCVASRQDED	Integrase

mapping approach expanded asynchronously, reaching peak frequencies at different times FOSx and then declining (examples of data from eight patients are shown in Fig. 3). We typically observed rapid expansion of a first wave of responses to a subset of viral epitopes that underwent a sharp decline in frequency following their peak and were followed by the slower expansion of responses of additional specificities that tended to be of lower magnitude than the first-wave response. As a consequence of the waves

Table III. *Asynchronous expansion of epitope-specific T cell responses during acute and early HIV-1 infection*

Patient	Total No. of Responses followed over 6 mo FOSx	Cumulative No. of Responses That Had Reached Peak Recorded Magnitude by Specified Time Period				
		≤15 DFOSx	16–30 DFOSx[a]	31–45 DFOSx	46–60 DFOSx	≥61 DFOSx[a]
MM12	4	n/a[b]	0	2	2	4
MM13	4	n/a	3	3	3	4
MM19	7	0	0	7	7	7
MM23	8	1	5	8	8	8
MM25	8	1	2	8	8	8
MM28	8	6	7	8	8	8
MM33	7	3	3	4	7	7
MM38	2	n/a	0	0	1	2
MM39	7	2	2	2	4	7
MM40	3	0	1	1	1	3
MM42	6	n/a	2	6	6	6
MM43	15	n/a	1	6	6	15
MM45	10	n/a	0	1	7	10
MM46	10	10	10	10	10	10
MM47	8	n/a	2	3	5	8
MM48	8	n/a	1	1	5	8
MM49	18	n/a	0	1	1	18
MM50	13	n/a	n/a	5	13	13
MM51	6	0	2	5	5	6
MM55	8	n/a	4	4	8	8
MM56	17	1	11	15	17	17

[a] The difference in the average cumulative number of responses that had reached their peak by ≤30 DFOSx or ≥61 DFOSx was statistically significant ($p = 0.00001$; paired Stuent's t test).

[b] n/a, No PBMC samples were available for measuring response magnitude during specified time period.

of expansion and contraction of HIV-specific T cell responses, the hierarchy of T cell responses typically shifted several times during the course of acute and early infection. This pattern of successive expansion and contraction of HIV-specific T cell responses was observed irrespective of the methodology used to detect Ag-specific T cells and irrespective of whether epitopic regions/optimal epitopes had been mapped using clade B consensus sequence or autologous sequence reagents.

In patients sampled sufficiently early to capture events occurring at or just after the peak in plasma viremia (e.g., MM39, MM28, MM33), a subset of epitope-specific T cell responses was seen to reach its peak magnitude very early after symptomatic presentation (in temporal association with the peak in acute viremia), or was detectable at its highest magnitude at the first available sampling time point and declined in magnitude thereafter, suggesting that they reached peak frequency before this time.

In the patient group as a whole, the earliest T cell responses we were able to detect using the samples we had available peaked between 5 and 51 DFOSx (mean = 23 DFOSx; median = 22 DFOSx), at a mean of 5.5 days (range = 0–33 days) following the highest recorded acute-phase plasma virus titer. However, this average is likely to be an overestimate, because in many patients the first time point available for study was after the peak in acute-phase viremia, and hence, may have been too late to have captured the most rapidly expanded responses (which may have declined to undetectable levels by the time of the first available PBMC sample).

In many patients, the earliest detectable T cell responses were of extremely high magnitude. In several individuals, we observed responses of the order of several thousand IFN-γ^+ spot-forming cells per million PBMC (as measured by peptide-stimulated IFN-γ ELISPOT assay; Fig. 3). Notably, in patient SUMA0874, >20% of total $CD8^+$ T cells were specific for the HLA-B14-MTKGLGISY epitope at 8 DFOSx, as detected by tetramer immunolabeling (Fig. 2).

Limited epitope breadth and protein specificity of the earliest peaking T cell responses detected

Given that the first T cell responses to emerge in acute infection will exert the initial T cell-mediated pressure on virus replication, we were interested to characterize these responses in more detail. In the majority of patients, the earliest peaking T cell responses we studied in acute infection were directed against just one or two epitopic regions (for all 21 patients; median = 2; mean = 2.2 epitopic regions; Table II). This was not always the case, however: the first detectable wave of HIV-specific T cell responses in patients MM46 and MM50 exhibited broader specificity, targeting 10 and 5 epitopic regions, respectively. Furthermore, we previously showed that the primary HIV-specific T cell response in patient SUMA0874 targeted >20 epitopes (17), responses to many of which peaked simultaneously in close temporal association with the peak in acute viremia (data not shown). Notably, we found no significant correlation between the number of epitopic regions targeted by the earliest detectable wave of T cell responses in individuals and their set-point viral load ($p = 0.76$; $R^2 = 0.0048$), suggesting that factors other than the epitope breadth of the first detectable T cell responses may impact on early containment of viral replication.

In contrast to the highly focused nature of the first wave of T cell responses observed in most individuals, responses of much broader epitope specificity were detected by 2–3 mo FOSx because additional wave(s) of T cell expansion had occurred by this time, adding to the overall response breadth. For instance, in the 16 subjects mapped using clade B consensus sequence-based peptides, a mean of 9.4 epitopic regions was targeted by the T cell response at this time (median = 8; range = 2–21 epitopic regions). Table III summarizes for each patient the number of T cell responses followed over the first 6 mo after symptomatic presentation and the relative kinetics with which responses of different epitope specificities peaked after symptom onset, and demonstrates the gradual

increase in the epitope breadth of the HIV-specific T cell response over this time.

We were also interested in the specificity of the T cell populations constituting the earliest detectable wave of responses in our acutely infected subjects. For the T cell responses that we observed to peak earliest in each individual patient (including multiple epitope-specific responses in the same patient if they peaked simultaneously), the three most frequently targeted HIV-1 proteins were Nef, Gag, and Env, accounting for 73% of the total repertoire of epitopic regions targeted (28, 26, and 19%, respectively). When we restricted our analysis to responses detected ≤16 DFOSx, responses to Nef were most common, accounting for nearly 40% of all responses detected at this time.

Mechanisms underlying the decline in magnitude of epitope-specific T cell responses during acute/early HIV-1 infection

The epitope-specific T cell responses that were rapidly expanded in the first-wave response in acute/early HIV-1 infection typically underwent a marked decline in frequency after their peak. In no patient did we see an early T cell response plateau and sustain its peak magnitude for any considerable length of time. Several possible mechanisms (which may not necessarily be mutually exclusive) may drive this decline, including the following: 1) decreasing antigenic load as acute viremia is brought under control; 2) virus sequence variation and replacement of the wild-type epitope with variant(s) that is recognized less well by $CD8^+$ T cell responses (escape); and/or 3) exhaustion and deletion of the early responding T cell population(s). We addressed whether or not there was evidence to suggest that each of these mechanisms may be contributing to the contraction of epitope-specific $CD8^+$ T cell responses during acute and early infection.

Plotting the magnitude of epitope-specific T cell responses over time against plasma viral load revealed that the kinetics of expansion and contraction of certain T cell responses in some subjects closely paralleled their viral load dynamics, as would be expected if fluctuations in magnitude were driven by Ag abundance. In patient MM51, for example (Fig. 4, *upper panel*), we observed an initial decline in the magnitude of rapidly expanded responses concomitant with the decrease in viral load, and then an increase in the frequency of epitope-specific T cell coincident with a blip in viral replication at ~40–50 DFOSx. In patient MM42 (Fig. 4, *middle panel*), the initial decline in the frequency of T cells responsive to the earliest detectable peaking T cell response was also temporally associated with a decline in acute-phase viremia, although in this case, a subsequent increase in the plasma viral titer was not associated with a corresponding expansion of the epitope-specific T cell population. Likewise, in patient MM39 (Fig. 4, *lower panel*), although a Nef-specific response expanded coincident with a blip in viral replication at ~10 DFOSx, T cells responsive to another viral epitope (in Gag) declined in frequency at this time. Thus, whereas viral dynamics do appear to impact on the magnitude of HIV-specific T cell responses, variation in viral titer does not fully explain the pattern of expansion and contraction of HIV-specific T cell responses in acute/early HIV-1 infection.

However, plasma viremia reflects only the overall viral burden, not the presence of specific epitope sequences. Additional experiments were thus conducted in a subset of patients to address the contribution of sequence variation and escape to driving the decline of T cell responses in acute/early HIV-1 infection. Using longitudinal virus sequence data, we determined whether a decline in the magnitude of the first detectable peaking T cell responses was temporally associated with amino acid mutation within and/or surrounding the targeted epitopic region. Of a total of 28 earliest

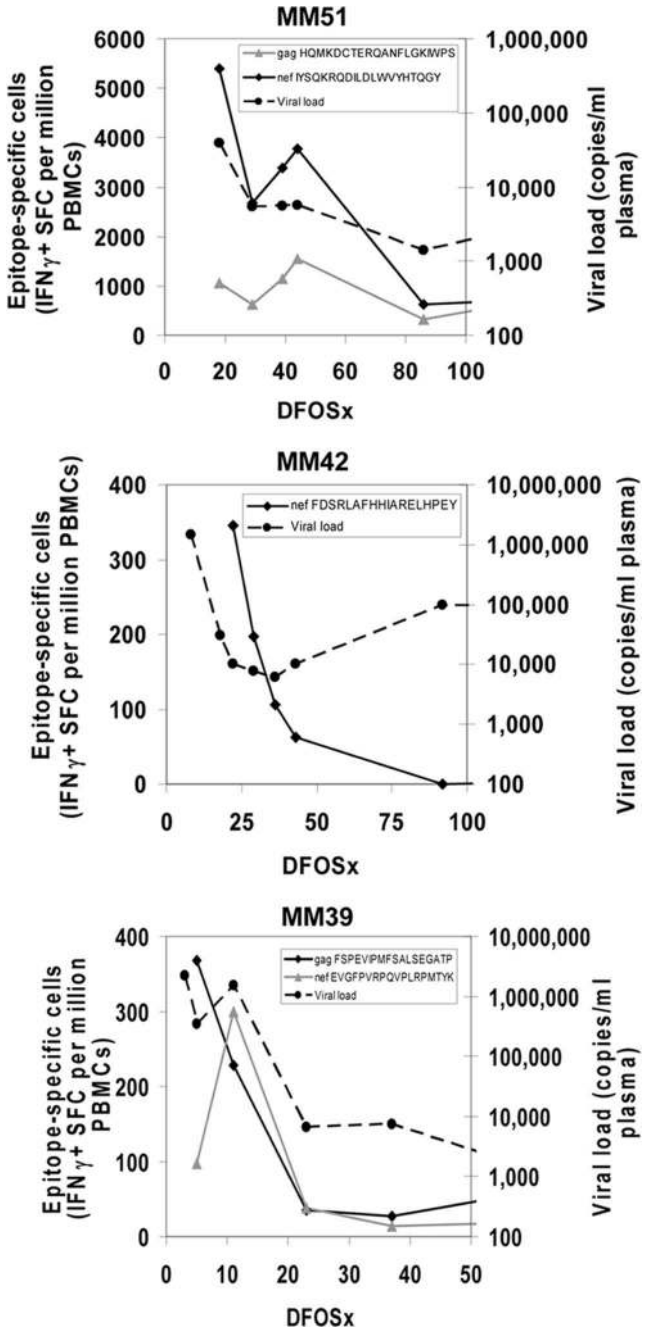

FIGURE 4. The magnitude of certain HIV-specific T cell responses closely follows viral load dynamics in acute/early HIV-1 infection. The magnitude of epitope-specific T cell responses was followed at sequential time points over the first 50–100 DFOSx by peptide-stimulated IFN-γ ELISPOT assay in patients MM51, MM42, and MM39. The frequency of T cells specific for the indicated epitopic region (solid lines) and plasma viral load (dashed lines) is plotted against time (DFOSx).

detectable peaking T cell responses studied, only 4 (14%) showed sequence variation within the epitopic region around the time the response peaked in magnitude and/or was in decline. In 4 of 4 cases, the sequence variants that evolved to dominate the quasispecies were recognized less well than the corresponding wild-type sequences by PBMC from time points around the time of response decline, and were therefore deemed to represent T cell escape variants (Fig. 5). Interestingly, in the five patients whose autologous early HIV-specific T cell responses were studied over the first 6 mo FOSx, we found that only ~20% of all targeted

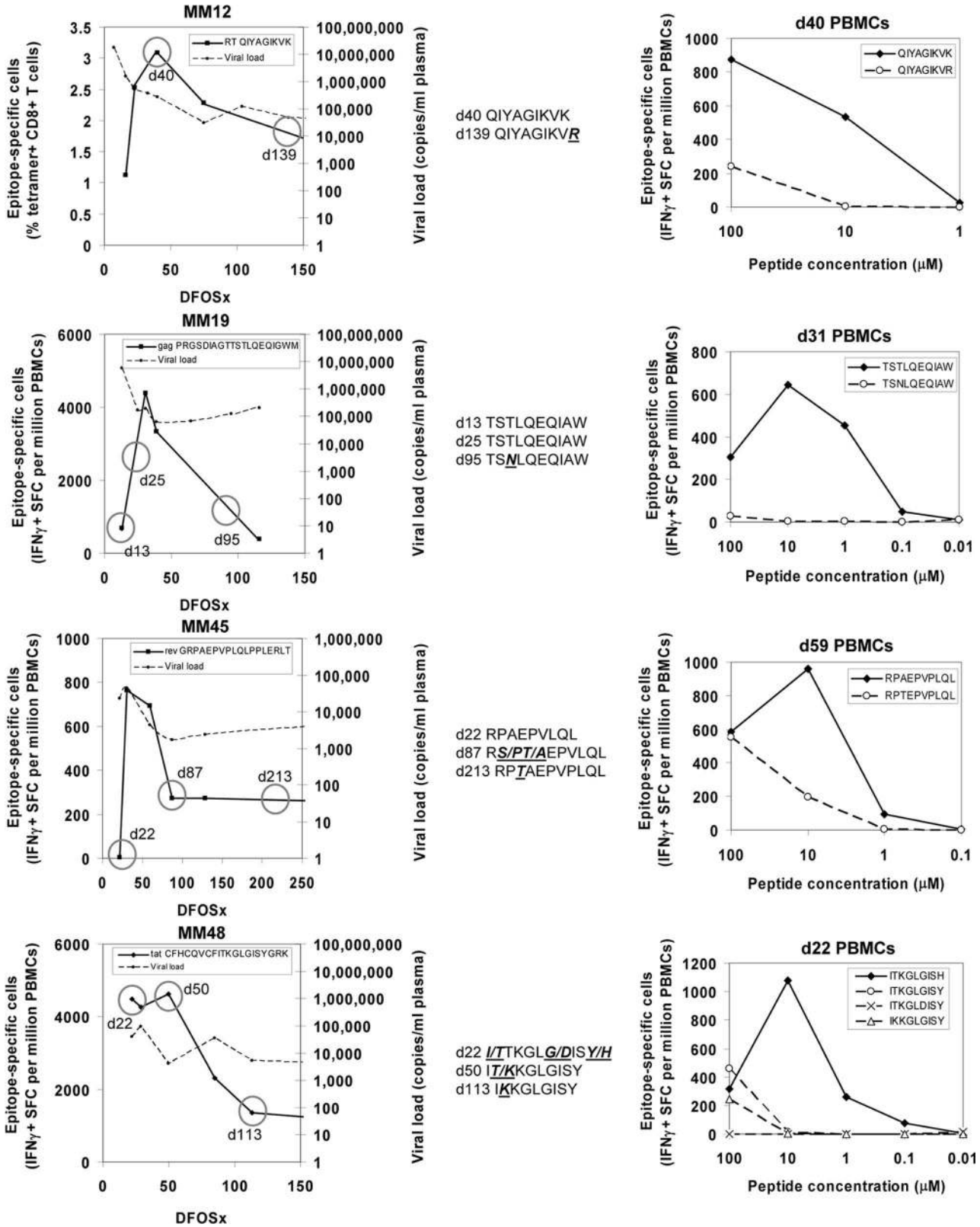

FIGURE 5. The decline in magnitude of a proportion of the earliest detectable peaking T cell responses in acute/early HIV-1 infection is temporally associated with mutational escape. The magnitude of epitope-specific T cell responses was followed at sequential time points over up to 250 DFOSx by tetramer immunolabeling in patient MM12 and by peptide-stimulated IFN-γ ELISPOT assay in patients MM19, MM45, and MM48 (*left panels*). The frequency of T cells specific for the indicated epitopic region (solid lines) and plasma viral load (dashed lines) is plotted against time (DFOSx). The time points at which autologous virus sequencing of the epitope-containing region was performed are indicated (gray circles), and the sequence of the optimal epitope peptide at these time points is shown (*center*). *Right-hand panels*, Show data from IFN-γ ELISPOT assays examining the ability of PBMC cryopreserved at around the time point at which the T cell response was found to decline in magnitude to recognize the index epitope peptide sequence (determined at the earliest available plasma time point; solid lines) and the mutant epitope peptide sequence that evolved to dominate the quasispecies (dotted lines).

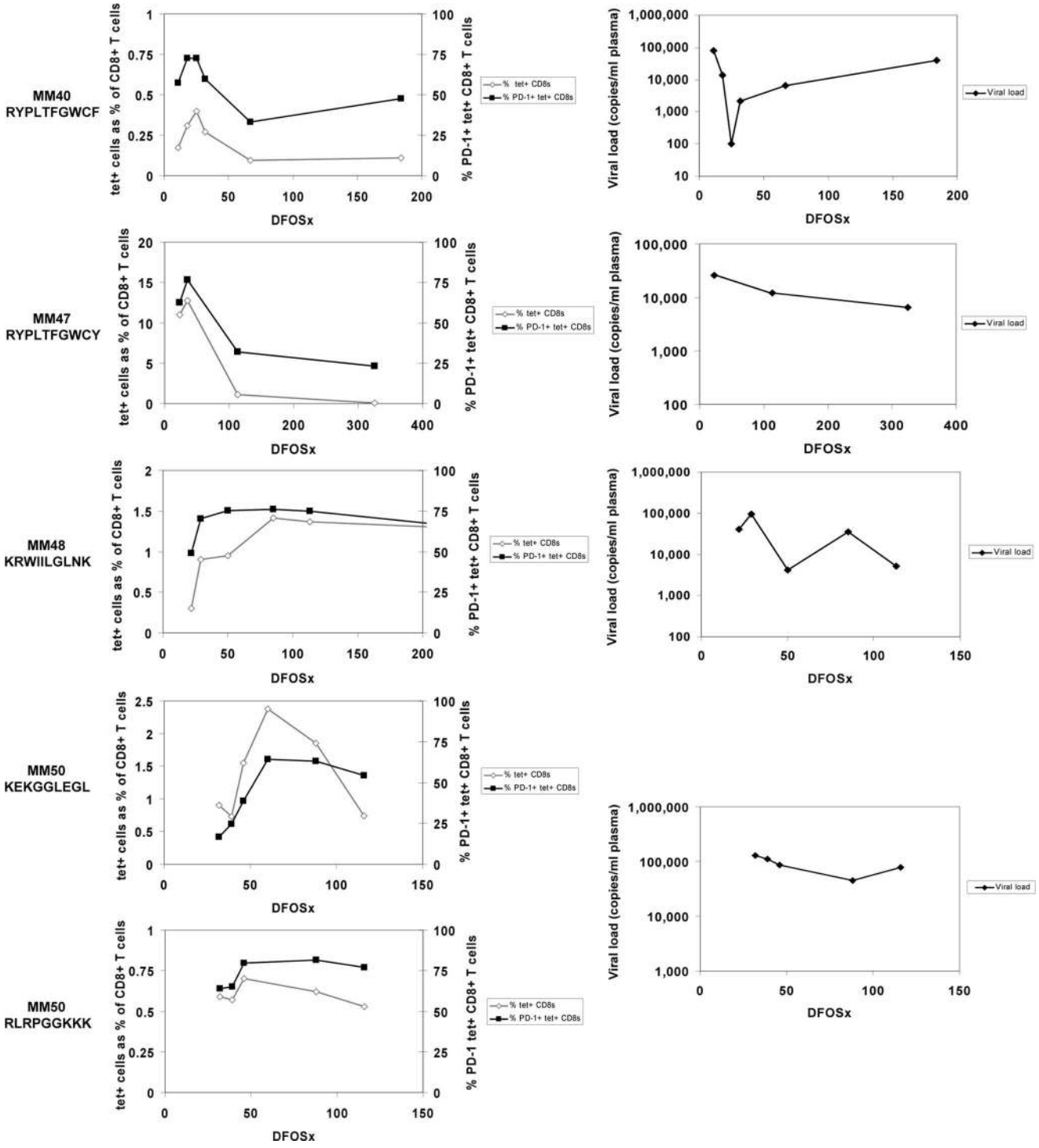

FIGURE 6. Longitudinal analysis of PD-1 expression by HIV-specific $CD8^+$ T cells. Longitudinal analysis of PD-1 expression was performed for the following patients' early-phase T cell responses: MM40 (HLA-A24 RYPLTFGWCF), MM47 (HLA-A24 RYPLTFGWCY), MM48 (HLA-B27 KRWIILGLNK), and MM50 (HLA-B40 KEKGGLEGL and HLA-A3 RLRPGGKKK). PBMC from sequential time points during infection were stained with the relevant tetramer/pentamer, and mAbs to CD3, CD8, and PD-1. PD-1 expression by total $CD3^+CD8^+$ T cells and tetramer$^+$ $CD8^+$ T cells was subsequently analyzed. Fluorescence minus one controls were performed at each time point to set the negative gate. Graphs in the *left column* show the percentage of tetramer$^+$ $CD8^+$ T cells at each time point and the corresponding proportion of tetramer$^+$ cells expressing PD-1. Graphs in the *right column* show viral load (copies/ml plasma) over time (DFOSx) for each patient.

epitopic regions exhibited amino acid change around the time of response decline, suggesting that the majority of T cell responses decline in magnitude in the absence of T cell-driven escape. Furthermore, that only 1 of 28 epitopic regions targeted by earliest peaking T cell responses showed amino acid change within the N- and C-terminal flanking regions (10 aa either side of the epitope) argues against a major role for Ag processing-impairing mutations in driving the decline in magnitude of the first-wave responses (data not shown).

It is important to note that we may have underestimated the contribution that T cell-driven escape makes to the decline of epitope-specific components of the primary HIV-specific T cell

Table IV. *Comparison of the proportion of epitopic regions showing sequence variation by 6 mo following symptomatic presentation, for T cell responses dominant or subdominant at different time intervals during acute and early HIV-1 infection*

	Proportion of Epitopic Regions Targeted Showing Sequence Variation by 6 mo FOSx during Specified Time Period					
	0–1 mo FOSx		1–2 mo FOSx		2–6 mo FOSx	
Patient	Dominant Responses	Subdominant Responses	Dominant Responses	Subdominant Responses	Dominant Responses	Subdominant Responses
MM33	2/2 (100%)	1/3 (33.3%)	3/4 (75%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)
MM39	0/2 (0%)	ND[a]	0/1 (0%)	1/2 (50%)	0/1 (0%)	2/6 (33.3%)
MM42	2/2 (100%)	ND	1/1 (100%)	2/3 (66.7%)	1/1 (100%)	0/3 (0%)
MM43	2/2 (100%)	3/8 (37.5%)	2/2 (100%)	3/9 (33.3%)	3/5 (60%)	3/9 (33.3%)
MM45	3/5 (60%)	ND	2/2 (100%)	3/8 (37.5%)	0/1 (0%)	5/9 (55.5%)
Mean	72%	35%	75%	51%	45%	38%

[a] ND, No responses were detected in the specified patient during the specified time period.

response, because in several patients the earliest available plasma viral loads suggested that the first PBMC sample time points were well after peak of acute viremia. In these patients, it is possible that first-wave responses that declined very rapidly could have been missed because they had fallen below the level of detection by the time point when the first PBMC sample was obtained. Nonetheless, our results indicate that although sequence variation may contribute to the rapid decline in the magnitude of epitope-specific responses constituting the first detectable wave of T cell responses in acute HIV infection, escape is not a prerequisite for response decline.

To address whether T cell exhaustion may also be involved in driving the decline of epitope-specific T cell responses following their peak, we performed a longitudinal analysis of surface PD-1 expression (a marker of T cell exhaustion (20–22)) on Ag-specific $CD8^+$ T cells (Fig. 6). For all responses studied, we found that a proportion of HIV $tetramer^+$ cells was already $PD\text{-}1^+$ at the first sampling time point, and that the proportion of cells expressing PD-1 increased and decreased in a pattern that closely followed the response magnitude over time. This was an Ag-specific effect because of the following: 1) total $CD8^+$ T cells did not up-regulate PD-1 expression during the same study period (data not shown), and 2) different epitope-specific T cell responses in the same individual exhibited different kinetic patterns of PD-1 expression (as shown in patient MM50 for responses to the HLA-B40 KEKGGLEGL and HLA-A3 RLRPGGKKK epitopes). The percentage of PD-1-expressing total $CD8^+$ T cells was not related to the viral load. The observation of PD-1 expression on a high proportion of $tetramer^+$ cells at the time epitope-specific responses reached their peak suggests that T cell exhaustion may be among the mechanisms that contribute to the postpeak decline in Ag-specific T cell frequencies in acute/early infection.

If Ag-specific T cells are exhausted and subsequently deleted from the repertoire after excessive acute-phase stimulation, these cells should not be able to clonally expand upon re-exposure to Ag. We were therefore interested to test for the presence of memory T cell populations specific for epitopes to which T cell responses had declined to levels undetectable in ex vivo IFN-γ ELISPOT assays. We cultured patient PBMC from chronic time points ($\geq$1 yr FOSx) for 10 days with peptide and IL-2, and examined whether this had resulted in expansion of epitope-specific T cells by measuring the response of the cultured populations to peptide stimulation by IFN-γ ELISPOT assay. In five of seven cases tested, Ag-specific T cells were expanded after short-term culture (range = 389–10,903 IFN-γ^+ spot-forming cells per million PBMC; data not shown), implying that at least some of the T cell responses that reach peak magnitude during acute/early infection and subsequently decline to undetectable levels may not be terminally exhausted and deleted from the repertoire, but persist at low frequency and can undergo clonal expansion following peptide stimulation. However, other epitope-specific T cell populations may not persist and/or retain the capacity to expand on restimulation.

Extent and kinetics of viral sequence evolution within epitopic regions targeted by T cell responses in acute and early HIV-1 infection

Despite the relatively low proportion of T cell responses that declined in magnitude in temporal association with escape during acute/early infection, escape may nevertheless play an important role in determining T cell expansion and contraction in early infection, and we were therefore interested to study this further in our patient cohort. In five patients in which autologous virus sequence data were available from the first available plasma sampling time point ($t = 0$), and at 3 and 6 mo FOSx, we determined the extent of sequence variation occurring by 3 and 6 mo FOSx within all epitopic regions targeted by the T cell response during the first 6 mo following presentation. In these five patients, an average of 18% of the epitopic regions targeted by the early T cell response showed sequence variation by 3 mo FOSx. This was increased significantly to 42% of epitopic regions showing variation by 6 mo FOSx ($p = 0.03$; unpaired Student's t test). Thus, sequence variation is common in early HIV-1 infection.

To address how the relative immunodominance of T cell responses expanded with different kinetics during acute/early HIV-1 infection may affect the extent and/or rapidity of viral escape, we grouped epitope-specific T cell responses according to whether they were dominant or subdominant at 0–1 mo, 1–2 mo, or 2–6 mo FOSx (supplemental Table I)[4] and compared the percentage deviation from the $t = 0$ index sequence of the epitopic region recognized by 6 mo FOSx (Table IV). The greatest difference in the relative proportion of epitopic regions showing sequence variation was seen for those targeted by dominant and subdominant T cell responses during the 0- to 1-mo time period (72 vs 35%). The difference between epitopes that were dominant and subdominant at 1–2 mo FOSx was less pronounced, and it was even less so for dominant and subdominant epitopes targeted between 2 and 6 mo FOSx. Furthermore, a greater proportion of epitopic regions targeted by responses that dominated at 0–1 or 1–2 mo showed sequence variation by 6 mo FOSx as compared with responses that

[4] The online version of this article contains supplemental material.

were dominant between 2 and 6 mo FOSx. Although the differences between the groups did not reach statistical significance (probably due to the low number of patients studied), the trend suggests that epitope-specific responses that are more dominant during acute/very early infection may more frequently exhibit sequence variation (potentially indicative of escape) compared with responses that are subdominant within the same time period, and also compared with responses that may be dominant later on. This may be a reflection of the intense immune pressure exerted on the virus in acute/very early infection when the very earliest T cell responses are highly focused, targeting a very limited number of epitopes. As the epitope repertoire targeted by the T cell response broadens out over time, the immune pressure exerted on the virus to escape control from more dominant T cell response may be less overall.

Discussion

In this study, we have shown that virus-specific T cell responses expand in an asynchronous fashion in acute and early HIV-1 infection. The first emerging T cell responses can peak extremely rapidly following symptomatic presentation, tend to be focused on just one or two epitopic regions, and typically show a marked decline in frequency following their peak; this may be driven by a combination of decreasing viral load, viral escape, and T cell exhaustion. The decline in the most rapidly expanded responses coincides with the emergence of a second wave of T cell responses, which may in turn be followed by further responses, resulting in an overall broadening of the T cell response and successive shifts in the immunodominance hierarchy over time.

There are several caveats to the methodologies we used to map the specificity and kinetics of expansion of T cell responses in this study. First, mapping with clade B consensus sequence-based reagents may lead to a proportion of T cell responses (particularly in more variable regions of the virus) being missed due to lack of T cell recognition of the consensus sequence peptide (23, 24), an issue that we confirmed in analyses performed on a small subset of patients. Secondly, by mapping T cell responses at ~2–3 mo FOSx, we may have missed initially immunodominant responses that declined in frequency below the limit of detection by the time mapping was performed. However, in five individuals in which we mapped responses at both 1 and 6 mo FOSx, most T cell responses were detectable at the two time points. A third caveat relates to the methods used for evaluating the magnitude of epitope-specific T cell populations over time. Direct comparison of the magnitude of certain epitope-specific T cell responses using IFN-γ ELISPOT assay and tetramer immunolabeling confirmed previous reports that ELISPOT can substantially underestimate the true magnitude of responses (18, 19). This was particularly noticeable during acute infection when T cells are highly activated and may become exhausted or undergo apoptotic cell death on in vitro restimulation (25, 26). Indeed, in patient SUMA0874, the peak time point calculated for two early epitope-specific responses was earlier when tetramer immunolabeling rather than IFN-γ ELISPOT assay was used to track the Ag-specific population. Thus, where the kinetics of T cell responses were mapped solely by IFN-γ ELISPOT assay, the true peak of the first emerging T cell response(s) may have occurred earlier than we were able to detect.

Finally, in the absence of exact dates of HIV-1 infection for our patient group, infection duration was measured in DFOSx of the acute retroviral syndrome (which are subjective and could possibly confound our estimates of the timing of the first wave of responses induced in HIV-1 infection). However, it has been shown previously that the symptoms experienced by many acutely infected individuals are triggered concomitant with the surge of cytokine production induced as plasma viral load increases (27, 28), and that onset of symptoms occurs 6–15 DFOSx before the peak in acute phase viremia (29). DFOSx thus provide a reasonable surrogate for the temporal alignment of the kinetics of events in the acute phase of HIV-1 infection.

Despite the limitations of the approaches used in this study, we were nonetheless able to document some key features of the HIV-specific T cell response expanded in acute/early infection. First, we demonstrated that epitope-specific components of the primary HIV-specific T cell response can be expanded remarkably quickly in acute HIV infection, and may reach peak frequencies as early as 5 DFOSx, around the time of the peak in acute plasma viremia. In our study, it was not possible to address the relationship between the timing and/or magnitude of the first wave of T cell responses expanded in acute HIV-1 infection and the subsequent efficiency of control of plasma viremia, because in some individuals, the first time point available for study was during the viral contraction phase and the true peak in the first-wave response may well have been missed. Furthermore, even in those patients who were sampled before peak viremia and despite the fact that samples were obtained at weekly intervals for the first month following study enrollment, the highly dynamic nature of events occurring during acute infection means that true response peaks may have been missed. In the future, this will be an important issue to address, because studies in murine and nonhuman primate models have shown that small changes in the speed and efficiency of CTL-mediated inhibition of viral replication can have profound effects on viral control (30–32).

A second key feature of the virus-specific T cell response expanded in acute HIV-1 infection is that responses of different epitope specificities did not expand synchronously. This asynchronous expansion contrasts with what is typically observed in acute viral infections that are rapidly cleared, such as acute lymphocytic choriomeningitis virus or influenza virus infections in mice, in which responses of different epitope specificities expand and contract in parallel (33, 34). However, if lymphocytic choriomeningitis virus is not cleared following acute infection, immunodominant responses are successively exhausted and eventually deleted, leading to dominance of initially subdominant responses (35, 36). Shifts in immunodominance may also occur as a result of changes in Ag expression as infection progresses from the acute to the persistent phase (as in murine γ-herpes virus and human EBV infections (37–39)), or as a result of the priming and clonal expansion of naive T cells long after the initial infection (40). As in other persistent infections (e.g., hepatitis C virus), changing patterns of epitope immunodominance may be driven by viral mutational escape from dominant components of the virus-specific $CD8^+$ T cell response (41).

In this study, we show that the first wave of T cell responses to be expanded in the earliest stages of acute HIV-1 infection in the majority of patients is focused on just one or two epitope-containing regions, consistent with the observation of oligoclonal T cell expansions in the peripheral $CD8^+$ T cell pool at this time (12) and with previous reports that T cell responses in subjects acutely infected with HIV are of more limited epitope breadth than those observed in chronically infected individuals (16, 42–44). That the first-wave HIV-specific T cell responses were so restricted in their epitope specificity was very intriguing given that each patient clearly could respond to a variety of other epitopic regions. Although no single Ag entirely dominated the first detectable wave of T cell responses in our cohort (which is perhaps not surprising in a study population that is highly heterogeneous for HLA class I), in the patient group as a whole, responses to Nef and Gag were most common. When we restricted our analysis to those responses

peaking ≤16 DFOSx, responses to Nef were most common. This is consistent with observations from a previous study (45), in which Nef was also found to be preferentially recognized by $CD8^+$ T cells in primary infection.

We can only speculate that the following factors could all conceivably play a role in determining the limited specificity of the first wave of T cell responses in acute HIV infection: 1) Ag abundance; 2) the efficiency and kinetics of epitope peptide processing; 3) the affinity of the epitopic peptide for class I; 4) the avidity of the responding T cell population for the epitope peptide/HLA complex; and 5) the existence of cross-reactive memory T cell populations (46–49) (reviewed in Ref. 50). The emergence of a highly biased first-wave response in acute infection may also be facilitated by the fact that dominant responses can actually suppress the expansion of subdominant T cell responses of alternate specificities (51–54), or be exacerbated as a consequence of the limited availability of activated APCs and/or $CD4^+$ T cell help (55–61). Interestingly, a small subset of the patients studied in this work exhibited more broadly specific first-wave T cell responses, although there was not a significant correlation between response breadth and the efficiency of control of viral replication.

Those T cell responses that were expanded most rapidly in acutely infected individuals typically underwent a marked decline in frequency as plasma viremia was contained. Several factors may contribute to this decline. T cells are ultimately Ag driven, and a decrease in the frequency of Ag-specific T cells as infection is contained is a typical feature of many acute viral infections (33, 34). The rapid decline of the first detectable wave of T cell responses seen in this study may just reflect a normal homeostatic response to decreasing Ag load as acute viremia resolves. Our observation that declining epitope-specific T cell responses re-expanded coincident with blips in viral titer also lends support to the hypothesis that Ag load may help drive the expansion and contraction of T cell responses in acute/early HIV-1 infection. However, Ag load cannot be the only driving force, not least because a proportion of T cell responses still declined to very low levels despite persistent high-level virus replication.

It is possible that excessive acute-phase antigenic stimulation may drive T cells to exhaustion and/or apoptosis (25, 26, 62, 63), also contributing to the decline in the magnitude of T cell responses following their peak. We found that the proportion of epitope-specific $CD8^+$ T cells expressing the exhaustion marker PD-1 closely followed the magnitude of the response over time, with the highest proportion of $PD\text{-}1^+$ $tetramer^+$ cells being observed at the time point at which the response peaked. This may suggest that Ag-specific T cells gradually become impaired in function and/or proliferative capacity to the point in which the response declines as a result. Cause and effect are, however, difficult to ascertain. Data from others suggest that PD-1 expression on HIV-specific $CD8^+$ T cells may be intimately linked to Ag load (64); hence, the decrease in the proportion of $tetramer^+$ cells expressing PD-1 after the peak magnitude of the response may simply reflect a reduction in availability of the wild-type epitope and activation state of the responding T cell population. Furthermore, one would expect that if Ag-specific T cells were exhausted and/or deleted from the repertoire, they should not exist as a memory population that could clonally expand upon re-exposure to Ag. In this study, for several T cell responses that had declined in magnitude to levels undetectable by ex vivo ELISPOT assay, we found that IFN-γ-secreting Ag-specific T cells could be expanded by short-term culture with peptide and IL-2. This implies that at least in these cases, a decline in the magnitude of HIV-specific T cell responses may be driven by mechanisms other than exhaustion and deletion.

Selection for escape-conferring viral sequence changes is another mechanism that may contribute to the drop in frequency of initially immunodominant T cell responses in acute HIV infection. We found that the decline in the magnitude of the earliest detectable peaking T cell responses was temporally associated with sequence variation and escape in <20% of cases, fewer than we would have anticipated, given that escape is known to be promoted where the T cell response is highly biased (17, 65). However, the sampling regimen may have impacted on our ability to detect true first-wave T cell responses in a subset of the individuals studied in this work; hence, it is possible that we may have underestimated the contribution made by sequence variation and escape to the decline of the very first T cell responses mounted in acute infection. Escape may nevertheless play an important role in driving the subsequent pattern of kinetics of T cell responses in acute/early HIV-1 infection. In a subset of patients, we found sequence variation to occur within a relatively high proportion of epitopic regions targeted by initially immunodominant T cell responses, a result consistent with our previous findings (17). When dominant epitope-specific T cell responses are escaped, these may decline in frequency and be replaced by previously subdominant responses to other epitopes, and/or responses of different specificities may oscillate in frequency in an escape-driven fashion (66).

Our observation that the primary HIV-specific T cell response is highly dynamic has important implications for future studies of T cell responses in acute/early HIV-1 infection. The T cell responses that emerge earliest and peak around the time of the peak in acute-phase plasma viremia may be involved in resolving primary viremia and/or influencing the persisting viral load established. Notably, several previous studies have failed to document differences in qualitative characteristics of the HIV-specific T cell response in subjects controlling virus replication with differing efficiency (16, 67–70). However, the very first wave of T cell responses may have been overlooked in these studies due to lack of sufficiently early samples, fragility of cells at early time points, or a failure to map T cell responses sufficiently early in infection. Factors such as 1) the kinetics with which the first HIV-specific T cell responses are mounted, 2) the extent to which the first T cell responses are escaped and the cost to viral fitness of the escape mutations involved, and 3) qualitative aspects of the earliest T cell responses (e.g., avidity, cytolytic capacity, range of cytokines/chemokines produced) may all impact on the overall efficiency with which early virus replication is controlled, and consequently on the nature of the subsequent disease course, and therefore should be analyzed to identify correlates of good control of acute virus replication. Such information could be valuable for rational vaccine design.

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Disclosures

The authors have no financial conflict of interest.

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