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Evolution of the T Cell Repertoire During Primary, Memory, and Recall Responses to Viral Infection

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Many viral infections induce a broad repertoire of CD8⁺ T cell responses that initiate recognition and elimination of infected cells by interaction of TCRs with viral peptides presented on infected cells by MHC class I proteins. Following clearance of the infection, >90% of activated CD8⁺ T cells die, leaving behind a stable pool of memory CD8⁺ T cells capable of responding to subsequent infections with enhanced kinetics. To probe the mechanisms involved in the generation of T cell memory, we compared primary, memory, and secondary challenge virus-specific T cell repertoires using a combination of costaining with MHC class I tetramers and a panel of anti-Vβ Abs, as well as complementarity-determining region 3 length distribution analysis of TCR Vβ transcripts from cells sorted according to tetramer binding. Following individual mice over time, we found identity between primary effector and memory TCR repertoires for each of three immunodominant epitopes from lymphocytic choriomeningitis virus. During secondary responses, we found quantitative changes in epitope-specific T cell hierarchies but little evidence for changes in Vβ usage or complementarity-determining region 3 length distributions within epitope-specific populations. We conclude that 1) selection of memory T cell populations is stochastic and not determined by a distinct step of clonal selection necessary for survival from the acute responding population, and 2) maturation of the T cell repertoire during secondary lymphocytic choriomeningitis virus infection alters the relative magnitudes of epitope-specific responses but does not significantly modify the repertoire of T cells responding to a given epitope. The Journal of Immunology, 2000, 165: 6081–6090.

Immune responses to viruses and other intracellular pathogens are often characterized by a massive activation and expansion of CD8⁺ T lymphocytes (1–6). These CTL are responsible for the Ag-specific recognition and elimination of virus-infected cells (7). Following the initial expansion of Ag-specific CD8⁺ T cells and subsequent clearance of the viral infection, most CTL undergo apoptosis, leaving behind a stable pool of memory CD8⁺ T cells (1, 4, 8). These memory T cells are capable of responding to subsequent viral infections with enhanced kinetics due to both quantitative and qualitative changes and mediate correspondingly more rapid viral clearance (4, 9–11).

CD8⁺ T cell recognition of infected cells is accomplished by the interaction of clonally distributed TCRs on effector cells with the trimolecular complex of viral peptide/MHC class I (MHC I)β2-microglobulin on infected cells (12, 13). The TCR is a disulfide-linked, membrane-bound heterodimer of α- and β-chains, each the result of unique somatic rearrangements in variable (V), joining (J), and, in the case of the β-chain, diversity (D) segments, during T cell development and maturation (14, 15). These are in turn joined to one of a limited number of functionally similar constant (C) regions. The broad diversity of the naive TCR repertoire is a direct result of this recombination process along with random incorporation of junctional nucleotides. Further diversity is generated by the combinatorial pairing of different α- and β-chains (16).

Similar to what is seen in Ig proteins, the diversity of the TCR is focused into three complementarity-determining regions (CDRs), termed CDR1, CDR2, and CDR3. The CDR1 and CDR2 are encoded in the germline V segment genes, whereas the CDR3 encompasses the V-J, or in the case of the β-chain, the V-(D)n-J junction (14). Thus, the most diverse portion of the TCR is the CDR3 of the β-chain. Functional (17–19) as well as recent crystallographic data (20) have indicated that the CDR3 is centered over the peptide/MHC I complex and makes direct contact with the presented peptide, whereas the CDR1 and CDR2 are situated peripherally to the CDR3 and make more extensive contact with the MHC. Nevertheless, specificity of T cells for a particular MHC/peptide combination is, at least in part, determined by contacts made by the CDR1 and CDR2, and consequently, the V gene segment used (15). Analysis of the specificity of Ag recognition has shown restricted CDR3 lengths within particular Vβ-expressing subpopulations of responding cells, suggesting that the length of this region is a major determinant of TCR specificity (2, 21–23).

Studies of TCR repertoires within Ag-specific populations have shown that some populations of epitope-specific cells show restricted TCR diversity (2, 24, 25), whereas others are of a considerably broader distribution (26, 27). However, many of these analyses have relied upon in vitro expansion of Ag-specific subsets of cells, a process prone to experimental bias. TCR repertoires that have been analyzed ex vivo are limited to systems containing one immunodominant epitope possessing restricted diversity (24, 25).

The relationship between primary Ag-specific effector cells and memory cells in these systems supports a stochastic selection process in which the repertoire of the memory pool directly reflects...
that which is present in the effector population (22, 24, 25, 28). However, recent evidence suggests that, upon secondary exposure to the same antigenic determinants, there is a selective expansion or “focusing” of Ag-specific cells both in terms of diversity of their repertoires as well as affinity of the TCR for its ligand (29, 30). This focusing can be viewed from at least two different levels. Populations specific for one epitope may be privileged to expand over others (4), whereas within a given epitope-specific population, there may be preferential expansion of certain TCR subpopulations (29).

We have performed direct ex vivo analysis of CD8+ T cell populations specific for three concurrent immunodominant epitopes generated during infection of C57BL/6J (H-2b) mice with populations specific for three concurrent immunodominant epitopes generated during infection of C57BL/6J (H-2b) mice with lymphocytic choriomeningitis virus (LCMV) (4, 5). Two of these, NP396-404 and GP33-41, are presented in the context of the H-2Db MHC I molecule. The third immunodominant epitope, GP34-41, although a 1-aa truncation of the Db-restricted GP33-41 epitope, is presented by H-2Kb (31). Using soluble MHC I tetramer reagents, as well as a panel of Vb-specific Abs, we have analyzed the TCR Vb usage of these three epitope-specific populations. Sorting specific cells, we have also analyzed the CDR3 distribution of each of these populations. Due to the high degree of mouse-to-mouse variation in Vb usage found in this and other studies (28, 32, 33), we also followed the TCR repertoire of LCMV epitope-specific cells in individual mice within primary effector, memory cell, and secondary effector populations to more accurately determine the relationship between these populations.

Materials and Methods

Mice and virus challenges

Adult C57BL/6J mice (B6, H-2b) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facilities. For primary challenge and memory analyses, mice were infected i.p. with 2 x 106 PFU of the Armstrong strain of LCMV and were used at the indicated time post infection. For secondary challenge analyses, immune B6 mice primed >6 mo previously with LCMV Armstrong were infected i.v. with 2 x 105 PFU of the clone 13 strain of LCMV and used 4–5 days postinfection. Virus stocks were grown and maintained as previously described (34).

Cells and flow cytometry

Abs used in this study were purchased from Pharmingen (San Diego, CA) with the exception of the anti-CD8a Ab (clone CT-D8a; Caltag, Burlingame, CA) used to reveal Kb-restricted CD8+ T cells and 2-μm microspheres (37). Following amplification, an eight-round run-off linear amplification step was performed using an internal 6-carboxyfluorescein-labeled Cb primer. Products and m.w. standards were run on a 4.0% polyacrylamide denaturing gel on an Applied Biosystems (Foster City, CA) 377 automated sequencer. Peak intensities were extracted using Applied Biosystems sequence analysis 3.0 software and analyzed using Immunoscope 1.0 software (37).

Results

Vβ usage of LCMV-specific primary effector cells

Adult B6 mice infected with the Armstrong strain of LCMV exhibit a dramatic increase (>10-fold) in the total number of activated CD8+ T cells during the first 8 days following infection (4). During this expansion, the number of CD8+ cells within each of the Vβ-expressing populations also increased dramatically per spleen (Fig. 1). It has recently been shown that the large expansion of CD8+ T cells seen during LCMV infection is primarily due to an increase in the number of virus-specific CD8+ T cells (4, 5). Considering the number of epitope specificities represented in these cells (4, 31), the antiviral response includes CD8+ T cells using TCR that encompass virtually all of the expressed Vβ gene segments.

It has been well established that clearance of LCMV within this period is dependent on virus-specific CD8+ CTL (7). The epitope specificity and hierarchy (in terms of frequency) of these cells have also been extensively studied by functional assays as well as by MHC I tetramer analysis (4). Briefly, the immunodominant H-2Db-restricted NP396-404 and GP33-41-specific responses account for ~20 and 15%, respectively, of all activated CD8+ T cells at the height of the immune response. However, functional analysis of this specificity is confounded by the presentation of the GP34-41 peptide in the context of H-2Kb, accounting for roughly half of the IFN-γ response elicited by the GP33-41 peptide (J. D. Altman, manuscript in preparation; Ref. 38).

We analyzed the TCR-Vβ repertoire of CD8+ T cells specific for each of these three epitopes by flow cytometry, containing CD8+ T cells with Db and Kb MHC I tetramers (DNP396-404, DGP33-41, KGP34-41), and a panel of Vβ-specific Abs. A representative analysis of Vβ/MHC I tetramer co-stained cells of CD8+ T cells from an individual LCMV-infected mouse is shown in Fig. 2. Abs to Vβ regions not indicated in the figure were either not available at the time of analysis or did not contribute to the epitope-specific populations examined; with the indicated reagents, we were able to detect 80–85% of the total CD8+ population. Although we did not attempt to determine the relative distribution of the remaining 15–20% of CD8+ T cells, immunoscope analysis of TCR transcripts from tetramer-sorted cells (described below) indicates that this population did not contain a significant fraction of LCMV-specific cells. There were no significant differences in the number of CD8+ cells that specifically bound the MHC I tetramers in the presence or absence of any of the anti-Vβ Abs used or in
The entire Db NP396-404-specific repertoire. Commitment of Vβ T lymphocytes and are calculated using 5% (Db NP396-404) or 2% (Db GP33-41 and Kb GP34-41) probability curves. Due to inherent differences in fluorescence intensities of the different Vβ-specific Abs, regions were accommodated to fit cell density patterns.

assays measuring functional responses to synthetic viral epitopes (24, 25).

Analysis of the Vβ usage within these three epitope-specific populations from five representative individual mice at the peak of the primary immune response to LCMV is shown in Fig. 3. Consistent with a previous report (28), a portion of each of the epitope-specific populations consisted of Vβ8.1,8.2+ CD8+ cells (Fig. 3). The magnitude of this contribution to each epitope-specific populations varied not only between epitopes (comparing vertically, Fig. 3, A–E, F–J, and K–O, or comparing horizontally, Fig. 3, A–E, F–J, and K–O), but also between mice (comparing Fig. 3, A–E, F–J, and K–O). In addition to Vβ8.1,8.2+ cells, conserved “signature” Vβ usage profiles were seen for each of the epitopes examined. Characteristically, usage of Vβ6 and Vβ9 gene segments was observed by DNP396-404-specific cells (Fig. 3, A–E) and, with Vβ8.1,8.2+ cells, comprised greater than two-thirds of the entire DNP396-404-specific repertoire. Commitment of CD8+ T cells within the Vβ8.1,8.2+ population to this response was high, as ~30% of all Vβ8.1,8.2+ cells were specific for the DNP396-404 epitope. Similarly, there was a high level of commitment within Vβ6- and Vβ9-expressing CD8+ T cells to the DNP396-404-specific response as ~25% of these cells were specific for this epitope. Commitment within other Vβ+ lineages to the DNP396-404 response was low.

CD8+ T cells specific for the DGP33-41 epitope preferentially bound anti-Vβ8.1,8.2 or Vβ7 Abs and comprised ~50% of the entire epitope-specific population (Fig. 3, F–J). There was also variable recruitment of Vβ6, Vβ9, Vβ10, Vβ11, or Vβ13 positive cells, with these subpopulations contributing ~30% of the total DGP33-41-specific response. Similar to what was seen with DNP396-404-specific cells, commitment of Vβ7-expressing cells to the DGP33-41 response was high, as >20% of all CD8+ Vβ7+ cells were specific for this epitope. In contrast, although a large number of Vβ8.1,8.2+ cells were recruited into the DGP33-41 response, <6% of CD8+ Vβ8.1,8.2+ cells were specific for this epitope. Commitment within Vβ populations constituting minor portions of the DGP33-41 response was also low when compared with that seen for Vβ7-expressing cells.

A much more restricted pattern of Vβ usage was seen in CD8+ T cells specific for the KGP34-41 epitope (Fig. 3, K–O). In this case, Vβ8.1,8.2+CD8+ T cells represented a minor portion of the

FIGURE 1. Broad expansion of Vβ subpopulations of CD8+ T cells during the immune response to LCMV infection. A. Mean number of CD8+ T cells expressing each of the Vβ gene segments indicated per spleen. Single cell suspensions of splenocytes taken from naive (□), primary challenged (8 days postinfection, ■), or rechallenged (4 days postsecondary challenge, □) mice were stained with the indicated Vβ-specific as well as anti-CD8α Abs and analyzed by flow cytometry. Data are representative of >10 mice per Vβ per time point. B. Relative increase in total number of CD8+ T cells expressing each of the indicated Vβ gene segments during primary effector (●), memory (△), or secondary effector (□) to that seen in naive populations (dashed line). Error bars indicate SD from the mean.

FIGURE 2. Representative flow cytometric analysis of the Vβ TCR repertoire for three LCMV-specific CD8+ T cell populations during infection of B6 mice. Splenocytes taken from an individual B6 mouse 8 days postinfection with the Armstrong strain of LCMV were stained with anti-CD8 Ab as well as the indicated tetramer and anti-Vβ-specific reagents. A–C, Representative tetramer/Vβ staining for the DNP396-404- (A), DGP33-41- (B), and KGP34-41- (C) specific CD8+ populations are shown with the percentage of each epitope-specific population being recognized by the indicated Vβ Ab shown in the upper right quadrant. Where no value is given, <1% of the epitope-specific population is represented. Contour plot analyses are gated on CD8+ T lymphocytes and are calculated using 5% (DNP396-404) or 2% (DGP33-41 and KGP34-41) probability curves. Due to inherent differences in fluorescence intensities of the different Vβ-specific Abs, regions were accommodated to fit cell density patterns.
response, whereas the major Vβ gene segment used, Vβ11, constituted >20% of the KβGP34-41-specific population (Fig. 3, K–O). Commitment within Vβ11-expressing cells to the KβGP34-41 response was high, as ~25% were specific for this epitope. However, similar to DβGP33-41-specific cells, there was a low commitment within the Vβ8.1.8.2+ population to this response. Importantly, the Vb usage profiles between the DβGP33-41 and KβGP34-41-specific CD8+ were drastically different, providing further evidence of the distinct nature of these two populations (Fig. 3, F–J, K–O).

For each of the epitopes examined, many of the mice recruited T cells expressing Vβ regions that were variably observed. These included Vβ7 and Vβ13 in DβNP396-404-specific populations; Vβ6, 9, 10, 11, and 13 within DβGP33-41-specific populations; and Vβ10,12, and 13 in KβGP34-41-specific cells. The most diversity in Vβ gene segment use was seen in DβGP33-41-specific populations (Fig. 3, F–J). Preliminary analysis also indicates significant semiconservative expansion of Vβ8.3-expressing cells within DβGP33-41-specific populations as well as Vβ5, Vβ8, Vβ10, and Vβ14 within populations specific for another LCMV epitope, DβGP276-286. Taken together, these analyses account for the broad expansion in virtually all of the different TCR-Vβ CD8+ T cell populations during the primary response to LCMV (Fig. 1A).

**Immunoscope analysis of LCMV-specific primary effector cells**

An alternative method for the elucidation of TCR repertoires has been the RT-PCR-based analysis of CDR3 length distributions known as immunoscope or spectratyping analysis (23, 25, 28, 39). Primarily, this approach has been used to identify responses based on the skewing of CDR3 length distributions within bulk T cell-Vβ-expressing subpopulations due to expansions of restricted precursors. We modified this approach by first sorting Ag-specific populations, thereby reducing “background” peaks. This enabled us to further resolve each of the signature responses even within small populations using a broad range of Vβ gene segments. In addition, this method allowed us to identify Ag-specific expansions within Vβ subpopulations for which there were no commercially available Abs at the time of analysis. Using this approach, we found systematic usage of certain CDR3 lengths within each of the signature Vβ responses previously identified (Fig. 4, Table I). For the conserved Vβ responses within DβNP396-404-specific populations, there was a systematic usage of TCR having CDR3 lengths of 9 aa by Vβ6+ cells, 11 aa by Vβ7+, 9 aa by Vβ8.1+, and 9 and 10 aa by Vβ9+ cells. Within DβGP33-41-specific populations, there was conserved usage of TCR with CDR3 lengths of 9 aa by Vβ7+, 8 and 9 aa by Vβ8.1+, and 8 aa by Vβ8.3+ cells. KβGP34-41-specific populations reproducibly used TCR having CDR3 lengths of 9 aa by Vβ8.1+, 10 or 11 aa by Vβ11+, and 8 aa by Vβ12+ cells. In addition to these conserved CDR3 lengths, other CDR3 length responses were sparcely observed in individual mice as shown in Table I. Within variable Vβ responses identified by Vβ/MHC I tetramer costs, no predictable pattern of CDR3 length usage was observed. CDR3 responses were similar between primary effector, memory cell, and secondary effector populations (data not shown).

**Vβ usage within LCMV-specific memory cells**

Following the initial expansion of the immune response and clearance of the virus, a death phase ensues in which >90% of activated CD8+ cells undergo apoptosis, leaving behind a stable pool of memory cells (Fig. 5) (4). The distribution of Vβ usage within bulk CD8+ T cells from immune mice showed no differences when compared with naive animals (Fig. 1B). This is due to both the number of epitopes at which the CD8+ response is directed as well as the diversity in Vβ usage within each of those epitope-specific populations, effectively diluting the memory scar over a larger CD8+ T cell repertoire. Approximately 3–5% of CD8+ cells in immune mice were specific for the DβNP396-404 epitope, whereas the DβGP33-41- and KβGP34-41-specific cells each constituted ~1–2% of CD8+ cells. Our analysis of LCMV epitope-specific CD8+ cells showed the same mean distribution of Vβ usage between primary effector and memory populations (Fig. 6A).
and D; B and E; and C and F). In addition, the signature Vβ responses that were seen during primary responses were always recapitulated in memory populations. However, there was still a high degree of variation in the magnitude of these signature responses between mice (Fig. 6, A and D; B and E; and C and F).

Vβ usage within secondary effector cells

To examine the Ag-specific T cell repertoires during a recall response, mice that had been primed i.p. with \(2 \times 10^5\) PFU of LCMV Armstrong 3–6 mo earlier were challenged i.v. with \(2 \times 10^6\) PFU of LCMV clone 13. In contrast to well established models for the study of recall T cell responses to influenza A, in the LCMV model it is not possible to obtain reassortment viruses that share T cell epitopes but not determinants for neutralizing Abs. Therefore, the dose and route for the challenge were chosen to permit establishment of infection in mice that had substantial cellular and humoral immunological memory to LCMV. Although we did not attempt to account for the possible influence that circulating virus-specific Ab might have on the T cell repertoires during the recall response, experiments in CD4 knockout mice, which lack an Ab response, revealed very similar LCMV-specific T cell repertoires compared with wild-type mice (data not shown).

During secondary infection with LCMV there was a preferential expansion of D\(^b\)NP396-404-specific CD8\(^+\) T cells when compared with either the D\(^b\)GP33-41 or the K\(^b\)GP34-41 epitopes (Fig. 5). Expansion of D\(^b\)NP396-404-specific cells was observed to be 30-fold, whereas expansion of the K\(^b\)GP34-41- and D\(^b\)GP33-41-specific populations showed 10- and 5-fold expansion. Thus the ratio of epitope-specific cells changed from 1.5:1:1 (D\(^b\)NP396-404:D\(^b\)GP33-41:K\(^b\)GP34-41) during primary responses and memory phase to 6:1:2 during secondary responses. Recent studies on the relationship of secondary to primary effector pools have shown that there is a narrowing of the TCR repertoire due to selective expansion of subpopulations of cells (29, 30). Total CD8\(^+\) T cell expansion of Vβ subpopulations upon secondary expansion was similar to that seen during primary expansion (Fig. 1A). However,

| Table I. Conserved CDR3 lengths within each of the signature Vβ responses to three immunodominant epitopes of LCMV\(^a\) |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | D\(^b\)NP396-404\(^+\) | D\(^b\)GP33-41\(^+\) | K\(^b\)GP34-41\(^+\) |
| CDR3 length (amino acids)       | Conserved       | Sporadic        | Conserved       | Sporadic        | Conserved       | Sporadic        |
| Vβ 6                            | 9               | 11              | 9               | 11              | 11              | 9               |
| 7                               | 11              | 7, 9            | 7               | 9               | 7, 10           | 10              |
| 8.1                             | 9               | 7, 11           | 9               | 8.1             | 8, 9            | 7               |
| 8.2                             | 9, 11           | 8.2             | 8.3             | 8               |                 | 10              |
| 9                               | 9, 10           |                 |                 |                 |                 |                 |
| 13                              |                 |                 |                 |                 |                 |                 |

\(^a\) CD8\(^+\) splenocytes from LCMV-immunized B6 mice were sorted as indicated, and RNA was extracted as described in Materials and Methods. First strand cDNA synthesis was performed using an oligo-dT\(_\mu\) primer, and the resulting product was used in 24 Vβ specific-C\(_\beta\)PCR reactions. Products were then labeled with an internal C\(_\beta\) primer and run on an automated sequencer. Peaks were extracted with ABI Sequence Analysis 3.0 software and analyzed using Immunoscope 1.0 software.
there appeared to be privileged expansion of Vβ6 and Vβ9 (two of the TCR-Vβ represented in DbNP396-404-specific populations) as well as Vβ10 cells, part of the DbGP276-286 response, which also becomes more prominent during secondary responses (Fig. 1B). Thus the differences that were observed in the hierarchy of CD8+ Vβ1 subpopulations were attributable to the preferential expansion of DbNP396-404- or DbGP276-286-specific populations.

Upon comparison of many mice, we saw evidence that within the DbNP396-404-specific population, there was a minor preferential expansion of Vβ8.1,8.2 cells when compared with Vβ6- or Vβ9-expressing populations (Fig. 6G). Additionally, within the KβGP34-41-specific population, there appeared to be a preferential expansion of Vβ11-expressing cells (Fig. 6I) and, to a lesser degree, Vβ7-expressing cells within the DbGP33-41-specific population (Fig. 6H), over Vβ8.1,8.2+ cells. However, due to the high degree of diversity seen in the magnitude of these signature responses during both primary and secondary expansions, it is impossible to determine whether the observed differences in Vβ usage in these rechallenged mice were originally skewed within individual mice during the primary effector and memory phases.

Longitudinal analysis of Vβ usage

Experiments using both PBMC and splenocytes from individual mice showed no differences in the repertoires of Ag-specific cells represented within these compartments (data not shown). Therefore, to more accurately determine the relationship between primary effector, memory, and secondary effector TCR repertoires, we followed LCMV-specific CD8+ T cells present in PBMC of individual mice over time. As the limited blood volume available from each mouse permitted us to perform only six FACS stains, we were able to determine the Vβ repertoire for only one specificity per mouse, where each stain contained one FITC- and one PE-labeled Vβ Ab. Shown in Fig. 7 are the Vβ usage profiles for each of the epitopes examined of five individual mice during primary and secondary responses to challenge with LCMV, as well as the mean of two memory phase time points. It is obvious from this analysis that within DbNP396-404- (Fig. 7A), DbGP33-41- (Fig. 7B), or KβGP34-41- (Fig. 7C) specific populations, there is near identity in the Vβ usage profile between primary effector and memory time points, supporting a model of stochastic selection for the memory pool. Minor evidence was seen for selection during recall responses to LCMV infection within DbGP33-41- or
KbGP34-41-specific populations in terms of Vβ usage (Fig. 7, B and C). However, differences were observed in the Vβ usage profiles of DNP396-404-specific cells analyzed during secondary challenges with LCMV. This focusing of the repertoire was most apparent in mice in which the primary response had a broad distribution of Vβ usage (Fig. 7, A and 3).

**Discussion**

In this report we have analyzed the CD8+ T cell repertoire of responses directed against three immunodominant epitopes of LCMV during infection of B6 mice. Our results clearly show that within each of these epitope-specific populations there existed conserved patterns of TCR Vβ usage comprising 60–100% of the total specific response. For each of the Db- and Kb-restricted LCMV epitopes there was prevalent usage of Vβ8.1 gene segments among Ag-specific populations. This is due, at least in part, to the large proportion of CD8+ T cells in a naive mouse repertoire using Vβ8.1 or Vβ8.2 (up to 25% of all CD8+ T cells). This increases the probability of these V gene segments being used by specific precursors. However, this does not adequately explain this prominence, as there is a similar frequency of Vβ5.1.5.2+ cells in naive mice, but these cells are not part of the epitope responses analyzed here. In addition to the Vβ8.1 response, DNP396-404-specific populations also reproducibly contained cells using Vβ6 and Vβ9. For DbGP33-41-specific cells, the signature response always contained Vβ8.1 and Vβ13 using cells, whereas KGP34-41-specific populations always contained Vβ11- and Vβ8.1-expressing cells. These conserved responses are likely due to interactions involving the CDR1 and CDR2 germline-encoded regions within each Vβ segment, providing a decreased stringency or increased probability of specificity for each of the particular MHC/peptide combinations (13). In addition to conserved patterns of Vβ usage within these epitope-specific populations, there were also conserved CDR3 lengths used within each conserved Vβ subpopulation. The sum of these signature responses accounted for roughly 60% of each of the epitope-specific populations.

There was considerable diversity in both the magnitude of the signature responses as well as in the Vβ usage and CDR3 lengths within the remaining sporadic portion of the Ag-specific populations. The variability in the sporadic responses most likely reflects low frequencies of recruitable precursors. In such a model, the probability of having an Ag-specific cell present in the naive pool and in the correct environment to expand in response to an Ag is near some finite threshold required for recruitment into the response. Because there is a distribution of CDR3 lengths within any Vβ+ population in the naive pool, the absolute number of cells using a particular Vβ-CDR3 combination can be limiting. Likewise, a more stringent CDR3 sequence requirement can select for a low number of precursors eligible for a given sporadic response. When the frequency of such rare recruitable precursors falls below one per mouse, the corresponding response will not be observed in all individuals and might even only be sporadically observed. Under an ergodic hypothesis of repertoire selection (40), the naive repertoires found in different mice at any given time are equivalent to the naive repertoires found within a single mouse at different time points. The proportion of individuals undergoing a given sporadic response is a measurement of the probability of the presence of the corresponding precursors in a normal naive T cell mouse repertoire.

Prior analyses of a combined epitope repertoire using this system has led others to postulate that it is "impossible" to compare the repertoires of different mice taken during different times following infection (28). This was based upon analysis of the Vβ8.1 response to LCMV, which contains many different specificities. We show here that, in fact, when we deconvolute the Vβ8.1 response, there are predictable repertoires that emerge and are comparable. In addition, as we have directly shown that Vβ8.1+ T cells are recruited into the responses specific for each of at least four epitopes, the analysis of the CDR3 length distribution for Vβ8.1 from LCMV-infected mice must be performed on an epitope-specific basis as with MHC I tetramer-sorted populations shown here. This becomes especially important during chronic viral infections in which there are dramatic changes in the hierarchy of epitope-specific populations.

We have also shown that the repertoires of the CTL populations present within the primary response and memory pool are similar. This further supports a stochastic model for selection of the memory pool (21, 22, 24, 25, 28, 32). This was true both when looking at the sum of many mice or when comparing repertoires of effector and memory CD8+ cells from individual mice. The small differences seen between the Vβ usage of effector and memory pools from individual mice are within limits of experimental incertitude and could be due to constant errors being applied to populations of decreasingly small size.

During secondary recall responses, there was a selective expansion of DNP396-404-specific cells, followed by KGP34-41-, and DbGP33-41-specific populations. The change in the ratio of these populations as compared with the primary response is likely due to a difference in the kinetics of epitope presentation. Because there is a greatly reduced lag phase before the secondary response, cells responding first will be expanded preferentially. During the repli- cative cycle of LCMV, the nucleoprotein is produced first and is abundantly present in the cytoplasm of infected cells. Therefore, cells specific for nucleoprotein epitopes will be privileged to expand and kill infected cells, hampering secondary responses to other epitopes. This does not adequately explain the differential expansion of KGP34-41- and DGP33-41-specific cells as these overlapping epitopes are derived from the same portion of the glycoprotein. However, there may be differences in the amount or kinetics of presentation of these different epitopes due to differences in the way each is processed and presented.

Minor differences were observed in the Vβ usage profiles of DNP396-404-specific cells expanded during secondary responses when compared with primary effector or memory pools. However, this was not the case for either the DGP33-41- or KGP34-41-specific populations. It is possible that the strong antigenic challenge administered in this case was sufficient to recruit the entire memory pool for these epitopes. However, it is probable that the lesser expansion observed in these populations during secondary responses minimized any observable selection events between primary and secondary effector repertoires. Previous reports comparing primary and secondary effector populations have shown conflicting results, with some showing dramatic focusing of the secondary repertoire due to expansion of only a portion of the memory pool (11, 29, 30), whereas others have shown that there were little or no differences between these populations (22, 41, 42). It is possible that during the LCMV immune response, most of the selection for high affinity TCR has taken place during the primary response. Thus, we would not be able to detect differences between primary and secondary repertoires in this system.

It is still unclear whether there is any immunologic benefit gained from focusing of T cell responses. Maturation of B cell responses, with associated changes in the affinity of Ig molecules, occurs during the initial response when Ag becomes limiting. Thus, following each response there is an improvement in the functional ability of Ig molecules to bind and neutralize virus particles preventing infection. However, the results presented here show that, regardless of the diversity of the T cell response, the
A. D^bNP396-404

Mouse #1  Mouse #2  Mouse #3  Mouse #4  Mouse #5

Primary

Immune

Secondary

B. D^bGP33-41

Mouse #6  Mouse #7  Mouse #8  Mouse #9  Mouse #10

Primary

Immune

Secondary
repertoire of memory cells reflects that of the preceding effector population. Selection of T cell populations occurs during periods of expansion when Ag is not limiting, so pathogens are able to establish themselves before the selection event. The relative importance of selection within T cell responses needs to be determined.

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References

FIGURE 7. Analysis of epitope-specific populations over time within individual mice reveals that primary and memory Ag-specific populations have identical Vb usage repertoires. PBMC from individual mice were prepared as described in Materials and Methods. At the indicated times post infection, PBMC were stained as above. CD8+, DNP396-404- (A), DGP33-41- (B), and KGP34-41- (C) specific populations were analyzed for usage of Vβ gene segments by flow cytometry. Shown are the percentages of each epitope-specific population that are recognized by the indicated anti-Vβ Abs. Graphs for individual mice are arranged vertically.


