Lack of Original Antigenic Sin in Recall CD8+ T Cell Responses

Dietmar Zehn, Michael J. Turner, Leo Lefrançois and Michael J. Bevan

J Immunol 2010; 184:6320-6326; Prepublished online 3 May 2010;
doi: 10.4049/jimmunol.1000149
http://www.jimmunol.org/content/184/11/6320

References This article cites 32 articles, 11 of which you can access for free at:
http://www.jimmunol.org/content/184/11/6320.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Lack of Original Antigenic Sin in Recall CD8+ T Cell Responses

Dietmar Zehn,*† Michael J. Turner,‡§ Leo Lefrançois,‡ and Michael J. Bevan*

In the real world, mice and men are not immunologically naive, having been exposed to numerous antigenic challenges. Prior infections sometimes negatively impact the response to a subsequent infection. This can occur in serial infections with pathogens sharing cross-reactive Ags. At the T cell level it has been proposed that preformed memory T cells, which cross-react with low avidity to epitopes presented in subsequent infections, dampen the response of high-avidity T cells. We investigated this with a series of related MHC class-I restricted Ags expressed by bacterial and viral pathogens. In all cases, we find that high-avidity CD8+ T cell precursors, either naive or memory, massively expand in secondary cross-reactive infections to dominate the response over low-avidity memory T cells. This holds true even when >10% of the CD8+ T cell compartment consists of memory T cells that cross-react weakly with the rechallenge ligand. Occasionally, memory cells generated by low-avidity stimulation in a primary infection recognize a cross-reactive epitope with high avidity and contribute positively to the response to a second infection. Taken together, our data show that the phenomenon of original antigenic sin does not occur in all heterologous infections. The Journal of Immunology, 2010, 184: 6320–6326.

In an individual, the TCR repertoire is clonally distributed among Ag-inexperienced naive cells and Ag-experienced memory cells (1, 2). The ratio of naive to memory T cells skews in favor of memory cells as animals age, as animals accumulate a history of infectious challenges, and the atrophied thymus exports fewer naive T cells (3). Because conventional memory cells have arisen by expansion and differentiation from naive T cells driven by foreign Ag, the diversity of the TCR repertoire in the memory population is far less than that in the naive subset. After a new antigenic challenge, the T cell response may be influenced by previous Ag encounters and cells may be recruited from the memory as well as the naive T cell pool. Because all animals, even inbred lines, have “private” TCR repertoires, and cross-reactivity between MHC-peptide ligands is unpredictable, prior antigenic history can alter the pattern of response to a new pathogen in unforeseeable ways (4). For example, the pattern of epitope immunodominance as well as the clonal heterogeneity of the response that is normally observed in naive mice may be altered because of the contribution of cross-reactive memory cells that have arisen from a previous encounter with a different pathogen (5). An extreme example of how previous antigenic encounters can alter the response to a cross-reactive infection is embodied in the term “original antigenic sin” (6–8). In this scenario, the immune response to a current infection may be dominated by T cells or Abs that have higher avidity for epitopes encountered on a previous pathogen than for cross-reactive epitopes in the current infection. This term was first applied to the humoral response to current and past influenza epidemics (6, 7), but has also been observed in the CD8+ T cell response to lymphocytic choriomeningitis virus (LCMV) and its variants (9), and to the sequential CD8+ T cell response to a different serotype of dengue virus (10, 11).

To explain original antigenic sin it has been argued that the response by an increased precursor frequency of memory T cells with high avidity for the initial Ag but low avidity for the current variant dominates the response by suppressing the primary response of naive T cells with high avidity for the second infection.

How does the dominance of certain T cell clones come about in an immune response? T cells compete for access to APCs after immunization (12–14) and the results of this competition determine which cells are recruited into the ongoing response. T cell precursor frequency is one factor that contributes to the success in being recruited into a response and in suppressing the response of other T cell clones (13, 15). In addition, TCR affinity or T cell avidity for the epitope displayed on the APCs plays an important role, with T cells bearing high-avidity receptors able to out-compete cells with lower-avidity TCR (16). This is in contradiction to the original antigenic sin concept that proposes that low-avidity memory cells out-compete the response of high-avidity naive T cells.

We have recently generated a large panel of recombinants of the Gram-positive pathogen, Listeria monocytogenes that express and secrete OVA containing single residue variants of the major MHC class I-restricted epitope, OVA257-264 (SIINFEKL), which bind H-2Kb with equal affinity (17). All of the variants serve as strong immunogens for endogenous, polyclonal CD8+ T cells in B6 mice. We reasoned that these strains would provide an ideal way to examine the influence of cross-reactive memory on the specificity of a subsequent CD8+ T cell response. In these experiments, we found no evidence that pre-existing memory cells with low to medium avidity for a challenge Ag could suppress the response of higher-avidity naive T cells. Moreover, because the memory population consists of clones with a broad range of avidity for the first Ag, in some prime/challenge combinations a subset of the memory cells generated in the priming infection recognize the second Ag with

* Department of Immunology, Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195; †Service of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Swiss Vaccine Research Institute, Lausanne, Switzerland; ‡Department of Immunology, University of Connecticut Health Center, Farmington, CT 06030; and §Immunotherapy Research, Genzyme Corporation, Framingham, MA 01701

Received for publication January 19, 2010. Accepted for publication March 30, 2010.

This work was supported by the Howard Hughes Medical Institute and National Institutes of Health Grants AI19335 and U54 AI081680 and by a fellowship from the German Research Foundation (Deutsche Forschungsgemeinschaft) to D.Z.

Address correspondence and reprint requests to Michael J. Bevan, Department of Immunology, University of Washington, Box 357370, Seattle, WA 98195. E-mail address: mbevan@u.washington.edu

Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; SPF, specific pathogen-free; VSV, vesicular stomatitis virus; wt, wild-type.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1000149
high avidity and actually enhance the response. How these findings relate to our interpretation of models of clonal competition and the concept of original antigenic sin will be discussed.

Materials and Methods

Mice

C57BL/6 and CD45.1 congenic C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) or from Taconic Farms (Germantown, NY). OT-I TCR transgenic mice (18) are derived from our own colony. All mice were maintained in specific pathogen-free (SPF) facilities at the University of Washington.

Listeria monocytogenes and vesicular stomatitis virus infections

Recombinant L. monocytogenes strains secreting OVA or OVA containing altered peptide ligands were previously described (17). Frozen stocks of recombinant and wild-type (wt) L. monocytogenes were grown in brain-heart infusion broth to mid log phase. Bacteria numbers were determined by measuring the OD at 600 nm and, for naive mice, 1000 CFU were injected in PBS i.v. For rechallenging Listeria immune mice 50,000 CFU were applied i.v.

Wt Indiana strain vesicular stomatitis virus (VSV) and recombinant strains expressing OVA or V4-Ova were generated as previously described (19). The viruses were grown and titrated on BHK-21 cells using standard methods. Frozen stocks were diluted in PBS and injected at 2 × 10⁶ PFU per mouse i.v. Rechallenge experiments were performed at >35 d postpriming.

Adoptive OT-1 T cell transfer

Single-cell suspensions from the spleen of OT-1 TCR transgenic mice were obtained by mashing organs through a 100 μm nylon cell strainer (BD Falcon, Bedford, MA). RBCs were eliminated from splenocytes using ACK lysis buffer. Mouse CD8+ T cell isolation kit and LS separation columns (Milenyi Biotec, Bergisch Gladbach, Germany) for untouched CD8+ T cell isolation were used according to the manufacturer’s protocols, but supplemented with titrated dose of anti-CD44 biotin Ab (eBioscience, San Diego, CA) to remove previously activated T cells. Between 5 × 10⁶ and 5 × 10⁷ CD45.1 congenically marked OT-1 T cells were injected into C57BL/6 mice as noted in the text. The mice were infected at 24 h after the transfer.

Detection of Ag-specific T cells

Cells were harvested at 7 d after primary or secondary infections. Up to 4 × 10⁶ splenocytes were transferred into 96-well plates in DMEM medium (Invitrogen, San Diego, CA) containing 10% FCS, antibiotics and 50 μM 2-ME. Titrated doses of OVA257–264 SIINFEKL peptide or altered peptide ligands were added and the samples were incubated at 37°C. Thirty to 45 min after adding peptide, the samples were supplemented with 7 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO) and incubated for an additional 4.5 h. Then the samples were surface stained with anti-CD4, CD8, B220, and CD45.1 (all BD Biosciences, San Jose, CA or eBioscience), fixed and permeabilized with Cytofix/Cytoperm Kit (BD Biosciences), and stained intracellularly with anti–IFN-γ (BD Biosciences or eBioscience).

In some cases, the cells were simultaneously stained by SIINFEKL and the SIIVFEKL APL. This was performed to enumerate the total number of cells that could respond to only one or both peptides. To calculate the approximate number of naive T cells recruited during a V4 rechallenge infection, the number of T cells that responded to the N4 stimulation was subtracted from the number of cells seen after simultaneous N4 and V4 stimulation.

Data analysis

Flow cytometry data were acquired on a FACS-Canto machine (BD Biosciences) and analyzed with FlowJo software (TreeStar, Ashland, OR). The percentage of cells specifically producing IFN-γ was determined by subtracting the response seen with the no peptide control from the percentage of IFN-γ CD8+ T cell at a given peptide concentration. Graphs were prepared using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). When indicated, data were normalized to the level of maximum response seen at high peptide concentration. EC₅₀ data for the doses needed to induce a half-maximum response were calculated using an algorithm provided by GraphPad Prism.

Results

Cross-reactive T cells in the primary response

Mice infected with the Gram-positive intracellular bacterium L. monocytogenes develop strong CD4+ and CD8+ T cell responses. Infecting C57BL/6 mice with a L. monocytogenes strain that stably expresses a secreted form of chicken OVA induces a potent CD8+ T cell response specific for the H-2Kb-restricted SIINFEKL epitope (referred to as N4). The frequency of these N4-specific effector T cells peaks at around 7 d postinfection when ~5–15% of total CD8+ T cells produce IFN-γ in response to brief ex vivo restimulation with the N4 peptide. We generated a panel of novel recombinant Listeria strains that stably express OVA containing altered peptide ligands, such as SIIVFEKL (V4), SIIQFEKL (Q4), or SIIFFEKL (T4), in place of the N4 epitope. The OVA-related epitopes encoded by these Listeria strains are also strongly immunogenic for CD8+ T cells in B6 mice. On day 7, after immunization with recombinant L. monocytogenes strains, 2–15% of the endogenous polyclonal CD8+ T cells respond to the altered peptide ligand (data not shown).

To study T cell cross-reactivity, we screened the T cells induced by Listeria expressing one OVA-related ligand for their ability to be activated by other OVA-related peptide ligands. We infected naive mice with Lm-N4, harvested effector T cells on day 7 postinfection and stimulated the cells briefly in vitro with high concentrations of a panel of SIINFEKL-derived altered peptide ligands. In the example shown in Fig. 1A, 7.7% of CD8+ T cells made IFN-γ in response to N4 stimulation, whereas ~0.5% of CD8+ T cells cross-reacted with the V4 or the G4 peptide. Naive mice or mice immunized with nonrecombinant L. monocytogenes showed no response above background in this assay. Similar analyses of a number of mice showed that ~5–10% of N4-specific T cells cross-react with the V4 and G4 peptide. Conversely, mice immunized 7 d previously with Lm-V4 had a strong IFN-γ response to the V4 peptide and smaller responses to N4 or V3 peptide stimulation (Fig. 1B).

The results shown in the dotplots of Fig. 1A and 1B were obtained using micromolar concentrations of peptides for the in vitro stimulation. We also determined the functional avidity with which the cross-reactive ligands stimulate the T cells by exposing day 7 effector T cells to titrated amounts of peptide and measuring the magnitude of IFN-γ synthesis. With day 7 effectors from Lm-N4 primed mice, N4 peptide was recognized at low peptide concentrations, whereas it required much higher concentrations of V4 or G4 peptide to activate the cross-reactive cells. Thus, these ligands were recognized with greatly reduced functional avidity (Fig. 1C). Similarly, in Lm-V4 infected mice, the V4 peptide stimulated IFN-γ synthesis at low concentration, whereas the cross-reactive N4 and the V3 peptides were recognized with low functional avidity (Fig. 1D).

Heterologous recall responses are dominated by high-avidity cells

We asked how a prior infection with Listeria expressing one OVA-related epitope would influence the CD8+ T cell response to a subsequent challenge with Listeria expressing a related, cross-reactive epitope. Mice primed by infection with Lm-N4 (subsequently shown as Lm-N4˚1) were rechallenged with Lm-V4 (shown as Lm-V4˚2). Interestingly, the effector T cells detectable at the peak of the rechallenge response demonstrated high avidity for the second ligand, V4, and much lower avidity for the first ligand, N4 (Fig. 2A). Moreover, the avidity of the V4-specific T cells detected in Lm-N4˚1/Lm-V4˚2 mice was identical to the avidity of T cells found in mice that were primed only by Lm-V4, or in mice that were initially primed by empty, wt Listeria (Lm-wt) and recalled by Lm-V4 (Fig. 2A, 2B). We also determined the absolute numbers of V4-specific T cells in Lm-N4˚1/Lm-V4˚2 mice and compared them with the numbers seen in mice initially infected by Lm-wt and then recalled by Lm-V4. In all cases, Lm-N4˚1/Lm-V4˚2 mice showed the same or higher numbers of V4-specific T cells as seen in the Lm-wt˚1/Lm-V4˚2 mice (Fig. 2C). With other prime-recall combinations, such as Lm-N4˚1 and Lm-G4˚2 (Fig. 3A) or Lm-V4˚1 and Lm-N4˚2
we again observed a high-avidity T cell response specific for the recall ligand.

We extended these experiments by using different pathogens expressing OV A-related epitopes, such as infecting mice with Lm-N4, followed by an infection with VSV encoding the V4 epitope (VSV-V4) or vice versa (Fig. 3C, data not shown). In this experimental setup, the secondary pathogen were confronted with cross-reactive memory CD8+ T cells specific for the OV A epitope, whereas the mouse was immunologically naive toward all the other Ags derived from the secondary pathogen. Once again, we observed high-avidity T cell responses to the V4 peptide in the secondary challenge, equal to the avidity of T cells in animals confronting the V4 peptide for the first time. Together, the data presented so far show that elevated numbers of low-avidity cross-reactive memory T cells do not diminish the recruitment and expansion of cells that respond to the same ligand with high avidity.

High-avidity cross-reactive memory cells may contribute to recall

Our results may imply that in the heterologous recall responses described so far, the CD8+ T cell response to the OVA-related peptide originated entirely from naive precursors. This is not always the case, however. We noticed that the pattern of T cell cross-reactivity on OVA-related peptides after the recall infection differed from that observed in primary infections. For example, in mice that were previously primed with Lm-N4 and recalled with Lm-V4, >50% of the effectors responded to the N4 peptide (Fig. 4A). This level of cross-reaction is much higher than the 10–25% level seen after a single immunization with Lm-V4 (Fig. 1B). All or most of the T cells from Lm-N4/Lm-V4 mice that respond to the N4 peptide are included in the V4-reactive population, as demonstrated by stimulating the cells with a mixture of both peptides (Fig. 4A). Of particular note is the observation that the majority of T cells that respond to N4 in this Lm-N4/Lm-V4 prime/recall situation have low avidity for the N4 peptide (Fig. 2A). In these situations, it appears that a fraction of the memory cells generated by prior immunization with Lm-N4, cross-react with high avidity on V4 and on recall immunization with Lm-V4, these memory cells, plus naive T cells specific for V4 contribute to the response. We consider that the population of CD8+ T cells that responds only to the V4 but not to the N4 peptide are the progeny of naive T cells recruited in the Lm-V4 challenge. By subtracting the number of N4-reactive CD8+ T cells from the total number of V4- or N4-reactive T cells, we were able to calculate that in all Lm-N4/Lm-V4 mice, naive T cells specific for only for V4 were recruited to the response. Interestingly, the number of effectors cells derived from noncross-reactive naive cells was very similar to that seen in primary Lm-V4 infections (Fig. 4B).
Large numbers of cross-reactive memory cells do not impair the recruitment and expansion of high-avidity T cells

We asked whether very high numbers of low-avidity memory CD8\(^+\) T cells would impact the secondary response to a related ligand. To achieve this situation, B6 mice were grafted with a small number of naive OT-1 T cells and infected with Lm-N4. About 150 d later, 10–22\% of total CD8\(^+\) T cells in the blood of these mice were memory OT-1 cells (Fig. 5A). These memory mice were recalled by immunization with Lm-V4. V4 is a very low-avidity ligand for OT-1 T cells and the EC\(_{50}\) concentration for stimulating OT-1 T cells by V4 of around 4 \(\times\) 10\(^{-9}\) M is 700-fold higher than for N4 (17). Despite the presence of high numbers of low-avidity V4-reactive memory OT-1 cells, we observed a robust response by the endogenous CD8\(^+\) T cells that showed high avidity for the V4 ligand (Fig. 5B) and was of high magnitude (Fig. 5C). However, when 5 \(\times\) 10\(^5\) memory OT-1 T cells are transferred into naive mice that subsequently received Lm-N4 challenge, these relatively low numbers of memory T cells efficiently blocked the response of endogenous CD8\(^+\) T cells to the N4 epitope. Thus, memory OT-1 T cells can block the recruitment of endogenous T cells in Listeria infections but only after high-avidity stimulation (data not shown).

In another series of experiments, we used mice grafted with OT-1 that were primed by infection with VSV-N4. In these mice, 11–34\% of CD8\(^+\) T cells in the blood were OT-1 memory cells at 110 d postinfection (Fig. 5D). Nonetheless, after a recall immunization with Lm-V4, large numbers of endogenous CD8 T cells with high avidity for the V4 ligand were observed (Fig. 5E, 5F).

Taken together, these data show that even very high numbers of low-avidity cross-reactive memory T cells do not interfere with the recruitment and expansion of T cells that show high functional avidity for a cross-reactive ligand expressed in a recall response.

Memory cells suppress the response of low-avidity T cells

We recently reported that, in a naive B6 host, OT-1 cells respond with initial vigorous expansion to low-avidity ligands, such as T4 and V4 peptides, expressed in Listeria. To examine whether low-avidity responses were controlled by previous exposure to other epitopes of the pathogen, we transferred naive, congenically marked OT-1 cells into B6 mice that were either naive or had been immunized with wt Listeria 40 d previously. The Lm-wt primed mice contain an expanded pool of memory cells specific for endogenous epitopes encoded in the Listeria genome. These OT-1 chimeric mice were challenged by infection with either Lm-T4 or Lm-N4. T4 is another low-avidity ligand for OT-1 T cells. Consistent with our previous observations, both Lm-N4 and Lm-T4 immunization induced the activation and expansion of OT-1 T cells in naive B6 hosts (Fig. 6). In contrast, in Lm-wt immune mice, Lm-T4 immunization completely failed to induce any expansion of OT-1 cells in these hosts, whereas Lm-N4 immunization induced robust OT-1 expansion.

These results indicate that there are important differences in T cell recruitment between naive and immune mice (i.e., although low-avidity T cells are efficiently primed in naive mice, they do not expand in mice that are immune to other epitopes on the pathogen). It appears that mechanisms are in place during a recall response that precludes the expansion of low-avidity T cells.

Discussion

The magnitude of the effector CD8\(^+\) T cell population that forms in response to foreign Ag challenge is impacted by the precursor frequency (15, 20, 21), the inflammatory environment (22, 23),

FIGURE 3. High-avidity T cells dominate the recall response in cross-reactive heterologous infections. A. Naive and Lm-N4 primed mice were challenged with Lm-G4. B, Naive and Lm-V4 primed mice were challenged with Lm-N4. C, Lm-N4 primed and Lm-wt primed mice were challenged with VSV expressing V4-OVA. Seven days after the recall, cells were exposed to titrated doses of the recall peptide and the concentrations (EC\(_{50}\)) needed to achieve half-maximum INF-\(\gamma\) production by CD8\(^+\) T cells were determined.

Figure 3:

- **A**
  - Lm-G4 challenge
  - G4 EC\(_{50}\) [M] vs. Lm-N4
  - Lm-N4 challenge
  - Lm-N4 challenge

- **B**
  - Lm-N4 challenge
  - Lm-N4 challenge
  - Lm-N4 challenge

- **C**
  - Lm-N4 challenge
  - VSV-V4 challenge

FIGURE 4. Naive and cross-reactive memory T cells are recruited in heterologous rechallenges. A. Lm-N4 primed mice were challenged with Lm-V4. Seven days postinfection CD8\(^+\) T cells were harvested and either left untreated or briefly restimulated with a high concentration of V4 peptide, N4 peptide, or simultaneously with the V4 and N4 peptides. Representative flow cytometry plots are shown. Subtracting from the number of cells that produce IFN-\(\gamma\) after simultaneous N4 and V4 peptide stimulation (right panel) the number of cells that make IFN-\(\gamma\) after N4 peptide stimulation (middle right panel) reveals the fraction of cells that are specific for V4 only and not N4 specific. These V4-only–specific T cells originate from naive T cells that were recruited during the Lm-V4 rechallenge. B, The absolute numbers of calculated V4-only–specific T cells in Lm-N4**/Lm-V4** mice are compared with the number of V4-specific T cells seen in a primary Lm-V4 infection on day 7.
CD8+ T cells in later, the frequency of memory OT-1 cells among total specific T cells among total endogenous CD8+ T cells concentrations needed to induce a half maximum IFN-exposed to titrated doses of V4 peptide. The V4-peptide Lm-(A–C) or with VSV-N4 or wt VSV (D–F). More than 100 d later, the frequency of memory OT-1 cells among total CD8+ T cells in Ln-N4 (A) or VSV-N4 primed mice (D) was determined. All primed mice and naive control mice were challenged with Ln-V4. Seven days after the Ln-V4 challenge splenocytes were harvested and briefly exposed to titrated doses of V4 peptide. The V4-peptide concentrations needed to induce a half maximum IFN-γ response (EC50) in the endogenous CD8+ T cell population are shown (B) and (E). The frequency of V4-specific T cells among total endogenous CD8+ T cells at 7 d postinfection are presented in C and F.

and the affinity of the TCR for the stimulating Ag (17). Inbred mice housed under highly standardized SPF conditions show relatively predictable T cell responses with typical patterns of immunodominance to different epitopes presented by the infectious challenge. This is the case for infection of inbred mice with LCMV, VSV, or Listeria (24). This predictability indicates that inbred SPF mice have rather similar precursor T cell populations that respond to pathogen challenge.

In striking contrast to SPF mice, the T cell repertoire in animals in the wild and in humans is shaped throughout life by numerous pathogen challenges that leave their mark on the T cell repertoire in particular by expanding the number of memory T cells (25). Prior infections can generate memory T cells that cross-react with Ags presented in subsequent infections. This can augment the number of cells that respond to a particular Ag and it can lead to altered patterns of the immune response (4, 5). Pre-existing memory T cells are one important factor in explaining why animals that share the same genetics can respond very differently to the same pathogens.

An immunized animal will retain circulating Abs produced by long-lived plasma cells as well as memory T and B cells that were raised by a prior infection. These established memory cells or Abs may respond suboptimally and with low affinity or avidity to an epitope variant presented in a heterologous secondary infection. Moreover, it has been proposed that these suboptimally responding memory cells can diminish the recruitment or expansion of naive B and T cells with high avidity for the current Ag. This lack of adaptation to new Ag specificities has been termed “original Ag sin.” In our experimental setups, we created situations in which pathogen rechallenge was designed to show the original Ag sin phenomenon. However, on the contrary we found no evidence that the T cell response to the second epitope was impaired. Instead, in our heterologous prime-boost infections with wt or recombinant Listeria and VSV, we observed that the T cell repertoire readily adapts to the Ag motifs presented in secondary infections and that low-avidity cross-reactive memory T cells did not impair this adaptation. Even when low-avidity cross-reactive memory T cells accounted for >10% of the CD8+ T cell compartment, we failed to see any evidence of original antigenic sin.

Previous work on recall CD8+ T cell responses has implicated a role for original antigenic sin in secondary infections with cross-reactive pathogens. For example, animals primed with LCMV and rechallenged with a CTL-escape variant of LCMV expressing a mutated MHC class I-restricted epitope showed better CD8+ responses to the original epitope than to the one encoded by the challenge virus (9). Similarly, humans who confront a dengue virus serotype may generate a CD8+ response that reacts better with a previously encountered dengue virus serotype (11). These experiments differ from the ones we present in this study in many ways. Perhaps the most relevant in explaining why we did not observe original antigenic sin in our experiments is the degree of contribution by neutralizing or enhancing Abs in the secondary infection. LCMV-primed animals contained circulating Abs that cross-reacted with 100% efficiency on the virus expressing a CTL-epitope variant, and this may have squelched viral replication to such a degree that it resulted in a weak secondary challenge. Examination of the overall level of the recall response in these experiments suggests that this explanation applies to the results obtained in the LCMV study. In the case of humans pre-exposed to dengue serotypes, it is speculated that Ab-dependent enhancement of the secondary infection may lead to high viral titers and enhanced Ag presentation that drive high-avidity T cells to apoptosis, whereas sparing low-avidity T cells (26). In contrast to the dominant role of Ab in virus control, neutralizing or enhancing Ab responses play no or very little role in controlling Listeria infections in our experiments (27). In addition, when we prime and rechallenged mice with VSV and Listeria recombinants, OVA is the only Ag shared between the two pathogens. OVA is not incorporated into VSV particles so that Ab responses to OVA could not play a role. Thus, although our data clearly show that preimmunization with wt Listeria can impact the subsequent CD8+...
response to \textit{Lm-OVA}, the impact is seen only in blocking the response of low-avidity T cells without inhibiting the response of naive or memory T cells that recognize the new epitope with high avidity.

We have interpreted our results to mean that naive or memory T cells with high avidity for an Ag can compete effectively with low-avidity cells for recruitment and expansion after an infection, even when the latter are present in much larger numbers. We assume that this competition occurs at the level of the Ag-presenting dendritic cell. A surprising finding that distinguishes the response of naive and memory CD8\(^+\) T cells has recently been reported, which would also give an advantage to naive T cell recruitment over memory cell recruitment. Thus, a number of studies showed that after intranasal infection with influenza virus, a subset of dendritic cells in lung-draining lymph nodes was capable of stimulating the response of naive but not memory CD8\(^+\) T cells (28, 29). This remarkable finding would allow the diversity of cells recruited into a response to increase and could explain some of the results presented in this study. However, our T cell responses to \textit{Listeria} are initiated primarily in the spleen and not lymph nodes, and both of the pathogens we used were introduced by the i.v. route. Moreover, after transferring naive or memory OT-1 T cells into naive mice, we observed that both types of cells strongly reduced the magnitude of the endogenous response to the N4 peptide after i.v. challenge with \textit{Lm-N4} (data not shown). For these reasons we do not think that selective Ag presentation to naive over memory T cells explains our failure to observe original antigenic sin.

Prior exposure to pathogens expressing cross-reactive epitopes can influence the specificity of the CD8\(^+\) response to a secondary infection and lead to more rapid control of the second infection (4). We also observed differences in T cell responses to a pathogen depending on whether mice see the pathogen in a primary or in a secondary heterologous infection. For example, CD8\(^+\) T cells in \textit{Lm-N4} primed, \textit{Lm-V4}-challenged mice showed a much stronger cross-reaction on the N4 epitope (\(\sim 50\%\)) than was seen in naive mice immunized with \textit{Lm-V4} (\(\sim 10–25\%\), Figs. 1, 4). Importantly, however, in both cases, the V4-recalled T cells cross-reacting with N4 did so with low avidity. We interpret this finding to mean that during the initial priming with \textit{Lm-N4}, a subset of memory CD8\(^+\) T cells was generated by low-avidity interaction with N4 but this subset happens to cross-react with the V4 epitope with high avidity. The interpretation that low-avidity activation leads to functional memory T cells is in line with our recent demonstration that CD8\(^+\) T cells with a broad range of avidity for Ag are recruited into a primary response (17). Even though the expansion and contribution to memory by low-avidity cells is much less than that of high-avidity cells, their existence becomes apparent when the animal is challenged with a pathogen expressing their high affinity epitope. This could provide a rationale for the recruitment of a broad TCR repertoire with a range of avidities during a primary response because a diverse population of memory cells would be beneficial in coping with subsequent infections with related pathogenic strains expressing cross-reactive epitopes. Similarly, in various chronic infections it had been observed that mutations are more likely to occur in viral epitopes that are recognized by a narrow T cell repertoire than in those recognized by a broad repertoire (30–32). Thus, recruiting a broad range of T cell clones would safeguard against selecting virus variants that escape T cell recognition.

Our current data reveal interesting differences in terms of the ability of low-avidity T cells to expand in primary versus secondary infections. We previously reported that extremely low-affinity TCR ligation is sufficient to induce rapid T cell proliferation, resulting in the generation of functional effector and memory T cells. We showed that the strength of peptide MHC class I and TCR interaction critically determines how many cell divisions the progeny of an individual T cell goes through before the clone begins to decline in numbers (17). However, we now report (Fig. 6) that the same weak ligands that induce robust OT-1 expansion in primary infections, fail to do so in mice that are immune to \textit{wt Listeria}. The interesting question arises as to what prevents the response of low-avidity cells in this situation? Competition between T cells for the same Ag can be excluded as a cause because the mice shown in Fig. 6 were not previously exposed to the OVA Ag or any known cross-reactive epitope. This leaves open the possibility that memory CD8\(^+\) or CD4\(^+\) T cells specific for endogenous \textit{Listeria} Ags or maybe even NK cells are responsible for this effect. Preliminary data indicate that neither injecting anti-NK1.1 nor a CD4 depleting Ab restored the ability of the OT-1 T cells in \textit{Listeria}-primed mice to respond to the low-affinity ligand expressed in \textit{Listeria} (data not shown). In contrast, transferring high numbers of polyclonal CD8\(^+\) T cells from \textit{Lm-wt} immune mice into naive mice strongly diminished the expansion magnitude of OT-1 T cells in response to a \textit{Lm-T4} infection. This indicates that pre-existing memory T cells specific for other epitopes from endogenous \textit{Listeria} Ags can prevent the activation of low-avidity T cells in secondary infections.

Acknowledgments

We thank Martin Pricl for helpful discussions and help with some experiments and Xiaocun Pan for excellent technical support.

Disclosures

The authors have no financial conflicts of interest.

References


F. R. Carbone. 1994. T cell receptor antagonist peptides induce positive selec-
2008. Avidity maturation of memory CD8 T cells is limited by self-antigen 
T cell precursor frequency regulates primary and memory responses to infection. 
Immunity 28: 859–869.
cruitment of antigen-specific CD8+ T cells in response to infection is markedly 
22. Haring, J. S., V. P. Badovinac, and J. T. Harty. 2006. Inflaming the CD8+ T cell 
TCR stimulus controls the magnitude but not functionality of the CD8+ T cell 
totoxic T lymphocytes specific for different antigens during bacterial infection. 
D. Masopust. 2009. Memory CD8 T-cell compartment grows in size with im-
26. Dong, T., E. Moran, N. Vinh Chau, C. Simmons, K. Lahn, Y. Peng, B. Wills, 
cytokine secretion and loss of high avidity cross-reactive cytotoxic T-cells during 
the course of secondary dengue virus infection. PLoS ONE 2: e1192.
revisited: antibody provides resistance to Listeria infection. J. Immunol. 163: 
4087–4090.
Minimal activation of memory CD8+ T cell by tissue-derived dendritic cells 
29. Khanna, K. M., C. C. Aguilã, J. M. Redman, J. E. Suarez-Ramírez, 
by naïve and memory CD8 T cells to late antigen presentation by lymph node 
30. Meyer-Olson, D., N. H. Shoukry, K. W. Brady, H. Kim, D. P. Olson, K. Hartman, 
diversity of HCV-specific T cell responses is associated with CTL escape. J. Exp. 
D. R. Ambrozak, Y. Edghill-Smith, M. J. Kuroda, D. Bogdan, K. Kunstman, 
et al. 2004. T cell receptor recognition motifs govern immune escape patterns in 
2006. Narrowed TCR repertoire and viral escape as a consequence of heterol-