rational interventions that augment, depress, or deviate responses in ways that promote human health.

References and Notes

1. M. Cohn, R. E. Langman, *Immunol. Rev.* **115**, 11 (1990).
2. This description of the immune system is a paraphrase of the classic children's tale "Goldilocks and the Three Bears." Upon entering the empty bear's home in the forest, Goldilocks found the porridge on the table "Too hot," "Too cold," and "Just right."
3. R. P. Bucy *et al.*, *J. Exp. Med.* **180**, 1251 (1994); Y. Itoh, R. N. Germain, *J. Exp. Med.* **186**, 757 (1997).
4. A. Kelso, P. Groves, A. B. Troutt, K. Francis, *Eur. J. Immunol.* **25**, 1168 (1995).
5. S. L. Reiner, R. A. Seder, *Immunity* **11**, 1 (1999).
6. R. Callard, A. J. George, J. Stark, *Immunity* **11**, 507 (1999).
7. A. V. Gett, P. D. Hodgkin, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9488 (1998).
8. A. V. Gett, P. D. Hodgkin, *Nature Immunol.* **1**, 239 (2000).
9. W. E. Paul, Ed., *Fundamental Immunology*, (Lippincott-Raven, New York, ed. 4, 1999); C. A. Janeway Jr., P. Travers, M. Walport, J. D. Capra, Eds., *Immunobiology: The Immune System in Health and Disease* (Garland, New York, 1999).
10. D. L. Hartl, A. G. Clark, *Principles of Population Genetics* (Sinauer, Sunderland, MA, ed. 2, 1989).
11. P. Bretscher, M. Cohn, *Science* **169**, 1042 (1970).
12. R. H. Schwartz, *Science* **248**, 1349 (1990).
13. C. B. Thompson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1333 (1989).
14. D. A. Cantrell, K. A. Smith, *Science* **224**, 1312 (1984).
15. Supplementary data are available on *Science* Online at www.sciencemag.org/cgi/content/full/293/5528/240/DC1.
16. A. C. Mullen *et al.*, *Science* **292**, 1907 (2001).
17. D. A. Carson, *FASEB J.* **6**, 2800 (1992).
18. S. Sakaguchi, *Cell* **101**, 455 (2000).
19. J. M. Curtsinger, D. C. Lins, M. F. Mescher, *J. Immunol.* **160**, 3236 (1998); M. Tsukamoto, R. N. Germain, unpublished observations.
20. M. Croft, L. M. Bradley, S. L. Swain, *J. Immunol.* **152**, 2675 (1994); M. Pihlgren, P. M. Dubois, M. Tomkowiak, T. Sjogren, J. Marvel, *J. Exp. Med.* **184**, 2141 (1996); P. R. Rogers, C. Dubey, S. L. Swain, *J. Immunol.* **164**, 2338 (2000).
21. P. Matzinger, *Annu. Rev. Immunol.* **12**, 991 (1994).
22. P. V. Lehmann, T. Forsthuber, A. Miller, E. E. Sercarz, *Nature* **358**, 155 (1992).
23. C. B. Thompson, J. P. Allison, *Immunity* **7**, 445 (1997).
24. H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, *Immunity* **11**, 141 (1999).
25. J. D. Katz, B. Wang, K. Haskins, C. Benoist, D. Mathis, *Cell* **74**, 1089 (1993).
26. N. Barkai, S. Leibler, *Nature* **387**, 913 (1997).
27. M. Freeman, *Nature* **408**, 313 (2000).
28. Z. Grossman, W. E. Paul, *Semin. Immunol.* **12**, 197 (2000).
29. D. C. LaPorte, K. Walsh, D. E. Koshland Jr., *J. Biol. Chem.* **259**, 14068 (1984).
30. C. Y. Huang, J. E. Ferrell Jr., *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10078. (1996).
31. J. E. Ferrell Jr., E. M. Machleder, *Science* **280**, 895 (1998).
32. W. E. Paul, R. A. Seder, *Cell* **76**, 241 (1994).
33. J. Aliberti *et al.*, *Nature Immunol.* **1**, 83 (2000); O. Schulz *et al.*, *Immunity* **13**, 453 (2000); H. Hemmi *et al.*, *Nature* **408**, 740 (2000).
34. C. A. Wenner, M. L. Guler, S. E. Macatonia, A. O'Garra, K. M. Murphy, *J. Immunol.* **156**, 1442 (1996).
35. R. A. Seder, W. E. Paul, M. M. Davis, B. Fazekas de St. Groth, *J. Exp. Med.* **176**, 1091 (1992).
36. W. Ouyang *et al.*, *Immunity* **9**, 745 (1998).
37. R. N. Germain, I. Stefanova, *Annu. Rev. Immunol.* **17**, 467 (1999).
38. T. W. McKeithan, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5042 (1995).
39. S. C. Jameson, K. A. Hogquist, M. J. Bevan, *Nature* **369**, 750 (1994).
40. G. Iezzi, K. Karjalainen, A. Lanzavecchia, *Immunity* **8**, 89 (1998).
41. K. Yasutomo, C. Doyle, L. Miele, C. Fuchs, R. N. Germain, *Nature* **404**, 506 (2000).
42. K. F. Hoffmann, A. W. Cheever, T. A. Wynn, *J. Immunol.* **164**, 6406 (2000).
43. F. Sallusto, D. Lenig, R. Forster, M. Lipp, A. Lanzavecchia, *Nature* **401**, 708 (1999).
44. J. K. Whitmire, K. Murali-Krishna, J. Altman, R. Ahmed, *Philos. Trans. R. Soc. London Ser. B* **355**, 373 (2000); J. Jacob, D. Baltimore, *Nature* **399**, 593 (1999).
45. C. M. Snapper, F. D. Finkelman, W. E. Paul, *Immunol. Rev.* **102**, 51 (1988).
46. A. Lanzavecchia, F. Sallusto, *Science* **290**, 92 (2000).
47. I. Riviere, M. J. Sunshine, D. R. Littman, *Immunity* **9**, 217 (1998); M. Bix, R. M. Locksley, *Science* **281**, 1352 (1998); J. Hu-Li *et al.*, *Immunity* **14**, 1 (2001).
48. D. L. Cook, A. N. Gerber, S. J. Tapscott, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15641 (1998).
49. C. A. Biron, *Semin. Immunol.* **10**, 383 (1998).
50. G. von Dassow, E. Meir, E. M. Munro, G. M. Odell, *Nature* **406**, 188 (2000).
51. S. H. Strogatz, *Nature* **410**, 268 (2001).
52. H. Jeong, S. P. Mason, A. L. Barabasi, Z. N. Oltvai, *Nature* **411**, 41 (2001).
53. H. Jeong, B. Tombor, R. Albert, Z. N. Oltvai, A. L. Barabasi, *Nature* **407**, 651 (2000).

54. I regret that it was not possible to cite all relevant references owing to space limitations. I thank I. Stefanova, J. Dorfman, J. Delon, G. Bonnet, and M. Meier-Schellerscheim for helpful comments on the manuscript, A. Kirk for pointing out the similarities between the scenario for prophylaxis of autoimmunity and current research in transplantation combining lymphoid depletion and low-dose immunosuppression, and G. Bonnet for help with the figures.

VIEWPOINT

T Cell Death and Memory

Jonathan Sprent* and David F. Tough†

In typical immune responses, contact with antigen causes naïve T cells to proliferate and differentiate into effector cells. After the pathogen is destroyed, most effector T cells are eliminated—thereby preserving the primary T cell repertoire—but some cells survive and form long-lived memory cells. During each stage of this process, the life or death fate of T cells is strictly regulated.

Immune responses leading to rejection of infectious agents usually culminate in a state of specific T and B cell memory where secondary responses are more vigorous and effective than primary responses (*1–6*). Generation of memory T and B cells is the end result of a highly destructive process in which most of the responding lymphocytes are rapidly eliminated, and only a small proportion survive to become long-lived memory cells. This article reviews the life or death decision-making involved in the formation of memory T cells, as well as the role of certain cytokines in keeping these cells alive.

Longevity of naïve T cells. Naïve T cells are long-lived resting cells that reside in the recirculating lymphocyte pool and migrate continuously from blood to lymph through specialized T cell zones in the secondary lymphoid tissues, the spleen, lymph nodes (LNs), and Peyer's patches (*7*). The survival of naïve T cells requires continuous contact with self peptides bound to major histocompatibility complex (MHC) molecules combined with exposure to a cytokine, interleukin 7 (IL-7) (*6*). In consort, these two ligands are presumed to induce a form of low-level signaling that is sufficient to keep the cells alive but does not induce them to enter the cell cycle.

Life and death during the primary response. Primary T cell responses are initiated in secondary lymphoid organs by mature antigen-presenting cells, i.e., dendritic cells (DCs) (*8*). Recognition of immunogenic peptides bound to cell-surface MHC molecules on DCs in the T cell zone causes selective sequestration ("trapping") of antigen-specific recirculating T cells entering lymphoid tissues from the blood (*9*); the trapped cells are then induced to proliferate.

Because infectious agents often replicate at a prodigious rate, primary immune responses are geared to be as intense as possible. Division of antigen-reactive T cells during the height of the immune response is very rapid (three to four divisions per day for $CD8^+$ cells) and leads to >1000-fold expansion of the responding cells within a few days (*10*). After differentiating into effector cells, the progeny of the responding cells reenter the circulation through efferent lymph and disseminate throughout the body (*11–13*). By means of expression of new cell surface–homing molecules, the effector cells acquire the capacity to penetrate capillary blood ves-

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sels and thus come into direct contact with infected parenchymal cells, e.g., respiratory epithelial cells in influenza infection (*7*, *14*); these cells act as targets for cytotoxic cells and are killed. In bacterial infections, cells harboring intracellular bacteria are either killed by cytotoxic cells or, via cytokines, such as interferon-γ (IFN-γ), released from other effector cells, are instructed to destroy the pathogen (*15*).

Once the infection is cleared, the enormous numbers of effector cells generated during the immune response become superfluous. Allowing these cells to survive en masse would lead to congestion in the lymphoid tissues and thereby compromise subsequent immune responses. To cope with this problem, the vast majority (>90%) of effector cells are destroyed at the end of the primary response; only a few cells survive to become long-lived memory cells.

Death of effector cells: A default pathway or an instructional process? In considering how effector cells are eliminated, perhaps the simplest idea is that these cells are intrinsically short-lived and are programmed to die by a default pathway when the infection is cleared. Although appealing, the notion that effector cells are innately short-lived is difficult to reconcile with the finding that many different "gene-knockout" or mutant strains of mice spontaneously develop prominent and progressive hypertrophy of the secondary lymphoid tissues. As discussed elsewhere (*6*), this syndrome occurs with deletion or mutation of Fas/FasL, PD-1, CTLA4, NFAT, CD25, CD122, CD45, transforming growth factor–β and several other molecules. In these various situations, the immune system becomes overwhelmed with activated lymphoid cells, presumably reflecting unrestrained responses to various environmental antigens. Likewise, deletion or mutation of these molecules can substantially prevent the elimination of effector cells generated against defined antigens.

In light of the above findings, rather than reflecting a default pathway, the elimination of effector cells appears to reflect a tightly regulated instructional process involving multiple cell death–inducing mechanisms acting in consort. Even with inactivation of only a single component of one of these mechanisms, death is averted, and effector cells survive en masse. The sequence of molecular events required to initiate the death of effector cells is still unclear, but is thought to involve negative signaling by CTLA-4 and PD-1 receptors for costimulatory signals, activation of the Fas death pathway by dissociation of cFLIP from Fas, and onset of sensitivity to inhibition by several cytokines, notably IL-2 (*6*).

Generation of memory cells. In considering how memory T cells are generated, a key issue is whether these cells are derived from typical effector cells or represent a separate lineage. There is now firm evidence that memory T cells represent the descendants of proliferating cells, which, at least transiently, express effector functions such as perforin (*16*), Granzyme B (*17*), and cytokine (*18*) production.

As for the elimination of effector cells, generation of memory cells could be an instructional process: effector cells are coerced to die, whereas memory cells are taught to live. The obvious problem with this idea is that it fails to explain the radically different outcomes (life versus death) of the instructional process or processes. The opposing viewpoint is that memory cells are generated by a default pathway and represent a few escapees that somehow evade being instructed to die.

A difficulty with the latter idea is that, for $CD8^+$ T cells, it fails to explain the strong correlation between memory-cell production and the intensity of the primary response. Thus, irrespective of the dose of antigen eliciting the response and the T cell receptor (TCR)–MHC-peptide affinities involved, the proportion of $CD8^+$ cells that survive the response to become memory cells is remarkably constant, i.e., about 5 to 10% of the peak numbers of cells generated at the height of the response (*19*). One explanation for this apparent paradox is that all "successful" primary immune responses that lead to rapid rejection of pathogens may have essentially similar kinetics. Thus, whatever the initial dose or route of infection, the pathogen replicates until the concentration of antigen in the lymphoid tissues is sufficiently high to induce a powerful immune response, which then leads to rejection of the pathogen, usually after 7 to 10 days. As T cells are mobile cells, it is therefore quite likely that virtually all T precursor cells will come into contact with antigen, probably in high concentrations, before the pathogen is cleared. However, the duration of exposure to antigen is likely to vary considerably, depending upon the anatomical distribution of the precursor cells.

For typical infections occurring in mucosal sites, e.g., the lung and gut, the immune response is initiated in the draining LNs and involves blood-borne recruitment of recirculating lymphocytes from throughout the body. Here, it should be borne in mind that LNs are small structures with a limited blood supply and that the tempo of blood-to-lymph recirculation of lymphocytes is quite slow, 12 to 24 hours (*20*, *21*). Hence, the time taken for individual recirculating lymphocytes to enter an infected LN randomly varies enormously, from seconds for some cells to many days for others. Consequently, cells recruited late in the immune response will see antigen for a much briefer period than the cells initially recruited.

The duration of exposure to antigen could be an important factor in memory-cell generation. Thus, the cells destined to die at the end of the immune response could be the progeny of cells undergoing a prolonged response, protracted contact with antigen being required to switch on the death pathways. Conversely, for latecomer cells arriving near the end of the response, brief exposure to antigen could be sufficient to cause these cells to proliferate and differentiate into effector cells, but could be inadequate to activate the various death pathways that are required for effector-cell elimination.

According to the above model, generation of memory cells reflects incomplete differentiation of a subset of cells that avoids death (overstimulation) by arriving late in the immune response. It would follow therefore that subjecting all responding lymphocytes to prolonged antigenic stimulation would induce full differentiation and death, thereby precluding memory-cell generation. The finding that overwhelming viral infections can lead to total elimination ("exhaustion") of the responding T cells after a prolonged proliferative response is consistent with this idea (*22*).

Controlling the survival of long-lived memory cells. By analogy with naïve lymphocytes (see above), one can envisage that some form of signaling is required for the survival of memory cells. The original notion that memory is maintained through contact with trace amounts of antigen left over from the primary response and/or contact with cross-reactive environmental antigens (*1*) is unlikely because memory T cells survive well after transfer to $MHC^{-/-}$ hosts (*23*). Hence, the longevity of memory cells, in contrast to that of naïve T cells, does not seem to depend on TCR ligation.

A notable feature of memory T cells is that the normal rate of division (turnover) of these cells in vivo is substantially higher than for naïve T cells (*24*). Significantly, the relatively rapid turnover of memory T cells occurs after transfer into $MHC^{-/-}$ hosts, indicating that the stimulus for proliferation is MHC-independent (*23*). As discussed below, at least for $CD8^+$ T cells, the turnover and survival of memory cells are controlled by cytokines. The data refer to "memory-phenotype" ($CD44^{hi}$) T cells found in normal unstimulated hosts (*1*, *2*); these cells are presumed to represent the progeny of cells responding to various environmental antigens.

In mice, the notion that the normal turnover of $CD44^{hi}$ T cells is controlled by cytokines stemmed from the finding that proliferation of $CD44^{hi}$ $CD8^+$ cells increases sharply after injection of IFNs, either IFN-

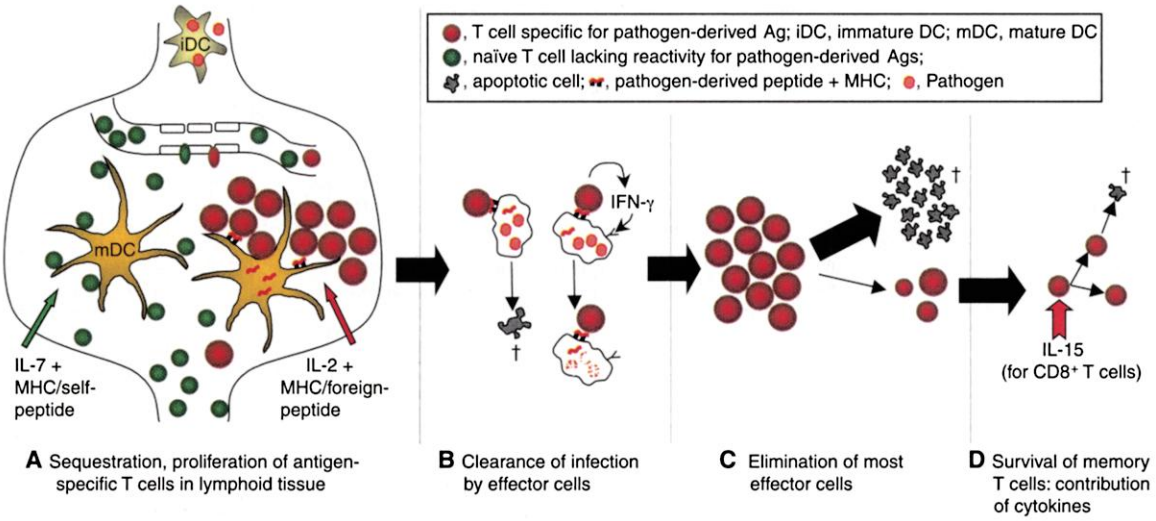

Fig. 1. Decision-making in the generation and maintenance of T cell memory. **(A)** In the absence of foreign antigen, naïve T cells (green) are kept alive in interphase by a cytokine, IL-7, plus TCR interaction with MHC + self peptides. During infection, recognition of pathogen-derived peptides in association with MHC on the surface of mature DCs leads to "trapping" and expansion of rare antigen-specific T cells (red) in secondary lymphoid tissues, while nonreactive lymphocytes (green) migrate through. Several cytokines, notably IL-2, support the proliferation of T cells responding to foreign antigen. **(B)** Effector T cells clear the infection through lysis of infected cells and/or release of cytokines that trigger intracellular destruction of the pathogen. **(C)** After removal of the pathogen, the vast majority of effector T cells are eliminated, whereas a portion of the activated cells survive as memory cells. **(D)** Long-term survival of memory T cells is partially dependent on contact with cytokines. Stimulation of memory $CD8^+$ T cells with IL-15 causes these cells to survive and divide intermittently; whether cytokines control the survival and/or proliferation of memory $CD4^+$ cells is unclear. At steady state, division of memory T cells is presumably balanced by cell death.

α/β or IFN-γ, or IFN-inducing agents (*25*, *26*). IFNs do not act directly on $CD44^{hi}$ $CD8^+$ cells but instead stimulate other cells, probably DCs and stromal cells, to produce an effector cytokine that is directly stimulatory for $CD44^{hi}$ $CD8^+$ cells (although not for $CD44^{hi}$ $CD4^+$ cells). As discussed elsewhere (*6*), the effector cytokine appears to be IL-15, an IL-2–like cytokine synthesized by a spectrum of cells, although not by T cells (*27*). Unlike IL-2, IL-15 selectively stimulates $CD44^{hi}$ $CD8^+$ cells, apparently because one of the components of the IL-15 receptor, CD122 (IL-2Rβ), is expressed at a much higher level on $CD44^{hi}$ $CD8^+$ cells than on other subsets, including $CD44^{hi}$ $CD4^+$ cells (*28*).

In addition to being the effector cytokine for IFN-induced proliferation of $CD122^{hi}$ $CD44^{hi}$ $CD8^+$ cells, IL-15 appears to control the normal background turnover of these cells (*29*). Moreover, as for proliferation, IL-15 plays a key role in maintaining $CD44^{hi}$ $CD8^+$ cell survival. Thus, $IL\text{-}15^{-/-}$ mice are almost devoid of $CD122^{hi}$ $CD8^+$ cells (*30*, *31*), and normal $CD122^{hi}$ $CD44^{hi}$ $CD8^+$ cells (but not other T cells) die rapidly when transferred to $IL\text{-}15^{-/-}$ hosts (*31*).

On the basis of the above findings, both the survival and turnover of $CD44^{hi}$ $CD8^+$ cells are under the strict control of a single cytokine, IL-15. These data refer to the memory-phenotype $CD8^+$ cells found in normal animals, and it remains to be proved whether IL-15 dependency applies to "real" memory cells generated in response to defined antigens. It is notable, however, that generation of long-lived antigen-specific memory $CD8^+$ cells derived from cells stimulated in vitro depends critically upon sustained up-regulation of CD122 after in vivo transfer (*32*).

By analogy with $CD8^+$ cells, it would seem likely that cytokines also control the survival and turnover of memory $CD4^+$ cells. Which particular cytokines act on these cells, however, is unclear, although γc-controlled cytokines do not seem to be crucial (*6*, *33*).

Summary and concluding comments. As outlined above, strict life or death decision-making is involved throughout the life-span of mature T cells. At each stage of the transition from naïve cells into memory cells, however, the decision-making is distinctly different. Four stages can be considered (Fig. 1):

1) Before contact with a foreign antigen, the survival of resting naïve T cells requires low-level signaling mediated by TCR contact with self-peptide–MHC complexes combined with stimulation via IL-7.

2) Contact with foreign antigen augments TCR signaling, thus driving T cells to proliferate and differentiate into effector cells. During this stage, the protective effect of IL-7 is replaced by other cytokines, especially IL-2, which act as growth factors for potentiating clonal expansion.

3) At the end of the immune response, loss of T cell contact with antigen and/or growth factors initiates qualitative changes in intracellular signaling: operationally, stimulatory signals now become destructive, and the majority of the cells are forced to die.

4) A minority population of effector cells avoids death, perhaps by arising from precursors that were recruited late in the immune response. During differentiation of effector cells into memory cells, the cells are reprogrammed to be independent of TCR signals for their survival. For $CD8^+$ T cells, memory-cell survival requires contact with IL-15 and hinges on up-regulation of receptors for this cytokine.

The underlying assumption in the above scheme is that T cells cannot survive without active signaling elicited by contact with external ligands. It is striking, however, that the ligands concerned are so different for naïve versus memory cells and even for $CD8^+$ versus $CD4^+$ memory T cells. Ultimately, these different ligands may all act via maintaining expression of various anti-apoptotic molecules. This issue, however, is far from resolved.

Understanding the steps involved in memory-cell generation is relevant to the important issue of vaccine design. As emphasized by others (*1*–*6*), successful vaccines induce strong and persistent humoral responses, and, at least for T cells, depend on strong initial clonal expansion of precursor cells. Further success in vaccine design may hinge on developing techniques for diminishing cell death at the end of the primary response, thereby augmenting entry of effector cells into the long-lived memory-cell pool. Whether this is a pipe dream or ultimately feasible remains to be seen.

References and Notes

1. R. M. Zinkernagel *et al.*, *Annu. Rev. Immunol.* **14**, 333 (1996).
2. J. Sprent, *Curr. Opin. Immunol.* **9**, 371 (1997).
3. R. W. Dutton, L. M. Bradley, S. L. Swain, *Annu. Rev. Immunol* **16**, 201 (1998).

4. J. K. Whitmire, R. Ahmed, *Curr. Opin. Immunol.* **12**, 448 (2000).
5. A. Lanzavecchia, F. Sallusto, *Science* **290**, 92 (2000).
6. J. Sprent, C. D. Surh, *Curr. Opin. Immunol.* **13**, 248 (2001).
7. L. J. Picker, M. H. Siegelman, in *Fundamental Immunology*, W. E. Paul, Ed. (Lippincott-Raven, Philadelphia, 1999), pp. 479–532.
8. R. M. Steinman, in *Fundamental Immunology*, W. E. Paul, Ed. (Lippincott-Raven, Philadelphia, 1999), pp. 547–573.
9. J. Sprent, *Immunol. Rev.* **42**, 108 (1978).
10. A. W. Goldrath, M. J. Bevan, *Nature* **402**, 255 (1999).
11. J. Sprent, *Cell. Immunol.* **21**, 278 (1976).
12. R. L. Reinhardt, A. Khoruts, R. Merica, T. Zell, M. K. Jenkins, *Nature* **410**, 101 (2001).
13. D. Masopust, V. Vezys, A. L. Marzo, L. Lefrançois, *Science* **291**, 2413 (2001).
14. R. Ahmed, C. A. Biron, in *Fundamental Immunology*, W. E. Paul, Ed. (Lippincott-Raven, Philadelphia, 1999), pp. 1295–1334.
15. S. H. E. Kaufmann, in *Fundamental Immunology*, W. E. Paul, Ed. (Lippincott-Raven, Philadelphia, 1999), pp. 1335-1386.
16. J. T. Opferman, B. T. Ober, P. G. Ashton-Rickardt, *Science* **283**, 1745 (1999).
17. J. Jacob, D. Baltimore, *Nature* **399**, 593 (1999).
18. A. Saparov *et al.*, *Immunity* **11**, 271 (1999).
19. D. J. Sourdive *et al.*, *J. Exp. Med.* **188**, 71 (1998).
20. J. L. Gowans, E. J. Knight, *Proc. R. Soc. London Ser. B* **159**, 257 (1964).
21. J. Sprent, *Cell. Immunol.* **7**, 10 (1973).
22. D. Moskophidis, F. Lechner, H. Pircher, R. Zinkernagel, *Nature* **362**, 758 (1993).
23. K. Murali-Krishna *et al.*, *Science* **286**, 1377 (1999).
24. D. F. Tough, J. Sprent, *J. Exp. Med.* **179**, 1127 (1994).
25. D. F. Tough, P. Borrow, J. Sprent, *Science* **272**, 1947 (1996).
26. D. F. Tough, X. Zhang, J. Sprent, *J. Immunol.* **166**, 6007 (2001).
27. T. A. Waldmann, Y. Tagaya, *Annu. Rev. Immunol.* **17**, 19 (1999).
28. X. Zhang, S. Sun, I. Hwang, D. F. Tough, J. Sprent, *Immunity* **8**, 591 (1998).
29. C. C. Ku, M. Murakami, A. Sakamoto, J. Kappler, P. Marrack, *Science* **288**, 675 (2000).
30. M. K. Kennedy *et al.*, *J. Exp. Med.* **191**, 771 (2000).
31. A. Judge, J. Sprent, unpublished observations.
32. L. R. Huang, F. L. Chen, Y. T. Chen, Y. M. Lin, J. T. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3406 (2000).
33. O. Lantz, I. Grandjean, P. Matzinger, J. P. Di Santo, *Nature Immunol.* **1**, 54 (2000).
34. Supported by grants CA38355, AI21487, AI46710, and AG01743 from the U.S. Public Health Service. For The Scripps Research Institute, this is publication no. 14126-IMM; for the Edward Jenner Institute for Vaccine Research, this is publication no. 45.

VIEWPOINT

Arrested Differentiation, the Self-Renewing Memory Lymphocyte, and Vaccination

Douglas T. Fearon,[1]* Peter Manders,[1] Simon D. Wagner[1,2]

Vaccination for persistent viral or bacterial infections must program the immune system for a lifelong need to generate antigen-specific effector lymphocytes. How the immune system does this is not known, but recent studies have shown that a subset of B lymphocytes, the germinal center B cell, is capable of self-renewal because it expresses a transcriptional repressor, BCL6, that blocks terminal differentiation. If a similar mechanism for arresting differentiation exists for long-lived, antigen-selected lymphocytes, a stem cell–like capacity for self-renewal could be the basis for the continual generation of effector lymphocytes from the memory pool. Understanding how to regulate the terminal differentiation of lymphocytes will improve immunotherapeutic approaches for chronic infectious diseases and cancer.

Vaccination is the attempt to mimic certain aspects of an infection for the purpose of causing an immune response that will protect the individual from that infection. Usually vaccination is performed for prophylaxis, but it may also have a therapeutic application, as, for example, in the treatment of patients with chronic infections or cancer. Empirical approaches to the development of vaccines have served us well in the past, but the "easy pickings" are over, and to meet current challenges requires a better understanding of the immune system. The starting point of this overview is a description of the aims of an immune response, as these are the endpoints for vaccination.

The immune system must accomplish three goals to protect the host from infectious disease. First is the generation of effector lymphocytes, such as plasma cells to secrete antibody, helper T cells to secrete cytokines and stimulate other immune cells by expressing CD40 ligand, and cytotoxic T lymphocytes (CTLs) to kill virally infected cells. Second is the development of the ability to generate rapidly these effector lymphocytes when antigen is encountered again in the future, a function that is ascribed to "memory" lymphocytes. Third is less explicit but is evident when one considers infections that are chronic, such as those caused by herpes viruses, hepatitis B, human immunodeficiency virus, *Mycobacterium tuberculosis*, etc. These require an ability to generate effector cells continually and over long periods, perhaps for the lifetime of the host. Here, we focus on recent studies that bear on the question of how the immune system might generate effector lymphocytes for the lifetime of the host, and on the possible relation of this process to what has been termed immunological memory.

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The Antigen-Dependent Phase of Lymphocyte Development and Continual Generation of Effector Cells

The adaptive immune system has antigen-independent and antigen-dependent phases of development. During the antigen-independent first phase, the immune repertoire of the immunologically naïve host is created by the generation of clones of B and T lymphocytes, each having a unique antigen receptor. For the most part, these antigen receptors appear not to have been selected for antimicrobial specificity; instead, they provide the host with a vast array of clonally distributed potential antigen-binding specificities. Thus, it becomes virtually certain that all infectious microorganisms will express antigens during some phase of their life cycle that will be recognized by at least a few of these clones. In humans, this phase of B cell development continues relatively unabated into adulthood, whereas the generation of new T cells is drastically reduced because of thymic involution. One might suspect, then, the existence not only of mechanisms to preserve naïve T cells, but also—and of particular relevance to this review—of mechanisms to maintain lymphocyte clones that have been selected during the antigen-dependent phase of development by microbial antigens. This would be important because such cells may be irreplaceable.

During this second phase of differentiation, lymphocytes bind antigen and, with the innate arm of the immune system promoting responses to antigens of microbial origin, initiate complex intracellular and intercellular processes leading to cellular proliferation and differentiation. The proliferation phase is an especially daunting task because the host starts with relatively few antigen-reactive clones in its naïve repertoire, but requires millions (or billions, depending on the size of the host) of terminally differentiated effector lymphocytes just to control the initial infection. This need, which becomes even greater if the infection persists into a chronic phase, is particularly stringent for T