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CUTTING EDGE

Cutting Edge: Regulation of CD8$^+$ T Cell Effector Population Size[1]

Roslyn A. Kemp, Timothy J. Powell, David W. Dwyer, and Richard W. Dutton[2]

Naive CD8$^+$ T cells are activated on encounter with Ag presented on dendritic cells and proliferate rapidly. To investigate the regulation of naive CD8$^+$ T cells proliferation, we adoptively transferred TCR-transgenic CD8$^+$ T cells into intact mice together with Ag-pulsed dendritic cells. Regardless of the number of cells initially transferred, the expansion of activated Ag-specific CD8$^+$ T cells was limited to a ceiling of effector cells. This limit was reached from a wide range of T cell doses, including a physiological number of precursor cells, and was not altered by changing the amount of Ag or APCs. The total Ag-specific response was composed of similar numbers of host and donor transgenic cells regardless of donor cell input, suggesting that these populations were independently regulated. Regulation of the transgenic donor cell population was TCR specific. We hypothesize that a clone-specific regulatory mechanism controls the extent of CD8$^+$ T cell responses to Ag. *The Journal of Immunology,* 2004, 173: 2923–2927.

Naive T cells expand from a small precursor frequency to a large number of activated cells upon exposure to specific Ag. Control of the expansion of effector cells is required, generating enough cells to clear Ag, but not so many as to induce damage. The expansion of T cells has been correlated with the amount of Ag presented and the number of APCs (1, 2). It has been proposed that T cells compete for physical access to Ag on APCs (3). There is also evidence for competition for Ag between T cells specific for different Ag/MHC complexes, which has been attributed to differences in Ag processing and competition between Ags for loading onto a limited number of MHCs (4–6).

The precursor frequency of naive CD8$^+$ T cells specific for a particular epitope has been estimated at ~100 per 100,000 CD8$^+$ T cells (7). It is difficult to track the response of a low number of Ag-specific precursor T cells, so many have used adoptive transfer of TCR-transgenic T cells to study T cell regulation and disease models (8). The number of T cells transferred is several orders of magnitude higher than the number of host precursors for the same Ag. These transfers do not reflect a normal physiological situation, and data obtained from such experiments may not reflect what happens to host cells naturally exposed to Ag. To compare the expansion of naive donor transgenic TCR cells and naive host cells in response to specific Ag, we used adoptive transfer of defined TCR-transgenic populations into wild-type hosts.

Materials and Methods

Mice

B6.PL-Thy1a/Cy (Thy1.1) mice were from The Jackson Laboratory (Bar Harbor, ME). BALB/c (By) Thy1.1 mice (from Dr. C. Surh, Scripps Research Institute, La Jolla, CA) were bred at the Animal Breeding Facility at Trudeau Institute. OT-1 mice (9) were originally obtained from Dr. M. Bevan (University of Washington, Seattle, WA). HY mice (10) were from Taconic (Albany, NY). The HA clone-4 transgenic TCR mice (11) were from Dr. L. Sherman. Animal procedures were conducted in accordance with institutional guidelines.

Peptides

SIINFEKL, IYSTVASSL, and WMHHNMDLI were from New England Peptide (Gardner, MA).

Cell isolation and in vivo transfer

CD8$^+$ T cells were prepared from mouse lymph nodes and spleens by positive selection with anti-CD8 beads using MACS columns (Miltenyi Biotec, Auburn, CA). Naive CD8$^+$ T cells were >95% pure. Cells were labeled with CFSE (Molecular Probes, Eugene, OR) as described (12). Bone marrow cells were cultured as described (13). Dendritic cells (DCs)[3] were incubated in medium with 0.01–10 μg/ml peptide for 2 h at 37°C as described (14). Mice received 10^2–10^7 purified naive CD8$^+$ T cells i.v.; 10^3 or 10^6 peptide-loaded bone marrow-derived DCs were injected at the same time as CD8$^+$ T cells. Mice were euthanized by cervical dislocation.

Influenza infection

Influenza Puerto Rico (PR8, H1N1) virus (from Dr. N. Klinman, Scripps Research Institute) was grown as described (15). Mice were infected with influenza by intranasal inoculation of 50 μl of 6000-egg infectious unit virus in PBS 24 h after adoptive cell transfer.

Flow cytometry

Single-cell suspensions were incubated with Abs and reagents for four-color analyses as indicated in the figures. Cells were analyzed on a Cyan (DakoCytomation, Carpinteria, CA).

Results and Discussion

The immune system cannot accommodate an unlimited number of effector cells. There must be a limit to the maximal size of the response of CD8$^+$ T cells to Ag, but a mechanism is unknown. To address this issue, we transferred titrated numbers of CFSE-labeled OT-1 transgenic TCR CD8$^+$ T cells into mice that were challenged with a high concentration of peptide on

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[3] Abbreviation used in this paper: DC, dendritic cell.

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bone marrow-derived DCs. By day 7, the number of tetramer-positive cells in the spleens of all mice that received cells was almost identical, despite the wide range of the initial transfer number, reaching a ceiling of 6.5×10^5 (SD, 1.5×10^5) effector cells (Fig. 1, *A* and *B*). Similar results were seen in pooled peripheral lymph nodes and, in small numbers, the peritoneal cavity, liver, lung, and mesenteric lymph nodes (data not shown). Mechanisms regulating the ceiling were already in effect by days 3–5, because the expansion of T cells was slower when higher numbers rather than lower numbers of donor cells were transferred (Fig. 1*A*, compare slopes of curves days 1–5). Experiments looking at earlier time points for each dose of cells showed that there was no earlier peak for the high number of transferred cells. A similar phenomenon has also been seen on transfer of high numbers of $CD4^+$ T cells in influenza-infected mice (15). In contrast, cells transferred in low numbers had all divided more than seven times at the peak of the response (Fig. 1*D*). We conclude that a naive mouse possesses sufficient naive $CD8^+$ T cells specific for a particular epitope to generate the maximum response. Adoptive transfer of any number of Ag-specific naive $CD8^+$ T cells cannot increase this maximum.

We next examined what limits the size of this epitope-specific maximal response and considered the following mechanisms: 1) availability of presented epitope (16), 2) availability of space within which cells can expand (17), 3) supply of factor(s) necessary to support proliferation of cells, 4) a non-epitope-specific regulatory mechanism that suppresses further proliferation, or 5) a TCR-specific regulatory mechanism that prevents further expansion.

Nonclonal regulation should lead to competition between different populations of responding cells. However, we found that the host response to the same Ag was not subject to competition with the donor cells even when large numbers of transgenic cells were present—the host response was equally large whether 10^2 or 10^7 cells were transferred—a ratio of 1.4:1 donor:host at all titrations considered (Fig. 1*E*). The regulation of expansion of host cells must occur independently of that of donor cells.

This observation argues against the possibility that cells compete for Ag, because host cells are responding to the same peptide-loaded APCs as donor cells. If there were competition for Ag, the host response would be compromised upon transfer of high numbers of donor cells, but this is not the case. To further address the issue of Ag competition, we repeated the analysis and found Ag to be limiting when we transferred lower numbers of DCs (Fig. 2*A*) or DCs loaded with lower concentrations of peptide (*B*), and the overall expansion was altered. Hence, there is competition for Ag, but only when this Ag is limiting, and not when it is present in excess amounts. We also repeated the experiment transferring DCs 1 day after naive $CD8^+$ T cell transfer and found similar results (Fig. 2*C*), implying that differential access to Ag between host and donor cells does not explain the observed regulation. It is known that naive and memory $CD8^+$ T cells respond differently to Ag (18, 19), and it could be argued that the host cells responding to Ag are from a memory $CD8^+$ T cell population. However, both the donor population and the host $CD8^+$ T cell pool contained <2% $CD44^{high}$ cells (data not shown).

It seemed most likely that competition or suppression between T cells regulates the expansion of $CD8^+$ Ag-specific T cell populations, and that the mechanism of control may operate differently on host vs donor cells. To analyze this, we first

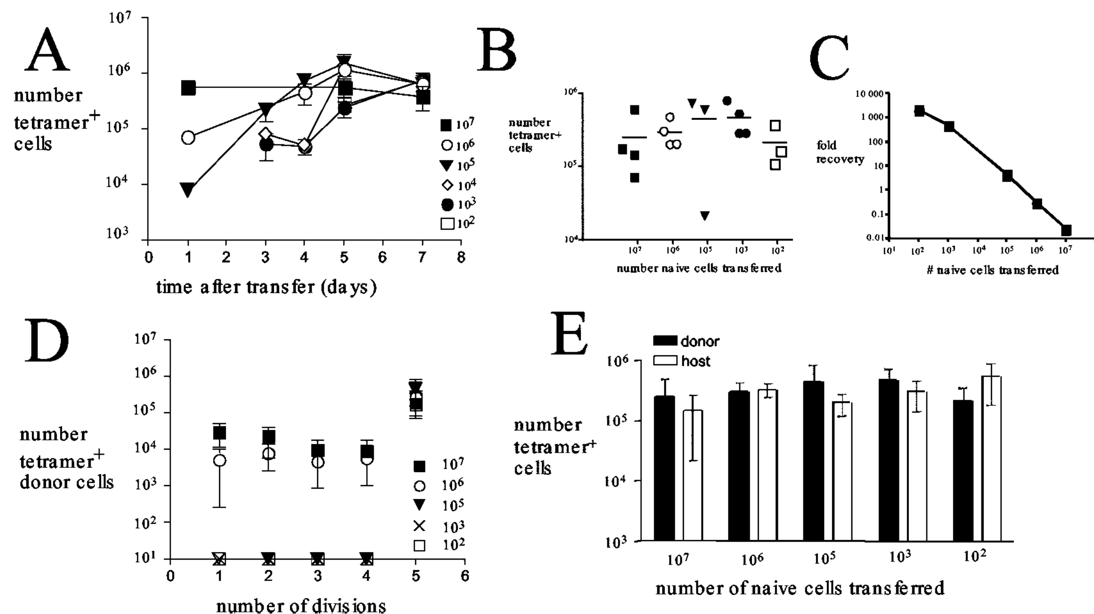

FIGURE 1. Naive $CD8^+$ T cells reach a maximal number upon exposure to Ag, regardless of precursor frequency; host and donor cell proliferation occur independently of each other. B6.PL mice were injected with 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, or 1×10^2 CFSE-labeled naive $CD8^+$ OT-1 transgenic TCR cells i.v. with 1×10^5 bone marrow-derived DCs that had been pulsed with 10 μg of SIINFEKL. At the indicated days after transfer, cells were recovered from the spleen and incubated with SIINFEKL tetramer and Abs to Thy1.2 and CD8. *A*, $Tetramer^+$ cells were enumerated at each time point. *B*, The number of donor cells of individual mice is shown at day 7 after transfer. *C*, The fold increase in cell number of $tetramer^+$ donor cells from each group of mice at day 7 after transfer is plotted against the initial cell input. *D*, $Tetramer^+Thy1.2^+CD8^+$ donor cells were enumerated from each group at the CFSE-measured division number. *E*, The number of tetramer-positive donor and host cells were measured at day 7 using Thy1.2. Each graph represents mean and SD of three to four mice per group and is representative of six independent experiments.

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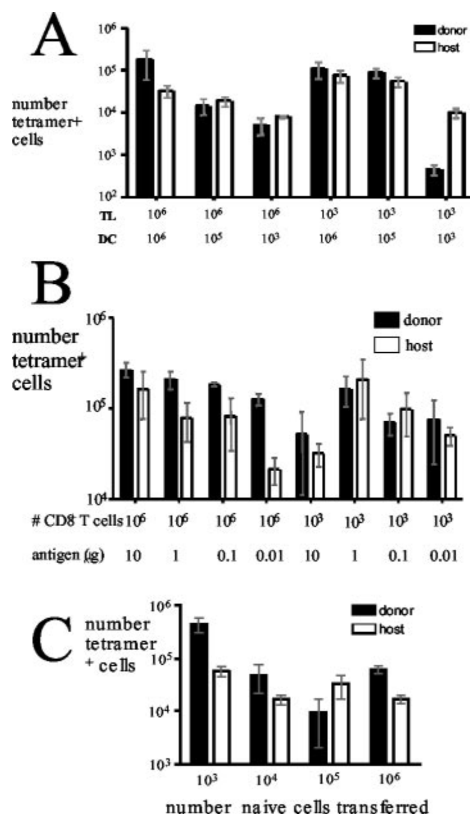

FIGURE 2. The maximum number of $CD8^+$ effector cells is not due to competition for Ag or APCs. *A*, B6.PL mice were injected with 1×10^6 or 1×10^3 naive $CD8^+$ OT-1 transgenic TCR cells i.v. with 1×10^6, 1×10^5, or 1×10^3 bone marrow-derived DCs that had been pulsed with 10 μg of SIINFEKL. At day 7 after transfer, donor and host $tetramer^+$ cells were enumerated. *B*, B6.PL mice were injected with 1×10^6 or 1×10^3 naive $CD8^+$ OT-1 transgenic TCR cells i.v. with 5×10^5 bone marrow-derived DCs that had been pulsed with 10, 1, 0.1, or 0.01 μg of SIINFEKL. At day 7 after transfer, donor and host $tetramer^+$ cells were enumerated. *C*, B6.PL mice were injected with 1×10^6, 1×10^5, 1×10^4, or 1×10^3 naive $CD8^+$ OT-1 transgenic TCR cells i.v. The following day mice were injected with 1×10^5 bone marrow-derived DCs pulsed with 10 μg of SIINFEKL i.v. At day 7 after transfer, cells were recovered from the spleen and incubated with SIINFEKL tetramer and Abs to Thy1.2 and CD8. Each graph represents mean and SD of three to four mice per group and is representative of three independent experiments each.

transferred titrated numbers of naive T cells from two genetically distinct strains of transgenic TCR OT-1 mice, one $CD45.1^+$ and one $CD45.1^-$. Transfer of either OT-1 or OT-1 $CD45.1^+$ cells resulted in a peak number of effectors that was composed of both donor and host cells, but the number of host cells was the same, regardless of donor cell number or source (Fig. 3*A*). Transfer of a high number of OT-1 $CD45.1^+$ cells inhibited proliferation of a low number of OT-1 cells transferred into the same host (Fig. 3*A*), indicating competition between the two donor cell populations of the same specificity. Thus, a population of donor OT-1 cells that did not inhibit proliferation of host SIINFEKL-specific cells did inhibit proliferation of another population of OT-1 donor cells, reducing their response by 100-fold. It does not seem likely that this difference is due to the minority population being outcompeted by the majority population, because the host response (an even smaller population) remains unaltered. Transfer of low numbers of both OT-1 and OT-1 $CD45.1^+$ cells resulted in equal proliferation of both donor populations. Hence, regulation due

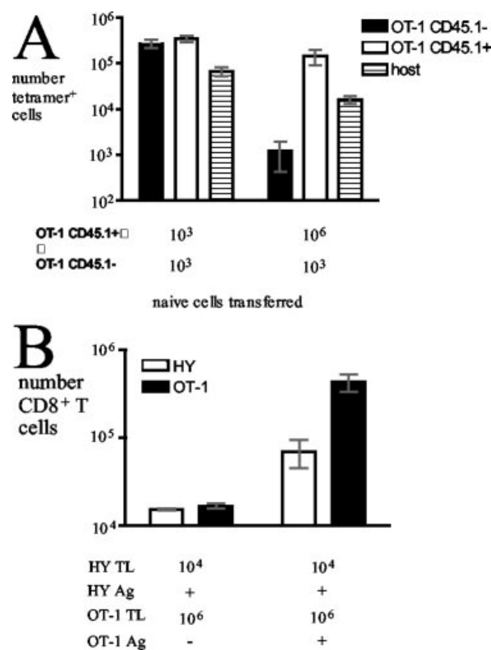

FIGURE 3. Regulation of effector $CD8^+$ T cell number is Id specific. B6.PL mice were injected with either 1×10^6 or 1×10^3 naive $CD8^+$ OT-1 $CD45.1^+$ transgenic TCR T cells plus 1×10^3 naive OT-1 $CD45.1^-$ transgenic TCR T cells with 1×10^5 bone marrow-derived DCs that had been pulsed with 10 μg of SIINFEKL. At day 7 after transfer, cells from each donor were enumerated. *B*, B6.PL mice were injected with 1×10^4 naive $CD8^+$ HY transgenic TCR T cells plus 1×10^5 bone marrow-derived DCs that had been pulsed with 10 μg of WHHNMDLI plus either 1×10^6 naive $CD8^+$ transgenic TCR OT-1 $CD45.1^+$ T cells, or 1×10^6 naive $CD8^+$ transgenic TCR OT-1 $CD45.1^+$ T cells plus 1×10^5 bone marrow-derived DCs that had been pulsed with 10 μg of SIINFEKL. At day 7 after transfer, donor cells were enumerated. The response of HY cells to WHHNMDLI and OT-1 cells to SIINFEKL alone was comparable. Each graph represents mean and SD of three to five mice per group and is representative of three independent experiments.

to identical TCR affinities was only observed in the donor transgenic TCR pool, and not between donor and host. These data imply that, once the clonal donor $CD8^+$ T cell population reaches a certain size, regulatory mechanisms control further expansion.

To investigate whether two populations of donor cells responding to different Ags would be similarly controlled, we transferred low numbers of HY transgenic naive $CD8^+$ T cells with high numbers of OT-1 naive $CD8^+$ T cells. We stimulated HY cells with or without concurrent stimulation of OT-1

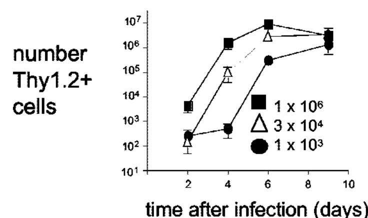

FIGURE 4. Adoptive transfer of small numbers of $CD8^+$ T cells results in delayed kinetics of $CD8^+$ T cell accumulation. BALB/cThy1.1$^+$ mice were injected with 1×10^6, 3×10^4, or 1×10^3 naive $CD8^+Thy1.2^+$ HA transgenic TCR T cells, and then infected with 6000-egg infectious unit influenza virus 24 h later. The graph represents mean and SD of donor cells in the lungs of three mice per group and is representative of two independent experiments.

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cells, using separate populations of DCs loaded with either SIINFEKL or WMHHNMDLI. If there were competition for space between donor cell populations specific for different Ags, the expansion of HY effectors would be inhibited by the expansion of OT-1 cells, as had been seen between OT-1 populations. In the presence of high numbers of OT-1 cells, the expansion of a low number of HY cells was not inhibited at the peak of the response (day 7; Fig. 3*B*), showing that the donor cell populations do not compete when their Ag specificity is different. In the presence of a concurrent response to OT-1 Ag, the HY response was enhanced compared with an HY response alone. We speculate that this may be due to prosurvival and proliferative factors produced during the OT-1 response. That there is competition between transgenic donor cells of the same specificity, but not different specificity, argues against regulation being a result of differences in a non-Ag-specific host vs donor niche.

We wanted to establish whether the maximal response seen in this system could be demonstrated in response to an infection. We injected titrated numbers of naive HA transgenic TCR $CD8^+$ T cells into BALB/c mice and infected them with influenza virus. At day 7 after infection, we calculated the number of donor cells in the lung (Fig. 4), airways, spleen, and draining lymph node (data not shown), and found that a ceiling of effector cells was reached, regardless of initial cell transfer number.

Hence, expansion of adoptively transferred cells is regulated in a clone-specific manner and is also controlled separately from the host response.

A correlation between the number of transferred cells and the extent of their proliferation had been shown previously by Lauouar and Crispe (20), who created bone marrow chimeras with different numbers of $CD4^+$ T cell precursors of known specificity. The presence of higher precursor numbers of cells led to a lower proportion of dividing cells than transfer of lower numbers. We created chimeras with peripheral $CD8^+$ T cells composed of HY, OT-1, and host. Following challenge with HY and OT-1 Ags, alone or together, the expansion of HY cells was the same in the presence or absence of a concurrent expansion of the OT-1 cells (data not shown).

Together, these data demonstrate a differential expansion of host and donor cells that is not due to Ag competition. This seems to be in contrast to the results of Kedl et al. (3), who showed that the transfer of high-affinity OT-1 cells inhibited the response of host Ag-specific T cells. However, these authors were comparing the response of T cells to two epitopes of the same protein and found that high-affinity responses competed out low affinity. It is clear that donor cells bearing the same TCR compete with one another (Fig. 3*A*), but cells with different TCRs do not (*B*). Probst et al. (21) showed an effect of donor transgenic cells on host responses; however, only at a single time point, and the only organ analyzed is the peripheral blood. Therefore, it is difficult to assess whether the same effects described in our system would have been seen.

To regulate clone-specific T cell competition and expansion, a mechanism must operate on a basis that distinguishes both TCR specificity and origin of the responding cells. The fact that regulation is clone specific rules out explanations such as competition or a role for differing physiological niches of host and donor populations. We have then a hypothesis that a TCR-transgenic specific regulatory population of cells in the host was induced (22–24). Alternatively, regulatory cells may have been introduced with the donor population; however, <0.05% of the transferred cells were $CD4^+CD25^+$. It is possible that the donor transgenic TCR cells have been compromised, and that regulation by the host is specific, not for the transgenic TCR, but for whatever marks the donor cells.

Expansion from a low precursor frequency, either from a normal host, or by transferring low numbers of cells, is efficient to generate a maximal response to Ag. However, we have shown that adoptively transferred transgenic cells expand differently to specific Ag than do host cells. This study questions the validity of comparing responses from wild-type host $CD8^+$ T cells and adoptively transferred transgenic TCR $CD8^+$ T cells.

In summary, we have shown that adoptively transferred naive TCR-transgenic $CD8^+$ T cells expand following Ag challenge to a ceiling level, regardless of the initial input cell number. The response of the host also remains the same irrespective of the donor population size. Low numbers of transferred cells are sufficient for a maximal response, while some inhibitory process restricts the expansion of higher numbers of transferred cells. This process does not inhibit the host response nor does it inhibit the response of adoptively transferred cells specific for a different Ag.

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References

1. Bousso, P., and E. Robey. 2003. Dynamics of $CD8^+$ T cell priming by dendritic cells in intact lymph nodes. *Nat. Immunol. 4:579.*
2. Bullock, T. N. J., D. W. Mullins, and V. H. Engelhard. 2003. Antigen density presented by dendritic cells in vivo differentially affects the number and avidity of primary, memory, and recall $CD8^+$ T cells. *J. Immunol. 170:1822.*
3. Kedl, R. M., W. A. Rees, D. A. Hildeman, B. Schaefer, T. Mitchell, J. Kappler, and P. Marrack. 2000. T cells compete for access to antigen-bearing antigen-presenting cells. *J. Exp. Med. 192:1105.*
4. Wolpert, E. Z., P. Grufman, J. K. Sandberg, A. Tegnesjo, and K. Karre. 1998. Immunodominance in the CTL response against minor histocompatibility antigens: interference between responding T cells, rather than with presentation of epitopes. *J. Immunol. 161:4499.*
5. Bullock, T. N. J., T. A. Colella, and V. H. Engelhard. 2000. The density of peptides displayed by dendritic cells affects immune responses to human tyrosinase and gp100 in HLA-A2 transgenic mice. *J. Immunol. 164:2354.*
6. Grufman, P., J. Sandberg, E. Wolpert, and K. Karre. 1999. Immunization with dendritic cells breaks immunodominance in CTL responses against minor histocompatibility and synthetic peptide antigens. *J. Leukocyte Biol. 66:268.*
7. Blattman, J. N., R. Antia, D. J. D. Sourdive, X. Wang, S. M. Kaech, K. Murali-Krishna, J. D. Altman, and R. Ahmed. 2002. Estimating the precursor frequency of naive antigen-specific CD8 T cells. *J. Exp. Med. 195:657.*
8. Pape, K., E. Kearney, A. Khoruts, A. Mondino, R. Merica, Z. Chen, E. Ingulli, J. White, J. Johnson, and M. Jenkins. 1997. Use of adoptive transfer of T-cell-antigen-receptor-transgenic T cell for the study of T-cell activation in vivo. *Immunol. Rev. 156:67.*
9. Hogquist, K., S. Jameson, W. Heath, J. Howard, M. Bevan, and F. Carbone. 1994. T cell receptor antagonist peptides induce positive selection. *Cell 76:17.*
10. Valujskikh, A., O. Lantz, S. Celli, P. Matzinger, and P. Heeger. 2002. Cross-primed $CD8^+$ T cells mediate graft rejection via a distinct effector pathway. *Nat. Immunol. 3:844.*
11. Morgan, D., R. Liblau, B. Scott, S. Fleck, H. McDevitt, N. Sarvetnick, D. Lo, and L. Sherman. 1996. $CD8^+$ T cell-mediated spontaneous diabetes in neonatal mice. *J. Immunol. 157:978.*
12. Kemp, R. A., and F. Ronchese. 2001. Tumor-specific Tc1, but not Tc2, cells deliver protective antitumor immunity. *J. Immunol. 167:6497.*
13. Garrigan, K., P. Moroni-Rawson, C. McMurray, I. Hermans, N. Abernathy,

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J. Watson, and F. Ronchese. 1996. Functional comparison of spleen dendritic cells and dendritic cells cultured in vitro from bone marrow precursors. *Blood 88:3508*.

14. Hermans, I., A. Daish, P. Moroni-Rawson, and F. Ronchese. 1997. Tumor-peptide-pulsed dendritic cells isolated from spleen or cultured in vitro from bone marrow precursors can provide protection against tumor challenge. *Cancer Immunol. Immunother. 44:341*.
15. Roman, E., E. Miller, A. Harmsen, J. Wiley, U. H. von Andrian, G. Huston, and S. L. Swain. 2002. CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *J. Exp. Med. 196:957*.
16. Kedl, R., B. Schaefer, J. Kappler, and P. Marrack. 2002. T cells down-modulate peptide-MHC complexes on APCs in vivo. *Nat. Immunol. 3:27*.
17. McNally, J. M., C. C. Zarozinski, M.-Y. Lin, M. A. Brehm, H. D. Chen, and R. M. Welsh. 2001. Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. *J. Virol. 75:5965*.
18. Veiga-Fernandes, H., U. Walter, C. Bourgeois, A. McLean, and B. Rocha. 2000. Response of naive and memory $CD8^+$ T cells to antigen stimulation in vivo. *Nat. Immunol. 1:47*.
19. Zimmermann, C., A. Prevost-Blondel, C. Blaser, and H. Pircher. 1999. Kinetics of the response of naive and memory CD8 T cells to antigen: similarities and differences. *Eur. J. Immunol. 29:284*.
20. Laouar, Y., and I. Crispe. 2000. Functional flexibility in T cells: independent regulation of $CD4^+$ T cell proliferation and effector function in vivo. *Immunity 13:291*.
21. Probst, H., T. Dumrese, and M. F. van den Broek. 2002. Competition for APC by CTLs of different specificities is not functionally important during induction of antiviral responses. *J. Immunol. 168:5387*.
22. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. de Vries, and M. Roncarolo. 1997. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature 389:737*.
23. Suvas, S., U. Kumaraguru, C. D. Pack, S. Lee, and B. T. Rouse. 2003. $CD4^+CD25^+$ T cells regulate virus-specific primary and memory $CD8^+$ T cell responses. *J. Exp. Med. 198:889*.
24. Jiang, H., and L. Chess. 2000. The specific regulation of immune responses by $CD8^+$ T cells restricted by the MHC class Ib molecule, Qa-1. *Annu. Rev. Immunol. 18:185*.

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