The Journal of Immunology

Cutting Edge: Antigen Is Not Required for the Activation and Maintenance of Virus-Specific Memory CD8$^+$ T Cells in the Lung Airways

Jacob E. Kohlmeier, Shannon C. Miller and David L. Woodland

J Immunol 2007; 178:4721-4725; ;
doi: 10.4049/jimmunol.178.8.4721
http://www.jimmunol.org/content/178/8/4721

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Antigen Is Not Required for the Activation and Maintenance of Virus-Specific Memory CD8$^+$ T Cells in the Lung Airways[1]

Jacob E. Kohlmeier, Shannon C. Miller, and David L. Woodland[2]

Respiratory virus infections establish a population of memory CD8$^+$ T cells in the lung airways that persist for months after infection. However, the relationship between Ag-specific memory T cells in the lung airways and the systemic memory T cell pool is not well understood. The majority of lung airway memory T cells express a highly activated phenotype (CD69$^+$/CD127$^-$), suggesting that recent Ag stimulation is required to drive T cell activation and recruitment to the lung airways. In this study, we demonstrate that the lung airway environment itself in the absence of cognate Ag alters the expression of acute activation markers such as CD69 and CD127 on memory CD8$^+$ T cells. Furthermore, the steady-state recruitment of virus-specific memory CD8$^+$ T cells to the lung airways from the circulation can occur without recent Ag stimulation. These findings alter the current perceptions concerning the contribution of Ag to the maintenance of peripheral T cell memory. *The Journal of Immunology,* **2007, 178: 4721–4725.**

Following the resolution of an adaptive immune response, memory T cells are established in secondary lymphoid organs and a broad range of peripheral tissues. In the case of influenza or parainfluenza virus infections of the respiratory tract, a population of memory CD8$^+$ T cells is also established in the lung airways, and this population is thought to play a key role in mediating early responses to secondary virus challenge (1). Studies in the mouse model have demonstrated that the absolute number of Ag-specific memory CD8$^+$ T cells present in the airways following respiratory virus clearance progressively declines over several months, before stabilizing for the life of the animal (2). Importantly, the number of cells correlates with the efficacy of cellular immune responses to secondary challenge (3). Although the precise mechanisms are not yet clear, recent studies have shown that memory T cells in the airways contribute to cellular immunity by limiting early viral loads in an Ag-specific manner (4). Given the contribution of peripheral memory T cells in the recall response, it is important that we understand the relationship between Ag-specific memory CD8$^+$ T cells in the lung airways and their systemic counterparts.

Recent work has demonstrated that CD8$^+$ T cells localized to the airways following Sendai or influenza infection are exclusively effector memory cells (5). Curiously, the majority of lung airway memory T cells also express CD69, a marker of acute activation (2). This observation suggested that stimulation by residual Ag in the draining lymph nodes was required to drive memory T cell activation and recruitment to the lung airways following clearance of a respiratory virus infection (6). However, earlier studies suggested that the localization of memory T cells to the lung airways can occur in an Ag-independent manner (7, 8).

To resolve these discrepancies and to better understand memory T cell maintenance at peripheral sites, we investigated the role of the lung airway environment on the activation status of airway memory CD8$^+$ T cells and the recruitment of memory CD8$^+$ T cells from the circulation in the presence or absence of residual Ag. We demonstrate that although memory CD8$^+$ T cells in the lung airways display an activated phenotype normally associated with recent Ag exposure, acquisition of this phenotype is Ag independent and is maintained on a subset of virus-specific cells for at least one year after initial infection. Furthermore, the regulation of these activation markers is a dynamic process that is influenced by the lung airway environment. Finally, we demonstrate that the steady-state recruitment of virus-specific CD8$^+$ memory T cells to the lung airways and subsequent phenotypic changes can occur in the absence of cognate Ag.

Materials and Methods

Viruses, mice, and infection

Sendai virus (Enders strain) and influenza virus A/PR8/34 (PR8, H1N1) were grown, stored, and titered as described previously (9). Female C57BL/6J, B6.Pl-*Thy1^a*/Cy (CD90.1), and B6.SJL-*Ptprc^a* Pep3/BoyJ (CD45.1) mice were purchased from The Jackson Laboratory. Mice (8–10 wk old) were anesthetized by i.p. injection of 2,2,2-tribromoethanol (200 mg/kg) and infected with 250 50% egg infectious doses (EID_{50})[3] of Sendai virus or 300 EID_{50} PR8 virus. All animal studies were approved by the institutional animal care and use committee.

Trudeau Institute, Saranac Lake, NY 12983

Received for publication December 22, 2006. Accepted for publication February 8, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants AI067967 and AI49823 and funds from the Trudeau Institute.

[2] Address correspondence and reprint requests to Dr. David L. Woodland, Trudeau Institute, 154 Algonquin Avenue, Saranac Lake, NY 12983. E-mail address: dwoodland@trudeauinstitute.org

[3] Abbreviations used in this paper: EID_{50}, 50% egg infectious dose; i.t., intratracheal; BAL, bronchoalveolar lavage.

Tissue harvest and flow cytometry

Tissues were harvested and processed as described previously (10). MHC class I-peptide tetramers specific for the Sendai virus nucleoprotein epitope (Sen-$NP_{324-332}/K^b$) or influenza nucleoprotein epitope (Flu-$NP_{366-374}/D^b$) were generated by the Trudeau Institute Molecular Biology Core. mAbs were purchased from BD Biosciences, eBioscience, or Caltag Laboratories. Samples were run on either a FACSCalibur cytometer (BD Biosciences) or CyAn ADP (DakoCytomation). All data were analyzed with FlowJo software (Tree Star).

Cell sorting and adoptive transfers

Splenic memory $CD8^+$ T cell subsets were sorted using a FACSVantage SE with DIVA option (BD Biosciences). A sample of the sorted cells was analyzed to confirm sort purity. Sorted cells were transferred intratracheally (i.t.) (1×10^5 cells in 100 μl) into anesthetized mice with a blunted and bent 20-gauge needle. Cells were injected i.p. (2×10^5 cells) or i.v. ($0.5–1.5 \times 10^6$ cells) in 200 μl.

Results and Discussion

To more thoroughly investigate the expression of activation markers on memory $CD8^+$ T cells in the lung airways, we compared the phenotype of Sendai-specific T cells in the lung airways and the spleen (Fig. 1*A*). In agreement with earlier work, Sendai-specific airway memory cells at 1 mo postinfection lacked expression of CD62L, and the majority expressed low levels of CD11a and Ly6C, and high levels of CD69, compared with splenic memory $CD8^+$ T cells (2). Additionally, the airway $CD8^+$ T cell population had decreased expression of CD27 and CD127, and was enriched for cells expressing CD103 and the activation isoform of CD43. This phenotype is very similar to that displayed by TCR-transgenic memory $CD8^+$ T cells found in the intestinal mucosa following systemic infection with lymphocytic choriomeningitis virus (11), suggesting that this phenotype may be a hallmark of T cells localized at mucosal surfaces as opposed to other peripheral sites (12). In support of this idea, Sendai virus-specific cells in the lung parenchyma, PBL, bone marrow, and peritoneal cavity had a phenotype similar to that observed in the secondary lymphoid organs (spleen and mediastinal lymph node) at 1 mo postinfection (data not shown).

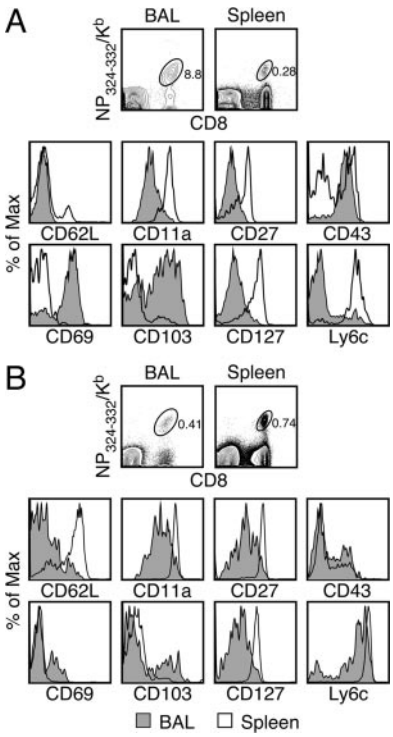

FIGURE 1. Ag-specific $CD8^+$ memory T cells in the lung airways display an activated phenotype. C57BL/6 mice were infected intranasally with 250 EID_{50} Sendai virus and sacrificed at 1 mo (*A*) or 12 mo (*B*) postinfection. Lung airway cells were obtained by bronchoalveolar lavage (BAL) pooled from 5 to 10 mice (1 mo) or 15–20 mice (12 mo) for analysis, and splenocytes were analyzed from individual mice. Representative tetramer staining is shown for the lung airways and spleen at each time point. The histograms shown are gated on Sen-$NP_{324-332}/K^{b+}$ cells from the lung airways (filled histogram) and spleen (open histogram). Data are representative of three individual experiments.

Because recent studies have shown that residual Ag is cleared by 2 mo following respiratory virus infection (6, 13), we also analyzed activation marker expression at 12 mo postinfection (when Ag is unlikely to be present). As shown in Fig. 1*B*, even at this late time point, many airway memory $CD8^+$ T cells displayed a highly activated phenotype, although fewer cells had decreased expression of Ly6C and increased expression of CD69 and CD103. Although fewer Sendai-specific cells expressed CD69 at 12 mo (29% $CD69^+$) compared with 1 mo (81% $CD69^+$) postinfection, it is surprising that any $CD69^+$ cells were found in the airways so long after virus clearance. The activated phenotype of airway memory $CD8^+$ T cells was not unique to Sendai virus infection because similar results were observed at both 1 mo and 12 mo following influenza A/PR8/34 virus infection (data not shown). Taken together, these results demonstrate that Ag-specific memory $CD8^+$ T cells generated following respiratory infections have a unique, activated phenotype within the lung airways that is not readily attributed to recent Ag exposure.

Our previous studies demonstrated that the lung airways could affect expression of an integrin molecule, CD11a, on memory T cells (8). Therefore, we hypothesized that the activation phenotype of memory $CD8^+$ T cells in the airways was also influenced by the local microenvironment. To investigate this hypothesis, total $CD8^+/CD44^{high}$ cells from the spleens of Sendai-immune mice were sorted on the basis of activation marker expression ($CD27^+$, $CD62L^+$, $CD69^-$, $CD103^-$, $CD127^+$, or $Ly6C^+$) and transferred i.t. into the airways of naive recipients (Fig. 2*A*). As a control, sorted populations were transferred into the peritoneums of naive recipients. Activation marker expression was assessed at 18, 48, and 96 h posttransfer, and in each experiment we also analyzed expression of molecules known to be influenced (CD11a) or unaffected (CD8) by the lung airway environment and measured the recovery of transferred cells (Fig. 2*B*).

As shown in Fig. 2*C*, the expression of CD27, CD127, and Ly6C was rapidly decreased on memory $CD8^+$ T cells following transfer into the lung airways but not the peritoneum. By 96 h, memory $CD8^+$ T cells transferred into the airways acquired a phenotype ($CD27^{low}$, $CD127^{low}$, or $Ly6C^{low}$) representative of the majority of Ag-specific memory cells present in the lung airways following respiratory virus infection. Importantly, these changes were observed for the entire memory ($CD8^+CD44^{high}$) population following transfer to a naive recipient, arguing against a role for Ag in driving these phenotypic changes. In contrast, CD62L expression was not decreased following i.t. transfer, indicating that the lung airway environment does not alter expression of this marker and suggesting that the cells present in the airways at early and late times postinfection arrive as effector memory ($CD62L^{low}$) cells. The expression of CD69 was also increased on a subset (25–42% of transferred

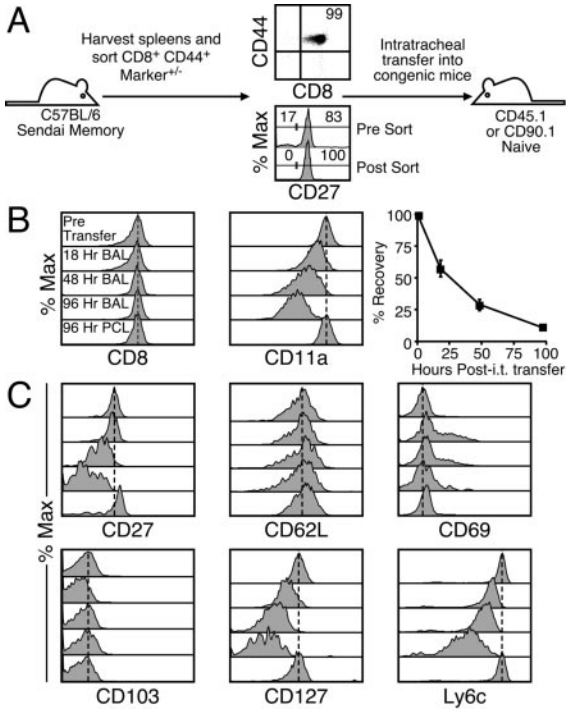

FIGURE 2. Activation marker expression is regulated by the lung airway environment. C57BL/6 mice were infected with 250 EID_{50} Sendai virus and rested for 45–60 days. Splenocytes were pooled from 10 to 15 mice and stained with Abs specific for CD8, CD44, and the activation marker of interest for sorting. Individual sorts were performed to isolate different memory $CD8^+$ T cell subsets ($CD27^+$, $CD62L^{high}$, $CD69^-$, $CD103^-$, $CD127^+$, or $Ly6C^+$). In *A*, $CD27^+$ sorted cells are shown as an example. Sorted cells were then transferred i.t. and i.p. into naive congenic (CD45.1 or CD90.1) recipients. Cells were recovered by BAL (for i.t. transfers) and peritoneal cavity (for i.p. transfers) at the indicated times and the donor cells ($CD45.2^+$ or $CD90.2^+$) were reanalyzed for expression of CD8, CD11a, and the activation marker used for sorting. *B*, Shows the expression of CD8 and CD11a on donor cells at 18, 48, and 96 h after i.t. transfer (BAL), or 96 h after i.p. transfer (peritoneal exudate lavage), as well as the percentage of donor cells recovered from the BAL at each time point. *C*, Shows transfer data from cells sorted on each of the indicated markers. Each panel is organized in the same manner as *B*. Data are representative of at least three independent experiments for each sorted memory subset.

cells became $CD69^+$) of memory $CD8^+$ T cells, although the kinetics were different from the other activation-associated molecules we examined. Maximal expression of CD69 was observed by 18 h posttransfer, and the percentage of $CD69^+$ cells steadily decreased between 18 and 96 h posttransfer. We considered the possibility that CD69 was rapidly up-regulated and then subsequently down-regulated causing us to underestimate the percentage of cells that expressed CD69. However, the percentage of $CD69^+$ cells at 6 h posttransfer was similar to that observed at 18 h, making this possibility unlikely (data not shown). The early expression of CD69 was not due to cellular stress during transfer, since cells transferred i.p. were still $CD69^-$ at 18 h (data not shown). These data demonstrate that the lung airway environment itself can dynamically alter surface marker expression on memory $CD8^+$ T cells, and this effect is sufficient to account for the activated phenotype characteristic of Ag-specific memory T cells in the lung airways following respiratory virus infection.

We were especially interested in the regulation of CD69 on cells in the airways, because this marker has classically been associated with recent Ag stimulation. Although CD69 expression was increased on cells transferred to the lung airways, the percentage of $CD69^+$ cells in these experiments was less than that observed in 1-mo memory mice (Figs. 1*A* and 2*C*). Therefore, we considered the possibility that the percentage of cells that became $CD69^+$ after i.t. transfer would be greater if the recipient mice had previously recovered from a Sendai virus infection (and may therefore retain viral Ag within the airways). To investigate this idea, we compared CD69 expression on Sendai-specific and nonspecific memory cells following i.t. transfer into naive mice or mice that had recovered from a prior Sendai virus infection (either 1 mo or 4 mo postinfection; Fig. 3). Comparisons between Sendai-specific (●) and nonspecific (○) memory $CD8^+$ T cells revealed no differences in CD69 expression, regardless of whether the recipient mice had been previously infected with Sendai virus. There was also no difference in the percentage of recovery of Ag-specific and nonspecific populations (data not shown). Along with Figs. 1 and 2, these data formally demonstrate that the activated phenotype of virus-specific memory $CD8^+$ T cells within the lung airways occurs independent of recent Ag stimulation.

Our observation that the lung airway environment itself can alter activation marker expression demonstrated that the phenotype of memory $CD8^+$ T cells in the lung airways is a poor indicator of recent Ag stimulation. These data prompted us to revisit the necessity of Ag for the recruitment of memory $CD8^+$ T cells to the lung airways. Resting ($CD69^-$) effector memory $CD8^+$ T cells were sorted from Sendai-immune mice 45–60 days postinfection and transferred i.v. into congenic mice infected with influenza PR8 virus 35 days earlier (Fig. 4*A*). This approach allowed us to investigate both the localization and phenotype of different respiratory virus-specific memory $CD8^+$ T cell populations in a setting where only one of the populations has access to residual Ag. In addition, we allowed donor memory cells to equilibrate within recipient mice for 14 days before analysis to control for the potential activation of cells during transfer. Fourteen days after transfer, both donor Sendai- and host Flu-specific memory cells were detected in the lung airways, as well as in the lung tissue, PBL, and spleen (tetramer staining shown is from the lung airways; Fig. 4*B*). Notably, the expression of CD11a, CD69, and CD127 was similar between Sendai- and Flu-specific memory cells within each of the tissues analyzed. As expected, memory $CD8^+$ T cells localized to the airways expressed low levels of CD11a (Fig. 4*B*). Importantly, only Sendai virus-specific memory $CD8^+$ T cells in the lung airways, not in the lung tissue, peripheral blood, or spleen, displayed an activated ($CD69^+CD127^{low}$) phenotype.

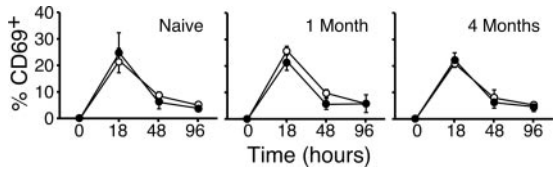

FIGURE 3. Ag specificity and infection history do not impact CD69 expression in the lung airways. Splenocytes were harvested from Sendai-immune mice as described in Fig. 2. A purified population of $CD8^+CD44^{high}CD69^-$ cells was obtained by cell sorting and transferred i.t. into naive (*left panel*), 1-mo Sendai memory (*middle panel*), or 4-mo Sendai memory (*right panel*) congenic recipient mice. The percentage of $CD69^+$ Sen-$NP_{324-332}/K^{b+}$ (●) and $CD69^+$ Sen-$NP_{324-332}/K^{b-}$ (○) donor cells was determined by flow cytometry. Data are representative of two independent experiments.

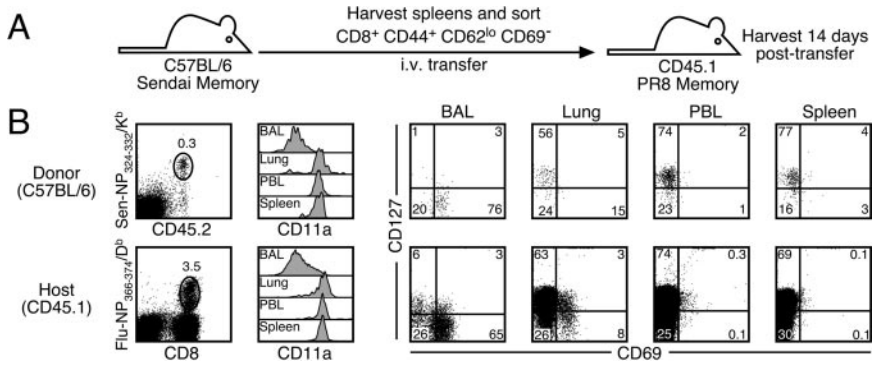

FIGURE 4. Ag is not required for the recruitment of virus-specific cells to the lung airways. Resting effector memory $CD8^+$ T cells ($CD8^+CD44^{high}CD62L^{low}CD69^-$) were sorted from C57BL/6 Sendai memory mice 45–60 days postinfection. Sorted cells were transferred i.v. into congenic $CD45.1^+$ mice that had been infected with 300 EID_{50} influenza PR8 virus 35 days earlier (*A*). Fourteen days posttransfer, BAL, lung tissue, peripheral blood, and spleen cells were stained with Sen-$NP_{324–332}/K^b$ or Flu-$NP_{366–374}/D^b$ tetramers and Abs specific for CD8, CD45.2, CD11a, CD69, and CD127. Tetramer staining for Sendai-specific (donor, gated on $CD8^+$) and Flu-specific (host, total lymphocyte gate) T cells in the BAL is shown (*B*). The expression of CD11a (histograms), CD69, and CD127 (dot plots) in the BAL, lung tissue, PBL, and spleen was analyzed on Ag-specific donor or host $CD8^+$ T cells. Data are representative of three independent experiments.

Furthermore, the percentage of $CD69^+$ Sendai-specific memory cells in the lung airways was similar to that observed in Fig. 1*A*, raising the possibility that migration across endothelial and epithelial barriers may be required for maximal CD69 expression. Taken together, these data clearly demonstrate that the recruitment of memory $CD8^+$ T cells from the circulation into the lung airways and their acquisition of an activated phenotype can occur independent of Ag.

Recent observations that residual Ag is maintained in the local draining lymph nodes for several weeks following respiratory virus infection, and that memory T cells within the airways have an activated phenotype, have suggested a model in which recent Ag stimulation is required for continual recruitment to the lung airways (6). However, this model was predicated on the reported observation that Ag-specific cells could not be detected in the airways at 4 mo following influenza virus infection, which is in disagreement with several previous studies (2, 8, 14, 15). Our data suggest an alternative, Ag-independent mechanism by showing that virus-specific cells can be found in the airways for at least 12 mo postinfection and also by demonstrating that recent Ag stimulation is not required for the recruitment or activated phenotype of airway memory $CD8^+$ T cells. It is important to note, however, that these new observations do not exclude a role for residual Ag in memory cell recruitment to the lung airways, particularly during the initial months postinfection. It is tempting to hypothesize that memory T cell recruitment to the lung airways is controlled by both Ag-dependent and independent processes immediately following virus clearance. As residual Ag is cleared and the gradual decline in the number of memory $CD8^+$ T cells within the lung airways stabilizes at a low level, Ag-independent processes are required to maintain this population over the long term.

Although it is unclear how the lung airway environment alters activation marker expression on memory T cells, recent work has demonstrated that signaling through the IFN-$\alpha\beta$ receptor is sufficient to induce CD69 expression (16, 17). It is possible that the continual presence of inhaled Ags and microorganisms results in a low level of persistent inflammation, which in turn alters the expression of activation markers on memory T cells within the respiratory tract. Continual inflammation may also affect local chemokine production, thereby influencing Ag-independent recruitment to the lung airways. Alternatively, the ability of virus-specific effector memory T cells to migrate to the airways under steady-state conditions could imply that tissue-specific homing receptors exist for recruitment to the lung airways (18–20).

In conclusion, our data demonstrate that Ag is not required for the activated phenotype of memory $CD8^+$ T cells within the lung airways or their recruitment from the circulation. Future studies will clarify the role of the local cytokine and chemokine milieu in altering memory T cell phenotype within the lung airways and in directing their recruitment from the circulation. Understanding the factors that control the maintenance of memory $CD8^+$ T cells in the lung airways will be important for future vaccine design.

Acknowledgments

We thank Brandon Sells for assistance with flow cytometry, the Trudeau Institute Molecular Biology Core Facility for producing multimeric reagents, and Drs. Marcia Blackman, Ken Ely, William Reiley, Shiki Takamura, and Eric Yager for critically reviewing the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Hikono, H., J. E. Kohlmeier, K. H. Ely, I. Scott, A. D. Roberts, M. A. Blackman, and D. L. Woodland. 2006. T-cell memory and recall responses to respiratory virus infections. *Immunol. Rev.* 211: 119–132.
2. Hogan, R. J., E. J. Usherwood, W. Zhong, A. D. Roberts, R. W. Dutton, A. G. Harmsen, and D. L. Woodland. 2001. Activated antigen-specific $CD8^+$ T cells persist in the lungs following recovery from respiratory virus infections. *J. Immunol.* 166: 1813–1822.
3. Liang, S., K. Mozdzanowska, G. Palladino, and W. Gerhard. 1994. Heterosubtypic immunity to influenza type A virus in mice: effector mechanisms and their longevity. *J. Immunol.* 152: 1653–1661.
4. Hogan, R. J., W. Zhong, E. J. Usherwood, T. Cookenham, A. D. Roberts, and D. L. Woodland. 2001. Protection from respiratory virus infections can be mediated by antigen-specific $CD4^+$ T cells that persist in the lungs. *J. Exp. Med.* 193: 981–986.
5. Hogan, R. J., L. S. Cauley, K. H. Ely, T. Cookenham, A. D. Roberts, J. W. Brennan, S. Monard, and D. L. Woodland. 2002. Long-term maintenance of virus-specific effector memory $CD8^+$ T cells in the lung airways depends on proliferation. *J. Immunol.* 169: 4976–4981.
6. Zammit, D. J., D. L. Turner, K. D. Klonowski, L. Lefrancois, and L. S. Cauley. 2006. Residual antigen presentation after influenza virus infection affects CD8 T cell activation and migration. *Immunity* 24: 439–449.
7. Topham, D. J., M. R. Castrucci, F. S. Wingo, G. T. Belz, and P. C. Doherty. 2001. The role of antigen in the localization of naive, acutely activated, and memory $CD8^+$ T cells to the lung during influenza pneumonia. *J. Immunol.* 167: 6983–6990.

8. Ely, K. H., T. Cookenham, A. D. Roberts, and D. L. Woodland. 2006. Memory T cell populations in the lung airways are maintained by continual recruitment. *J. Immunol.* 176: 537–543.
9. Hou, S., L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty. 1994. Virus-specific $CD8^+$ T-cell memory determined by clonal burst size. *Nature* 369: 652–654.
10. Roberts, A. D., K. H. Ely, and D. L. Woodland. 2005. Differential contributions of central and effector memory T cells to recall responses. *J. Exp. Med.* 202: 123–133.
11. Masopust, D., V. Vezys, E. J. Wherry, D. L. Barber, and R. Ahmed. 2006. Cutting edge: gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J. Immunol.* 176: 2079–2083.
12. Lefrancois, L., and L. Puddington. 2006. Intestinal and pulmonary mucosal T cells: local heroes fight to maintain the status quo. *Annu. Rev. Immunol.* 24: 681–704.
13. Jelley-Gibbs, D. M., D. M. Brown, J. P. Dibble, L. Haynes, S. M. Eaton, and S.L. Swain. 2005. Unexpected prolonged presentation of influenza antigens promotes CD4 T cell memory generation. *J. Exp. Med.* 202: 697–706.
14. Marshall, D. R., S. J. Turner, G. T. Belz, S. Wingo, S. Andreansky, M. Y. Sangster, J. M. Riberdy, T. Liu, M. Tan, and P. C. Doherty. 2001. Measuring the diaspora for virus-specific $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA* 98: 6313–6318.
15. Marsland, B. J., N. L. Harris, M. Camberis, M. Kopf, S. M. Hook, and G. Le Gros. 2004. Bystander suppression of allergic airway inflammation by lung resident memory $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA* 101: 6116–6121.
16. Alsharifi, M., M. Lobigs, M. Regner, E. Lee, A. Koskinen, and A. Mullbacher. 2005. Type I interferons trigger systemic, partial lymphocyte activation in response to viral infection. *J. Immunol.* 175: 4635–4640.
17. Shiow, L. R., D. B. Rosen, N. Brdickova, Y. Xu, J. An, L. L. Lanier, J. G. Cyster, and M. Matloubian. 2006. CD69 acts downstream of interferon-$\alpha\beta$ to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* 440: 540–544.
18. Ray, S. J., S. N. Franki, R. H. Pierce, S. Dimitrova, V. Koteliansky, A. G. Sprague, P. C. Doherty, A. R. de Fougerolles, and D. J. Topham. 2004. The collagen binding $\alpha_1\beta_1$ integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. *Immunity* 20: 167–179.
19. de Bree, G. J., E. M. M. van Leeuwen, T. A. Out, H. M. Jansen, R. E. Jonkers, and R. A. W. van Lier. 2005. Selective accumulation of differentiated $CD8^+$ T cells specific for respiratory viruses in the human lung. *J. Exp. Med.* 202: 1433–1442.
20. Kohlmeier, J. E., and D. L. Woodland. 2006. Memory T cell recruitment to the lung airways. *Curr. Opin. Immunol.* 18: 357–362.