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Temporal Dynamics of the Primary Human T Cell Response to Yellow Fever Virus 17D As It Matures from an Effector- to a Memory-Type Response

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The live attenuated yellow fever virus (YFV) 17D vaccine provides a good model to study immune responses to an acute viral infection in humans. We studied the temporal dynamics, composition, and character of the primary human T cell response to YFV. The acute YFV-specific effector CD8 T cell response was broad and complex; it was composed of dominant responses that persisted into the memory population, as well as of transient subdominant responses that were not detected at the memory stage. Furthermore, HLA-A2− and HLA-B7−restricted YFV epitope-specific effector cells predominantly displayed a CD45RA+CCR7−PD-1−CD27low phenotype, which transitioned into a CD45RA−CCR7−PD-1−CD27low memory population phenotype. The functional profile of the YFV-specific CD8 T cell response changed in composition as it matured from an effector- to a memory-type response, and it tended to become less polyfunctional during the course of this transition. Interestingly, activation of CD4 T cells, as well as FOXP3+ T regulatory cells, in response to YFV vaccination preceded the kinetics of the CD8 T cell response. The present results contribute to our understanding of how immunodominance patterns develop, as well as the phenotypic and functional characteristics of the primary human T cell response to a viral infection as it evolves and matures into memory. The Journal of Immunology, 2013, 190: 2150–2158.

The ideal immune response to a viral infection is initiated by a strong and appropriately balanced innate response that contains viral replication, followed by a highly specific adaptive response that seeks out and eliminates the invading pathogen. In the best of all situations, this occurs without persistent damage to the host, without development of chronic infection or inflammation, and with the establishment of protective immunological memory to subsequent encounters with the virus. In reality, however, many immune responses are less successful. Infections with pathogens, such as HIV-1 or hepatitis C virus, become chronic and are associated with persistent immune responses that contribute to immunopathology (1–3). In contrast, infections with viruses of the Flavivirus genus, such as the yellow fever virus (YFV), can be associated with acute immunopathology, including life-threatening viral hemorrhagic fever (4). YFV infection causes an estimated 200,000 illnesses and 30,000 deaths yearly, and ~90% of these infections occur in Africa (5). The vast majority of YFV cases are preventable, because an effective vaccine was developed in the 1930s after successful attenuation of the YFV Asibi strain (6). The YFV vaccine has received renewed attention because of its high efficacy and safety (5) and because it provides a model for studies of human immune responses to a mild, but nevertheless replication-competent, acute viral infection (7–9).

Primary antiviral T cell responses go through a phase of rapid proliferation and expansion of Ag-specific T cell clones. Murine models, such as the comprehensively studied immune response against lymphocytic choriomeningitis virus, showed that, during the peak of the response, a majority of CD8 T cells can be specific for the infecting virus (10, 11). This massive response then contracts into a much smaller memory population after viremia is controlled and the infection has been cleared. Murine models have also been instrumental in the understanding of the patterns of immunodominance in Ag-specific responses, where complex responses composed of several distinct epitope specificities establish hierarchies of dominant, subdominant, and cryptic responses (12, 13). To a large extent, studies of human T cell responses to viral infections have focused on pathogens causing chronic infections with wide implications for human health, such as HIV-1, EBV, hepatitis C virus, and CMV (14–20). In this context, surface receptors and markers, such as CD45 isoform, and homing receptors CCR7 and CD62L were proposed to identify T cell differentiation stages, identifying CD45RA−CCR7− cells as naive, CD45RA−CCR7−/− memory cells, and cells reverting to CD45RA expression without CCR7 as late-stage effector cells (21, 22). However, a recent study by Akondy et al. (23) showed that CD8 T cells against an HLA-A2−restricted YFV epitope displayed a predominantly CD45RA+ phenotype during the effector response, whereas it was CD45RA− at the memory stage. These findings illustrate the value of studies of primary infection models, such as the YFV 17D vaccine, for obtaining a better understanding of how immunodominance patterns develop, as well as the phenotypic and functional characteristics of the primary human T cell response to a viral infection as it evolves and matures into memory.
understanding of human T cell responses to this specific virus, as well as viral infections in general.

In the current study, we adopted the YFV 17D model to further study the specificity, breadth, functional and phenotypic characteristics, and immunodominance patterns in the primary T cell response to this acute viral infection. T cells in the CD8, CD4, and FOXP3+ regulatory T cell (Treg) compartments all responded to YFV, and the kinetics of CD4 and Treg responses preceded the CD8 T cell response. The CD8 T cell response against a range of YFV candidate epitopes was broad and complex, composed of both dominant responses that persisted into the memory population, as well as of transient subdominant responses that were not detected at the memory stage. Furthermore, HLA class I tetramer–defined YFV-specific effector cells predominantly displayed a CD45RA–CCR7–PD-1+ phenotype that transitioned into a CD45RA+CCR7–PD-1+ memory phenotype. The functional profile of the CD8 T cell response changed composition as it matured and tended to become less polyfunctional in the transition from the effector to the memory stage. In conclusion, the results presented in this article add to our understanding of how the primary human T cell response to viral infection evolves and matures into memory.

Materials and Methods

Study subjects and samples

Twenty-one healthy volunteers ranging from 18 to 50 y of age were recruited into the study after informed consent. Approval of procedures was obtained from the Regional Ethical Review Board in Stockholm. A single dose of yellow fever vaccine strain 17D (Stamaril; Sanofi Pasteur) was administered s.c., and blood samples were obtained just before vaccination (baseline) and at days 10, 15, and 90 after vaccination. PBMCs were purified by Lymphoprep gradient centrifugation (Axis-Shield, Dundee, U.K.), according to standard protocols, and cryopreserved in 90% FCS and 10% DMSO.

Epitope search and synthetic peptides

Candidate epitopes presented by any of the HLA-A1, -A2, -A3, -A24, -B7, and -B8 supertypes were predicted using the NetCTL search engine (version 2.1). Seventy-eight peptides were synthesized by standard 9-fluorenlymethyloxycarbonyl chemistry, purified to 90% purity by reverse-phase HPLC, and validated by mass spectrometry (JPT Peptide Technologies, Berlin, Germany).

Flow cytometry and mAbs

T cell responses to YFV were assessed using multicolor flow cytometry (26). The following mAbs and HLA-tetramers were used in flow cytometry: anti-CD4 Pacific Blue, anti-CD8 PerCP, anti-CD33 PE-Cy7, anti-CD27 FITC, anti-HLA-DR PerCP, anti-Ki67 FITC, anti-CCR7 PE-Cy7, anti-IFN-γ FITC, anti-MIP-1β PE, anti-TNF-α PE-Cy7, anti-CD107a biotin, anti-IFN-γ allophycocyanin, and anti-IL-2 allophycocyanin were all from BD Bioscience (San Jose, CA). Anti-FOX3 Alexa Fluor 660, anti-CD38 Alexa Fluor 700, anti-CD127 Alexa Fluor 700, anti–programmed death-1 (PD-1) (CD279) PE, and anti-CD25 PE-Cy7 were from eBioscience (San Diego, CA). Anti-CD14 Qdot 525, anti-CD19 Qdot 525, Qdot 605 streptavidin, anti-CD38 Qdot 705, Near-IR LIVE/DEAD, and Aqua LIVE/DEAD were from Invitrogen (Carlsbad, CA). HLA-A2 NS4b LLW, HLA-87 NS5 RPI, and HLA-A24 NS1 VYM tetramers in allophycocyanin and anti-CD3 ECD were from Beckman Coulter (Brea, CA). Anti-CD45RA was from BioLegend (San Diego, CA), and anti-CD3 Cascade Yellow was from Dako (Glostrup, Denmark). For phenotypic analysis of cells, incubation for 15 min at room temperature with HLA tetramer alone was followed by 30 min at 4°C in the dark with surface mAbs. This was followed by washing with PBS and fixing in 1% paraformaldehyde. For intracellular staining of Ki67 and FOXP3, cells were fixed and permeabilized with Fix/Perm (eBioscience) for 30 min at 37°C in the dark. Flow cytometry data were acquired on a CyAn ADP or BD LSRFortessa (BD Biosciences) and analyzed using FlowJo software version 8.8.6 or 9.1 (TreeStar, Ashland, OR) and FlowJo Software (San Jose, CA). Anti-CD107a biotin, anti–IFN-γ PE, anti–HLA-DR PerCP, anti-Ki67 FITC, anti-CCR7 PE-Cy7, anti–MIP-1β PE, anti-TNF-α PE-Cy7, anti-CD107a biotin, anti-IFN-γ allophycocyanin, and anti-IL-2 allophycocyanin were all from BD Bioscience (San Jose, CA). Anti-FOX3 Alexa Fluor 660, anti-CD38 Alexa Fluor 700, anti-CD127 Alexa Fluor 700, anti–programmed death-1 (PD-1) (CD279) PE, and anti-CD25 PE-Cy7 were from eBioscience (San Diego, CA). Anti-CD14 Qdot 525, anti-CD19 Qdot 525, Qdot 605 streptavidin, anti-CD38 Qdot 705, Near-IR LIVE/DEAD, and Aqua LIVE/DEAD were from Invitrogen (Carlsbad, CA). HLA-A2 NS4b LLW, HLA-87 NS5 RPI, and HLA-A24 NS1 VYM tetramers in allophycocyanin and anti-CD3 ECD were from Beckman Coulter (Brea, CA). Anti-CD45RA was from BioLegend (San Diego, CA), and anti-CD3 Cascade Yellow was from Dako (Glostrup, Denmark). For phenotypic analysis of cells, incubation for 15 min at room temperature with HLA tetramer alone was followed by 30 min at 4°C in the dark. Flow cytometry data were acquired on a CyAn ADP or BD LSRFortessa (BD Biosciences) and analyzed using FlowJo software version 8.8.6 or 9.1 (TreeStar, Ashland, OR) and SPICE 5.1 software provided by Dr. M. Roederer (National Institutes of Health, Bethesda, MD).

In vitro functional assays, CFSE labeling, and in vitro expansion of PBMCs

PBMCs were thawed and rested in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, 1% penicillin, and streptomycin (Invitrogen) overnight at 37°C. Cells were stimulated with 10 µg/ml peptides for 6 h in 96-well round-bottom plates in the presence of brefeldin A (Sigma-Aldrich, St. Louis, MO), monensin (BD Biosciences), and purified anti-CD28/CD49d (1 µg/ml; BD Biosciences) at concentrations selected for synthesis. Peptides were synthesized by standard 9-fluorenlymethylcarbonyl chemistry, purified to 90% purity by reverse-phase HPLC, and validated by mass spectrometry (JPT Peptide Technologies, Berlin, Germany).

A

B

C

**FIGURE 1.** Magnitude and kinetics of CD4 and CD8 T cell activation in response to the YFV vaccine. (A) CD38 and Ki67–coexpressing cells in CD8 and CD4 T cell populations over time in vaccinated subjects. (B) Median and 10–90th percentiles of CD38 and Ki67 coexpression in CD8 T cells at baseline and at days 10, 15, and 90 in vaccinated subjects. (C) Median and 10–90th percentiles of CD38 and Ki67 coexpression in CD4 T cells at baseline and at days 10, 15, and 90 in vaccinated subjects. Statistical analysis was performed using nonparametric repeated-measures ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001.
For mapping of T cell epitopes, freshly isolated or thawed PBMCs were labeled for 5 min with 1 μM CFSE (Molecular Probes) in PBS at 37°C. Cells were washed extensively with RPMI 1640 plus 10% FCS. CFSE-labeled cells were then incubated for 12 h with the same peptide (4 μg/ml) in the presence of IL-2 (20 IU/ml) for 6 d. At the end of culture, cells were restimulated for 12 h with the same peptide (4 μg/ml) in the presence of brefeldin A (Sigma-Aldrich) and anti-CD28/CD49d (1 μl/ml). Staining, flow cytometry, and analyses were performed as described above.

**HLA class I typing**

Genomic DNA was isolated from whole blood using the DNeasy kit (QIA-GEN). HLA typing was performed using a multiplexed reverse sequence-specific oligonucleotide probe method (LABType SSO; One Lambda), according to the manufacturer’s instructions.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism software 5.0 for Mac OS X (GraphPad Software, La Jolla, CA). Data were analyzed by nonparametric repeated-measures ANOVA. The p values < 0.05 were considered statistically significant.

**Results**

**Magnitude and kinetics of CD4 and CD8 T cell responses to the YFV 17D vaccine**

T cell activation and proliferation can be assessed simultaneously to estimate the overall magnitude of an ongoing T cell response (9, 27).

In this study, surface expression of CD38, in combination with intracellular expression of Ki67, was used to determine the frequency of responding CD8 and CD4 T cells before and on days 10, 15, and 90 after YFV vaccine administration to healthy, previously unvaccinated individuals (Fig. 1A). Baseline levels of CD38 and Ki67 coexpression were low; they expanded 10–15-fold on days 10 and 15 after vaccination and subsequently returned to baseline values by day 90, consistent with an effector-response population contracting into a memory population. Interestingly, the CD4 T cell response, as detected by CD38 and Ki67, preceded the CD8 T cell response (Fig. 1B, 1C). Thus, vaccination with the live attenuated YFV 17D vaccine, consistent with previous reports (9, 28, 29), generated sizeable CD8 T cell and CD4 T cell responses.

**Broad recognition of MHC class I–presented YFV epitopes**

To study the T cell response to YFV in more detail, we next set out to identify epitopes recognized in the primary response after vaccination. Seventy-eight possible YFV epitope candidates predicted to be presented by HLA-A1, -A2, -A3, -A24, -B7, or -B8 were identified using the NetCTL search engine and selected for further study (Supplemental Table I) (Fig. 2). To assess the existence of CD8 T cell responses against these peptide sequences, CFSE-labeled PBMCs from 13 of the 21 recently vaccinated
volunteers were cultured with the corresponding synthetic peptides for 6 d. By the end of day 6, these cultures were restimulated for 12 h in the presence of peptide, and responses were determined by intracellular staining for IFN-γ (Fig. 2). Responding cells were identified as double positive for CFSE dilution, indicative of proliferation during culture with peptide, and IFN-γ production > 0.5% by CD8 T cells in response to restimulation. By this measure, a CD8 T cell response was detected against 57 of the 78 tested (73%) candidate epitopes in at least one donor. Thus, the effector stage of the CD8 T cell response against YFV 17D is both broad and diverse.

Twelve of the seventy-eight candidate epitopes gave positive responses in ≥4 of the 13 subjects, and these 12 peptides were selected for further study in all 21 vaccinated subjects (Table I). Three of the more strongly targeted epitopes, which could also be assigned HLA restriction based on the HLA type of responding subjects, were selected for HLA-tetramer synthesis. Tetramer staining confirmed the presence of CD8 T cells specific for these epitopes in YFV-vaccinated donors (Fig. 2). Together, these results identify a set of YFV epitopes targeted in vaccinated healthy subjects and demonstrate that YFV generates a broad CD8 T cell response.

Dominant, subdominant, and transient subdominant specificities in the CD8 T cell response to YFV

The maturation of immunodominance patterns in primary CD8 T cell responses to acute virus infections in man is incompletely described. Thus, we set out to study this aspect of the response to YFV using the sensitive 7-d expansion and restimulation methodology to assess the presence of CD8 T cells specific for the 12

<table>
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<tr>
<th>Amino Acid Position</th>
<th>Amino Acid Sequence</th>
<th>Predicted HLA Binding</th>
<th>Location in Polyprotein</th>
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<td>KVKRVVASL</td>
<td>B7, B8, A24</td>
<td>C prot</td>
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<td>VYMDAVFELY</td>
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<td>RPIDDPRGEL</td>
<td>B7</td>
<td>NS5</td>
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FIGURE 3. The CD8 T cell response is composed of both dominant persisting and subdominant transient responses. PBMCs isolated from one representative donor at baseline and at days 10, 15, and 90 were cultured with single peptides for 6 d, followed by a 12-h restimulation with the same peptide in the presence of brefeldin A and purified anti-CD28/CD49d. Responding cells were identified as CFSE<sup>−</sup>IFN-γ<sup>+</sup>.
epitopes throughout the course of the study (Fig. 3). The magnitude of responses against targeted epitopes differed markedly, with the HLA-A2–restricted response to the NS4b LLW epitope standing out as the most strongly targeted, as observed previously (23). In addition, the HLA-B7–restricted response to the NS5 RPI epitope also stood out as dominant in both effector and memory stages. Many other specific responses were less strong, but still clearly detectable, during both effector and memory stages of the response. Interestingly, some responses displayed a transient effector nature: they were clearly detectable at day 15 but were undetectable at day 90. One such example is the NS1 VYM epitope in the representative subject shown in Fig. 3. Together, these results suggest that epitope-specific CD8 T cell responses generated against YFV display a range of differential magnitude and duration, with some specificities contributing strongly and others barely at all, to the YFV memory CD8 T cell pool.

CD45RA<sup>−</sup>CCR7<sup>−</sup>PD-1<sup>+</sup>CD27<sup>high</sup> YFV-specific effector cells transition into a CD45RA<sup>+</sup>CCR7<sup>−</sup>PD-1<sup>−</sup>CD27<sup>low</sup> memory population

To further characterize YFV epitope–specific CD8 T cells, we generated HLA class I tetramers for the HLA-A2–restricted NS4b LLW epitope, the HLA-B7–restricted NS5 RPI epitope, and the HLA-A24–restricted NS1 VYM epitope (Fig. 2). The HLA-A2 and -B7 tetramers were sufficiently stable and had detectable frequencies in a sufficient number of donors to allow careful enumeration and phenotypic characterization of specific CD8 T cells during the course of the response to YFV (Fig. 4). CD8 T cells identified with these two tetramers were characterized for their expression of CCR7, CD45RA, PD-1, and CD27 (Fig. 4A). The dominant NS4b LLW–specific response was clearly detectable at day 10 and reached frequencies of up to 7% of CD8 T cells (Fig. 4B). Furthermore, this response was observed by tetramer staining in all HLA-A2<sup>+</sup> donors. The NS5 RPI-specific
responses were detected in up to 0.35% of CD8 T cells in HLA-B7 donors (Fig. 4C). This response was observed by tetramer staining in all HLA-B7 donors tested. During the effector response at day 15, the most prevalent phenotype of tetramer-defined CD8 T cells of both specificities was positive for PD-1 and negative for CCR7 and CD45RA (Fig. 4B, 4C). At the memory stage at day 90, this PD-1+CD45RA- phenotype had shifted toward a loss of PD-1 and re-expression of CD45RA. Only a very small fraction of tetramer-positive cells expressed CCR7 throughout the course of the study. Establishment of this memory CD8 T cell phenotype was also accompanied by a degree of surface downregulation of CD27, measured as mean fluorescence intensity, although cells clearly remained positive. Together, these data demonstrate how the YFV-specific CD8 T cell response goes through maturation of the prevalent PD-1+CD45RA- effector character into a PD-1-CD45RA+ phenotype with the establishment of T cell memory.

Evolution of the functional profile during the YFV-specific CD8 T cell response

Next, we studied the detailed functional profile of responding cells during the course of the primary CD8 T cell response to YFV. We studied degranulation (CD107a), cytokine expression (IFN-γ, IL-2, and TNF-α), and chemokine expression (MIP-1β) in CD8 T cells specific for the dominant HLA-A2–restricted NS4b epitope (Fig. 5A). The response pattern was diverse and dominated by monofunctional cells (Fig. 5B). At the effector stage on day 10, bifunctional IFN-γ and MIP-1β–producing cells dominated the response (Fig. 5C). This was the most common combination of two functions through day 90, although their overall contribution to the response decreased over time. At day 90, MIP-1β monofunctional cells were the overall most common functional profile and had replaced degranulation as the dominant monofunctional subset (Fig. 5C). Triple-functional cells maintained a steady proportion of the response, although the composition changed over time (IFN-γ, IL-2, MIP-1β at day 10 to IFN-γ, MIP-1β, TNF-α at day 90) (Fig. 5C). A minor subset displayed four functions; the relative size of this subset was reduced, and it changed composition from IFN-γ, IL-2, MIP-1β, TNF-α at days 10 and 15 to CD107a, IFN-γ, MIP-1β, TNF-α at day 90 (Fig. 5C). Cells coexpressing five functions were rarely detected. These results indicate that the YFV NS4b–specific CD8 T cell response changes composition as it matures from an effector- to a memory-type response and, surprisingly, becomes a little bit less polyfunctional in this transition.

Activation of FOXP3+ Tregs at the peak of the T cell response

Tregs provide part of the natural regulatory-feedback loop to control an immune response. Therefore, we analyzed the CD4+ CD25+FOXP3+CD127- Treg population during the response to YFV (Fig. 6A). Treg levels were stable throughout the course of the study (Fig. 6B); however, there was clearly detectable activation, as measured by coexpression of CD38 and Ki67 in these cells at day 10 after vaccination (Fig. 6C). This activation was transient and essentially returned to background levels by day 15 and was accompanied by a decrease in PD-1 expression in Tregs (Fig. 6D). Thus, although the percentages of Tregs do not change during the primary response to YFV, there is transient activation of these cells.

Discussion

The YFV 17D vaccine has received renewed attention because it provides an excellent model for detailed studies of primary human immune responses against viral infections in a temporal manner (7–9). We adopted this model to study the specificity, functional profile, phenotypic characteristics, and immunodominance patterns in the primary T cell response to this acute viral infection. T cells in the CD8, CD4, and Treg compartments responded to YFV. The kinetics of CD4 T cell and Treg responses preceded the CD8 T cell response. The CD8 T cell response against a set of YFV epitopes was broad and complex and was composed of both dominant and subdominant responses that persisted into the memory population, as well as of transient subdominant responses that were undetectable at the memory stage. Furthermore, HLA-A2 and -B7 tetramer–defined YFV-specific effector cells predominantly displayed a CD45RA+PD-1+ phenotype that transitioned into a CD45RA-PD-1-memory phenotype. The functional profile of the CD8 T cell response changed composition as it matured; surprisingly, it tended to become somewhat less polyfunctional in the transition to the memory stage.
CD8 T cells can express a broad range of effector functions, which are rarely coexpressed in the same cell and with the same kinetics, resulting in considerable functional heterogeneity (18, 30–32). Studies of this heterogeneity are interesting because the qualities of T cell responses, as well as of individual T cells, may relate to the protection that they confer to the host. For instance, in the search for immunological correlates of immune control of chronic HIV-1 infection, it was observed that high levels of polyfunctionality in the CD8 T cell response to the virus were associated with slow disease progression (33, 34). However, the link between polyfunctionality and viral control is not straightforward. A recent report by Ferrari et al. (35) indicated that, during acute infection, the magnitude of the CD8 T cell response, rather than the level of polyfunctional activity, correlates with selection of viral-escape mutants. In this context it is interesting to note that, according to our results, the immunodominant YFV NS4b LLW response is not particularly polyfunctional but rather is dominated by mono- and bifunctional cells. Because the YFV 17D vaccine affords a high level of protection, this observation would be consistent with a model in which polyfunctionality is not a strict requirement for a protective CD8 T cell response. Furthermore, we observed that the exact repertoire of functions changes over time as the response matures from an effector to a memory response. However, the tendency is that the representation of four- or five-functional cells decreases from day 15 to 90. Overall, MIP-1β monofunctional cells become the most common functional profile at day 90, consistent with the findings by Kim et al. (36), who observed strong chemokine production in late-stage memory cells. This again argues against a central role of polyfunctionality as an essential character in a protective-immune response; perhaps the total magnitude or qualitative aspects, such as the strength of response at the single-cell level, are more important (35, 37).

A noteworthy characteristic of the CD8 T cell response to YFV is the breadth and diversity of the specificity of the effector response detected at day 15. To increase the likelihood of also detecting the weaker responses, we devised a culture-restimulation method by which we assessed responding cells that are CFSElow after the 6-d culture with peptide and IFN-γ. Of the 78 candidate epitopes initially identified using the NetCTL search engine, we observed a detectable response using this method in at least one donor to 57 of the 78 peptides. This probably reflects, in part, the diversity in HLA genotypes among donors, but it can also be viewed as an unexpected level of epitope diversity in the effector CD8 T cell response. For practical reasons, we limited the more extensive analysis to the 12 peptide epitopes targeted in several donors (Table I). Longitudinal analysis of these CD8 T cell responses revealed several interesting patterns. First, the immunodominant A2-restricted NS4b LLW epitope and the B7-restricted NS5 RPI epitope were recognized early and strongly in all donors carrying the respective HLA class I gene. These responses also persisted into the memory stage at day 90. For the NS4b LLW epitope, this pattern is consistent with that described by Akondy et al. (23), and we observed that the response to the NS5 RPI epitope, first described in this study to our knowledge, follows a similar pattern. Interestingly, we observed small, but detectable, responses to the NS4b LLW epitope in three of nine HLA-A2∗ donors at day 0 before vaccination (Fig. 3). This opens up the possibility that the immunodominant nature of this response might be influenced by cross-reactivity to other viruses. Second, responses to epitopes, such as NS4a AMD, were weak, but clearly detectable, throughout the course of study. This can be considered a classical subdominant response pattern. Finally, the NS1 VYM epitope appeared to be recognized transiently at the peak of the response but not at the memory stage; thus, it can be described as a transient subdominant epitope. It is important to note that responses not detected at the memory stage may still exist at very low frequencies or at a location other than in the peripheral blood.

In phenotypic studies of tetramer-defined CD8 T cells specific for the NS4b LLW and NS5 RPI epitopes, we observed that the dominant phenotype is PD-1−CD27low, whereas it is CCR7−CD45RA− during the effector response at day 15. At the memory
stage on day 90, this phenotype has shifted to a dominant CD45RA+PD-1+CD27lowCCR7+ phenotype. The major changes in the transition from effector to memory stages are CD45RA re-expression and loss of PD-1. Thus, it seems that PD-1 behaves primarily as an activation marker in this system. Furthermore, the previous description of CD45RA re-expression as a marker of late-stage effector cells is inconsistent with this pattern, because the YFV-specific populations detected at day 90 are, by definition, memory cells. In fact, we detected CD8 T cells with these specificities up to 4 y after vaccination, demonstrating that these cells can persist long-term (data not shown). The notion that CD45RA+CCR7+ cells may be involved in providing protective immunity is supported by observations in HIV-1 infection: HIV-specific CD8 T cells with this phenotype were associated with control of viremia (38, 39).

In addition to CD8 T cell responses, we measured responses to vaccination by CD38 and Ki67 coexpression in the CD4 T cell this phenotype were associated with control of viremia (38, 39). Observations in HIV-1 infection: HIV-specific CD8 T cells with this phenotype were associated with control of viremia (38, 39). Interestingly, responses in CD4 to vaccination by CD38 and Ki67 coexpression in the CD4 T cell response might be a central aspect of the high level of protection this phenotype was associated with control of viremia (38, 39). We thank the healthy volunteers who contributed time and effort related to sample donation for this study.

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Disclosures

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