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CUTTING EDGE

Cutting Edge: Emergence of CD127^{high} Functionally Competent Memory T Cells Is Compromised by High Viral Loads and Inadequate T Cell Help[1]

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***In this report we have inspected whether difficulties in controlling viral infections negatively impacts the generation of CD127^{high} memory T cells. Using both MHC class I and II tetramers we reveal that CD127^{low} T cells are not necessarily rapidly deleted but can persist in a pseudoeffector state in which they display the hallmarks of activated effector cells but are functionally inferior. CD127^{high} cells can, however, emerge if the infection is contained. We also show that in the absence of CD4 T cell help significant populations of CD127^{high} CD8 T cells fail to emerge. Analyses of cytokine-producing activities by both mouse and human CD8 T cells further document that the extended maintenance of T cells in a CD127^{low} state has functional consequences which manifest as an impairment of IL-2 production. The Journal of Immunology,* 2005, 174: 5926–5930.**

Effective T cell responses are pivotal for controlling many intracellular pathogens and contribute to immunological protection during secondary infections. Although these responses can be highly potent, qualitative differences between Ag-specific T cells have been observed. In the case of chronic viral infections, T cells can emerge which are incapable of elaborating the optimal array of functions necessary for controlling the infection (1–4). In addition, CD4 T cells have been proposed to play a key role in the maturation and maintenance of memory CD8 T cells (Ref. 5 and reviewed in Ref. 6).

Several reports have documented that IL-7 and IL-15 promote the survival and homeostatic proliferation of memory CD8 T cells (7, 8). Expression of the IL-7R α-chain (CD127) has been shown to mark a fraction of activated effector CD8 T cells that are more likely to survive and give rise to robust memory T cells (9–12). In addition, IL-7-dependent signals also support the emergence and survival of memory CD4 T cells (13, 14). Because Ag-specific T cells with reduced effector capacities have been observed in chronically infected hosts, and the absence of CD4 T cell help results in diminished memory CD8 T cell responses, it is of interest to determine whether these disparities are associated with alterations in CD127 expression.

In the present study we have evaluated whether difficulties in controlling viral infections as well as insufficient CD4 T cell help negatively impact the emergence of CD127^{high} T cells. We demonstrate that, in persistently infected hosts, Ag-specific CD127^{low} T cells remain detectable for extended periods but CD127^{high} cells can emerge if the viral load is contained. CD4 T cell help appears to be a critical fate determinant for Ag-specific CD8 T cells as "helpless" CD8 T cells which emerge in the absence of CD4 T cells also retain a CD127^{low} phenotype. We further show that the failure to express high levels of CD127 has functional consequences in both mice and humans which manifest as an inability to produce marked amounts of IL-2 at later timepoints following infection.

Materials and Methods

Mice and virus

C57BL/6J (B6) and C57BL/6-Cd4^{tm1Mak} (CD4$^{-/-}$) mice (The Jackson Laboratory) were bred and maintained in accredited facilities at the University of Alabama at Birmingham. Male and female mice between 6 and 10 wk of age were used. Acute, protracted, and chronic lymphocytic choriomeningitis virus (LCMV)[3] infections were established as previously described (3). For rechallenge studies, LCMV immune B6 mice, which had been infected >80 days previously with 2×10^5 PFU LCMV-Armstrong, were administered 2×10^6 PFU LCMV-clone 13 by i.v. injection.

MHC class I and II tetramer preparation and use

MHC class-I-peptide complexes were prepared and used as previously described (1, 3). MHC class II I-A^b LCMV gp61 tetramers were produced in High Five insect cells using a recombinant baculovirus expression system (15, 16). Cells were stained with I-A^b(gp61) tetramers (8 μg/ml) at 37°C for 75 min in R10. Subsequently, anti-CD4-allophycocyanin (clone RM4.5), anti-CD127-FITC (clone A7R34), and anti-CD16/32-biotin (clone 2.4G2) mAbs were added. After 45 min cells were washed and streptavidin-PerCP added. After an additional 30 min, two washes were performed and cells fixed before analysis.

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[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; SEB, *Staphylococcal* enterotoxin B; MFI, mean fluorescence intensity.

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Analyses of murine responses

Freshly explanted spleens were disrupted into single cell suspensions and responses analyzed as previously described (17).

Analyses of human responses

Blood was obtained from three healthy HIV-seronegative human volunteers and PBMC isolated by standard Histopaque density centrifugation and cryopreserved. Cytokine production by PBMC from human subjects was assessed by standard procedures. Thawed cells (10^6) were either left untreated or stimulated for 5 h at 37°C in the presence of anti-CD28 and anti-CD49d mAbs (1 μg/ml) together with staphylococcal enterotoxin B (SEB) (1 μg/ml). Monensin was added after the first hour of incubation. Following incubation for an additional 4 h, samples were stained for the expression of CD3, CD8, CD127, IL-2, and IFN-γ.

Flow cytometry

Samples were acquired using either a FACSCalibur or LSRII flow cytometer and data were analyzed with either CellQuest or FlowJo software.

Results and Discussion

Previous reports have shown that CD127^{high} T cells which arise during the effector phase of an immune response acquire the phenotypic and functional traits characteristic of memory T cells (9–12). During the course of infections that are difficult to resolve, virus-specific T cells do emerge but fail to attain the functional attributes which are representative of memory T cells (1–4). We used the LCMV system to address whether the expression of CD127 marked T cells that are maintained regardless of their functional competence or whether functionally compromised T cells which manifest in persistently infected hosts represent CD127^{low} remnants.

The expression of CD127 on virus-specific CD8 T cells was monitored during the course of acute LCMV-Armstrong infection of B6 mice, protracted infection of B6 mice with the clone 13 strain, and during the course of chronic clone 13 infection of CD4$^{-/-}$ mice (Fig. 1). Acute LCMV infection is rapidly controlled and serum viral loads were below the limits of detection (<50 PFU/ml) by 8 days after inoculation (Fig. 1*C*). Only a fraction (9–15%) of Ag-specific CD8 T cells were CD127^{high} at this timepoint (Fig. 1), but as previously shown, after viral clearance CD127^{high} T cells preferentially survive the contraction phase and dominate the memory pool (9–12).

We next determined whether extended exposure to viral Ags impacts the generation of CD127^{high} T cells. LCMV-clone 13 infection of B6 mice results in a protracted infection which is brought under control over a period of 2–3 mo (2, 3) (Fig. 1*C*). By 8 days after protracted LCMV infection, the responding antiviral CD8 T cells were predominantly CD127^{low}. Unlike acute LCMV infection during which a transition occurs as CD127^{high} memory T cells emerge, in protractedly infected hosts virus-specific T cells remain CD127^{low} for at least 4 mo (Fig. 1). Thus, CD127^{low} T cells have divergent fates that appear to depend upon whether the infection is controlled. Although the virus-specific CD8 T cells can remain CD127^{low} for an extended period it should be noted that during protracted and chronic LCMV-clone 13 infection a contraction of the overall response does occur during the first month following infection (3).

The notion that viral clearance can promote the development of CD127^{high} cells is supported by the patterns of CD127 expression on antiviral T cells at later timepoints. By 260 days postinfection, when protracted LCMV infection was markedly controlled, 78 ± 4% and 40 ± 9% of gp33 and np-396-specific

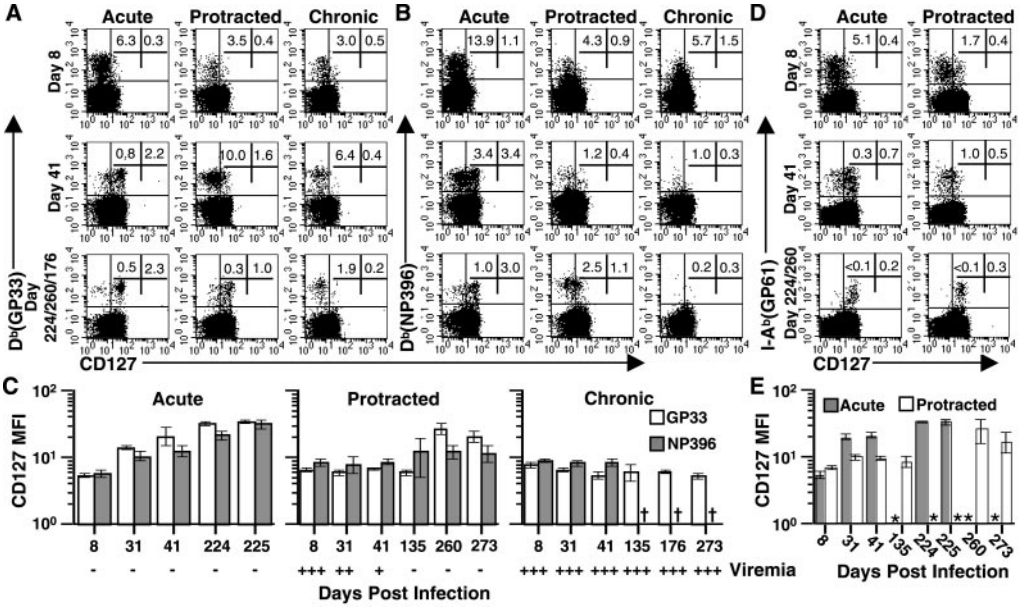

FIGURE 1. Dynamics of CD127 expression on virus-specific T cells. The expression of CD127 on virus-specific CD8 and CD4 T cells was evaluated by costaining mouse splenocytes with either MHC class I or II tetramers together with anti-CD8 or anti-CD4 mAbs as well as anti-CD127 mAbs. *A* and *B*, Representative flow cytometry plots showing gp33-specific (*A*) and np396-specific (*B*) CD8 T cells at various timepoints following acute, protracted, and chronic LCMV infections, as indicated. *C*, The geometric MFI of CD127 expression on either gp33- (□) or np396- (▨) specific CD8 T cells following acute, protracted, and chronic LCMV infection is shown. Similarly, *D* shows the staining profiles of gp61-specific CD4 T cells at various timepoints following infection, and *E* reports the MFI of CD127 expression on gp61-specific CD4 T cells following acute (▨) and protracted (□) LCMV infections. Values on flow cytometry plots state the percentages of gated CD8 (*A* and *B*) or CD4 (*D*) T cells that stain positively with the indicated tetramer and are CD127^{low} (*upper left*) or CD127^{high} (*upper right*). Mean values from two to four mice at each timepoint ± SD are plotted. †, Below limit of detection; ⋆, not done. The serum viral loads are indicated as follows: −, <50 PFU/ml; +, 50 to 1 × 10^3 PFU/ml; ++, 1 × 10^3 to 1 × 10^4 PFU/ml; +++, 1 × 10^4 to 1 × 10^5 PFU/ml.

CD8 T cells were $CD127^{high}$, respectively (Fig. 1). Thus, $CD127^{high}$ virus-specific T cells can emerge if the high grade infection is brought under control. Improvements in the functional quality of antiviral CD8 T cells following reduction of viral loads has now been demonstrated in several animal models of persistent viral infections as well as in human subjects. In the case of LCMV infection of mice and hepatitis C virus infections of humans at least a partial recovery of functional CD8 T cell activities has been reported as an initially high viral burden is brought under control (3, 18, 19). Decreased expression of CD127 on CD8 T cells has also been reported during HIV infection; however, levels of expression have been shown to increase if successful antiviral therapy is applied (20–23). The observations that $CD127^{high}$ cells can emerge if antigenic stimulation is withdrawn suggest that the maturation of antiviral T cells from a $CD127^{low}$ to $CD127^{high}$ state is associated with an enhancement in their ability to mediate long-term infection control.

Although, as shown above, $CD127^{low}$ CD8 T cells can remain detectable for prolonged periods, constant antigenic stimulation resulting from chronic viral infection is detrimental to the maintenance and function of antiviral T cells (1–4, 24). In $CD4^{-/-}$ mice chronically infected with LCMV-clone 13, the virus-specific CD8 T cells that develop fail to mature into $CD127^{high}$ memory phenotype cells (Fig. 1). By 8 days postinfection all specificities of antiviral CD8 T cells were $CD127^{low}$, and $CD127^{high}$ cells never became prevalent (Fig. 1). $CD127^{low}$ T cells are, however, not maintained indefinitely in the absence of CD4 T cell help as they succumb to deletion overtime (3–5, 24, and Fig. 1). These data highlight two key points regarding CD127 expression by antiviral CD8 T cells. First, the emergence of $CD127^{high}$ T cells is governed by both the withdrawal of antigenic stimulation and the presence of CD4 T cell help. Second, decreased IL-7 sensitivity due to the absence of receptor expression does not necessarily lead to rapid deletion (19); nevertheless the inability to up-regulate CD127 may result in an attrition of virus-specific T cell responses.

We next addressed whether high viral loads also impeded the emergence of $CD127^{high}$ antiviral CD4 T cells by assessing the expression of CD127 on virus-specific CD4 T cells during the course of acute and protracted infections (Fig. 1, *D* and *E*). The dynamics of CD127 expression on LCMV-specific CD4 T cells in acutely infected hosts paralleled that of CD8 T cell responses. By 8 days post-acute infection, expression was low (mean fluorescence intensity (MFI) = 5.4 ± 0.6); however, by 1 mo following infection $CD127^{high}$ virus-specific CD4 T cells predominated and levels of expression remained high (MFI = 19.8 ± 1.8) until the last timepoint checked, at 7.5 mo postinfection. Virus-specific CD4 T cells were also $CD127^{low}$ early following protracted LCMV infection and expression remained low while the virus persisted (Fig. 1, *D* and *E*). As the infection was contained $CD127^{high}$ CD4 T cells emerged and by >8 mo postinfection ~65% of virus-specific CD4 T cells were $CD127^{high}$ in protractedly infected hosts. Thus, during protracted LCMV infection the viral load appeared to act as a rheostatic regulator of CD127 expression on both virus-specific CD4 and CD8 T cells, which is consistent with a model that repetitive antigenic stimulation hinders the differentiation of the responding T cells.

We next monitored whether the expression of CD127 on virus-specific CD4 and CD8 T cells changed, as they participated in protective recall responses, to resemble the levels on primary effectors or whether the levels of expression more closely matched that of memory T cells. Rechallenge of LCMV immune mice resulted in downshifts in CD127 expression on both np396- and gp33-specific CD8 T cells (Fig. 2*A*) as well as on gp61-specific CD4 T cells (Fig. 2*B*). Thus, after memory is established the expression of CD127 is not permanently set at a high level and interchanges between $CD127^{high}$ and $CD127^{low}$ states can occur which reflect the T cells' maturation and activation state.

Numerous reports have documented that CD8 T cells, which develop in the absence of CD4 T cell help, exhibit a skewed phenotype, resembling effector rather than central memory T cells and display a diminished capacity to proliferate and function following antigenic re-exposure (6, 17). Strikingly, the presence of CD4 T cells appeared necessary for the rapid emergence of $CD127^{high}$ CD8 T cells. Following acute LCMV infection of $CD4^{-/-}$ mice the majority (56 ± 6% for gp33 and 69 ± 8% for np396) of virus-specific CD8 T cells retained a $CD127^{low}$ phenotype even by 4 mo postinfection (Fig. 3, *A* and *B*).

Because "helpless" CD8 T cells show impaired effector activities (6) and these cells remain $CD127^{low}$ for extended periods (Fig. 3, *A* and *B*), we compared the cytokine-producing capacity of $CD127^{low}$ and $CD127^{high}$ CD8 T cells (Fig. 3, *C* and *D*). At 8 days following acute infection the $CD127^{low}$ CD8 T cells which predominate at this timepoint can produce IFN-γ. In B6 mice the $CD127^{low}$ CD8 T cells are not maintained but the $CD127^{high}$ memory CD8 T cells which emerge over time can produce IFN-γ and a subset of these memory cells also produces IL-2. In acutely infected $CD4^{-/-}$ mice a significant population of $CD127^{high}$ CD8 T cells fails to emerge and the functional capacity of the $CD127^{low}$ CD8 T cells decays over time. Overall, these data illustrate the importance of CD4 T cell help for facilitating the development of functionally robust $CD127^{high}$

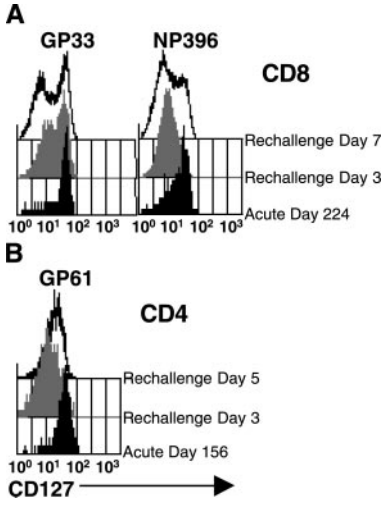

FIGURE 2. Changes in CD127 expression following viral rechallenge. *A*, CD127 expression on splenic gp33- or np396-specific CD8 T cells at day 224 post-acute LCMV-Armstrong infection of B6 mice and at 3 and 7 days following rechallenge of immune B6 mice with LCMV-clone 13. *B*, Using MHC class II tetramers, CD127 expression was assessed on gp61-specific CD4 T cells from LCMV immune mice at day 156 following infection and at 3 and 5 days following rechallenge. Gated $tetramer^{+}CD8^{+}$ or $tetramer^{+}CD4^{+}$ lymphocytes are shown in *A* and *B*, respectively. Representative data are shown from two to four mice analyzed at each timepoint.

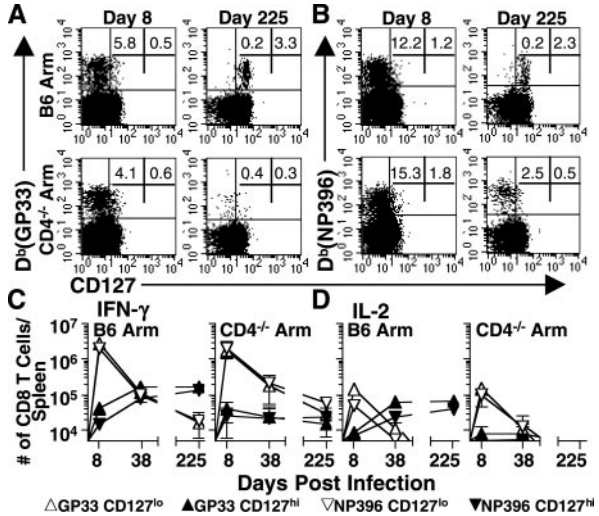

FIGURE 3. CD4-dependent emergence of functionally competent CD127^{high} virus-specific memory CD8 T cells. *A* and *B*, CD127 expression on splenic gp33- (*A*) and np396-specific (*B*) CD8 T cells was determined at days 8 and 225 following acute infection of B6 or CD4$^{-/-}$ mice. Values represent the percentage of gated CD8 T cells that stain with the respective tetramer and are CD127^{low} (*upper left*) or CD127^{high} (*upper right*). *C* and *D*, The numbers of CD127^{high} (closed symbols) and CD127^{low} (open symbols) gp33- (△, ▲) and np396-specific (▽, ▼) CD8$^+$ splenocytes which produce either IFN-γ (*C*) or IL-2 (*D*) following stimulation with peptide epitopes. Responses were evaluated at days 8, 38, and 225 following LCMV-Armstrong infection. Mean values ± SD from two to four mice at each timepoint are plotted.

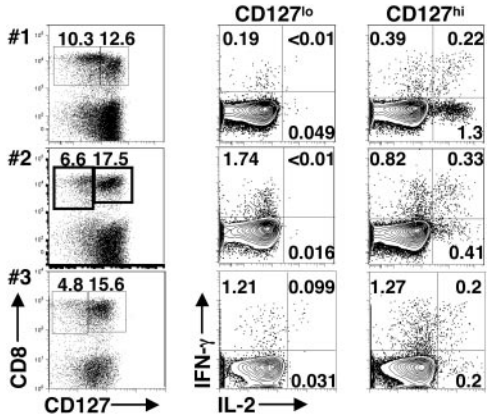

FIGURE 4. Cytokine expression by CD127^{high} and CD127^{low} CD8$^+$ T cells in humans. IFN-γ and IL-2 production by CD127^{low} and CD127^{high} CD8$^+$ human PBMC following stimulation with SEB was determined by intracellular cytokine staining. *Left*, Gated CD3$^+$ cells are shown and values given indicate the percentage of cells that are CD3$^+$ and CD8$^+$CD127^{low} or CD8$^+$CD127^{high}. *Middle and right*, Gated CD3$^+$CD8$^+$CD127^{low} (*middle*) and CD3$^+$CD8$^+$CD127^{high} (*right*) cells are shown and percentages of cells which produce either IFN-γ only, IL-2 only, or both IFN-γ and IL-2, are indicated in the *upper left*, *lower right*, and *upper right quadrants*, respectively.

memory CD8 T cells. In the absence of CD4 T cells the maturation of memory CD8 T cells stalls and CD127^{low} T cells which may survive under these conditions are functionally inferior, exhibiting a marked impairment of IL-2 production.

We next examined whether CD127 expression was similarly associated with IL-2 production by human CD8 T cells. CD127^{low} and CD127^{high} subsets of CD3$^+$CD8 T cells from the PBMC of three HIV-seronegative donors were analyzed for their ability to produce IL-2 and IFN-γ following stimulation with the superantigen SEB. In all samples examined the production of IL-2 was preferentially associated with CD127^{high} CD8 T cells (Fig. 4). This included T cells which coproduced IL-2 and IFN-γ as well as subpopulations that produced only IL-2 (Fig. 4, *upper* and *lower right quadrants*). Thus, in both humans as well as mice expression of CD127 marks functionally competent subsets of CD8 T cells.

A consistent theme of the results presented in this study is that CD127^{low} T cells which arise during the effector phase of an antiviral immune response are not necessarily rapidly deleted. The maturation of memory CD4 and CD8 T cells is, however, associated with a CD127^{high} phenotype and the expression of CD127 serves as a predictor of the functional quality of antiviral T cells in both mice and humans. Because the clearance of Ag, in addition to CD4 T cell help, promotes the development of memory CD8 T cells this suggests that responding T cells pass through two checkpoints as they attain the traits of memory T cells. Ag-dependent TCR signaling must be diminished, which is brought about if the overall immune response is sufficient to remove the inducing Ag. Also, a CD4 T cell-dependent licensing occurs that supports the emergence and maintenance of CD127^{high} T cell subsets. If conditions are unfavorable for the progression of memory cell development then CD127^{low} Ag-specific T cells may be retained and adopt a pseudoeffector phenotype. In the case of chronic viral infections, holding Ag-experienced T cells in a CD127^{low} state may be somewhat advantageous as if viral loads can be reduced within a sufficient timeframe then the maturation of CD127^{high} cells can proceed.

Disclosures

The authors have no financial conflict of interest.

References

1. Fuller, M. J., and A. J. Zajac. 2003. Ablation of CD8 and CD4 T cell responses by high viral loads. *J. Immunol.* 170: 477–486.
2. Wherry, E. J., J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed. 2003. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J. Virol.* 77: 4911–4927.
3. Fuller, M. J., A. Khanolkar, A. E. Tebo, and A. J. Zajac. 2004. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. *J. Immunol.* 172: 4204–4214.
4. Wherry, E. J., and R. Ahmed. 2004. Memory CD8 T-cell differentiation during viral infection. *J. Virol.* 78: 5535–5545.
5. Sun, J. C., M. A. Williams, and M. J. Bevan. 2004. CD4$^+$ T cells are required for the maintenance, not programming, of memory CD8$^+$ T cells after acute infection. *Nat. Immunol.* 5: 927–933.
6. Bevan, M. J. 2004. Helping the CD8$^+$ T-cell response. *Nat. Rev. Immunol.* 4: 595–602.
7. Schluns, K. S., W. C. Kieper, S. C. Jameson, and L. Lefrancois. 2000. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. *Nat. Immunol.* 1: 426–432.
8. Prlic, M., L. Lefrancois, and S. C. Jameson. 2002. Multiple choices: regulation of memory CD8 T cell generation and homeostasis by interleukin (IL)-7 and IL-15. *J. Exp. Med.* 195: F49–F52.
9. Kaech, S. M., J. T. Tan, E. J. Wherry, B. T. Konieczny, C. D. Surh, and R. Ahmed. 2003. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol.* 4: 1191–1198.
10. Huster, K. M., V. Busch, M. Schiemann, K. Linkemann, K. M. Kerksiek, H. Wagner, and D. H. Busch. 2004. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8$^+$ memory T cell subsets. *Proc. Natl. Acad. Sci. USA* 101: 5610–5615.
11. Madakamutil, L. T., U. Christen, C. J. Lena, Y. Wang-Zhu, A. Attinger, M. Sundarrajan, W. Ellmeier, M. G. von Herrath, P. Jensen, D. R. Littman, and H. Cheroutre. 2004. CD8αα-mediated survival and differentiation of CD8 memory T cell precursors. *Science* 304: 590–593.
12. Lenz, D. C., S. K. Kurz, E. Lemmens, S. P. Schoenberger, J. Sprent, M. B. Oldstone, and D. Homann. 2004. IL-7 regulates basal homeostatic proliferation of antiviral CD4$^+$ T cell memory. *Proc. Natl. Acad. Sci. USA* 101: 9357–9362.
13. Kondrack, R. M., J. Harbertson, J. T. Tan, M. E. McBreen, C. D. Surh, and L. M. Bradley. 2003. Interleukin 7 regulates the survival and generation of memory CD4 cells. *J. Exp. Med.* 198: 1797–1806.

14. Li, J., G. Huston, and S. L. Swain. 2003. IL-7 promotes the transition of CD4 effectors to persistent memory cells. *J. Exp. Med.* 198: 1807–1815.
15. Crawford, F., H. Kozono, J. White, P. Marrack, and J. Kappler. 1998. Detection of antigen-specific T cells with multivalent soluble class II MHC covalent peptide complexes. *Immunity* 8: 675–682.
16. Rees, W., J. Bender, T. K. Teague, R. M. Kedl, F. Crawford, P. Marrack, and J. Kappler. 1999. An inverse relationship between T cell receptor affinity and antigen dose during $CD4^+$ T cell responses in vivo and in vitro. *Proc. Natl. Acad. Sci. USA* 96: 9781–9786.
17. Khanolkar, A., M. J. Fuller, and A. J. Zajac. 2004. CD4 T cell-dependent CD8 T cell maturation. *J. Immunol.* 172: 2834–2844.
18. Lechner, F., D. K. Wong, P. R. Dunbar, R. Chapman, R. T. Chung, P. Dohrenwend, G. Robbins, R. Phillips, P. Klenerman, and B. D. Walker. 2000. Analysis of successful immune responses in persons infected with hepatitis C virus. *J. Exp. Med.* 191: 1499–1512.
19. Wherry, E. J., D. L. Barber, S. M. Kaech, J. N. Blattman, and R. Ahmed. 2004. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc. Natl. Acad. Sci. USA* 101: 16004–16009.
20. Carini, C., M. F. McLane, K. H. Mayer, and M. Essex. 1994. Dysregulation of interleukin-7 receptor may generate loss of cytotoxic T cell response in human immunodeficiency virus type 1 infection. *Eur. J. Immunol.* 24: 2927–2934.
21. MacPherson, P. A., C. Fex, J. Sanchez-Dardon, N. Hawley-Foss, and J. B. Angel. 2001. Interleukin-7 receptor expression on $CD8^+$ T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. *J. Acquired Immune Defic. Syndr.* 28: 454–457.
22. Mussini, C., M. Pinti, V. Borghi, M. Nasi, G. Amorico, E. Monterastelli, L. Moretti, L. Troiano, R. Esposito, and A. Cossarizza. 2002. Features of 'CD4-exploders', HIV-positive patients with an optimal immune reconstitution after potent antiretroviral therapy. *AIDS* 16: 1609–1616.
23. Paiardini, M., B. Cervasi, H. Albrecht, A. Muthukumar, R. Dunham, S. Gordon, H. Radziewicz, G. Piedimonte, M. Magnani, M. Montroni, et al. 2005. Loss of CD127 expression defines an expansion of effector $CD8^+$ T cells in HIV-infected individuals. *J. Immunol.* 174: 2900–2909.
24. Zhou, S., R. Ou, L. Huang, G. E. Price, and D. Moskophidis. 2004. Differential tissue-specific regulation of antiviral $CD8^+$ T-cell immune responses during chronic viral infection. *J. Virol.* 78: 3578–3600.