When would this scenario have happened? And how often? Are these merely viral anomalies, or do they have a more general lesson to teach? The most intriguing possibility would be one in which such events occurred in the RNA world (*3*), in which primitive self-replicating RNAs could have joined with equally primitive mRNA segments to form the first RNA genomes. If such mosaics survived until DNA arose, their preservation would lead to the presence of introns and split genes. This point of view could lead to the following: Today's intervening sequences or introns might have arisen from the viroidlike regions of early RNA mosaics from which modern DNA chromosomes may be descended. Although the independent replicating ability of these viroidlike sequences now embedded as introns in DNA-coded RNA precursors would soon have been lost, perhaps the RNA-catalyzed cleavage and ligation steps, characteristic of many viroidlike RNAs (*5*, *6*), have survived to become the mRNA splicing system now so widespread in eukaryotes (*8*).

References

1. W. Gilbert, *Nature* **319**, 618 (1986).
2. H. D. Robertson, *Curr. Top. Microbiol. Immunol.* **176**, 213 (1992).
3. R. F. Gesteland and J. F. Atkins, Eds., *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993).
4. R. Brazas and D. Ganem, *Science* **274**, 90 (1996).
5. J. S. Semancik, Ed., *Viroids and Viroid-Like Pathogens* (CRC, Boca Raton, FL, 1987).
6. A. D. Branch and H. D. Robertson, *Science* **223**, 450 (1984).
7. A. D. Branch *et al.*, *ibid.* **243**, 649 (1989).
8. M. J. Moore *et al.*, in (*3*), pp. 303–357.

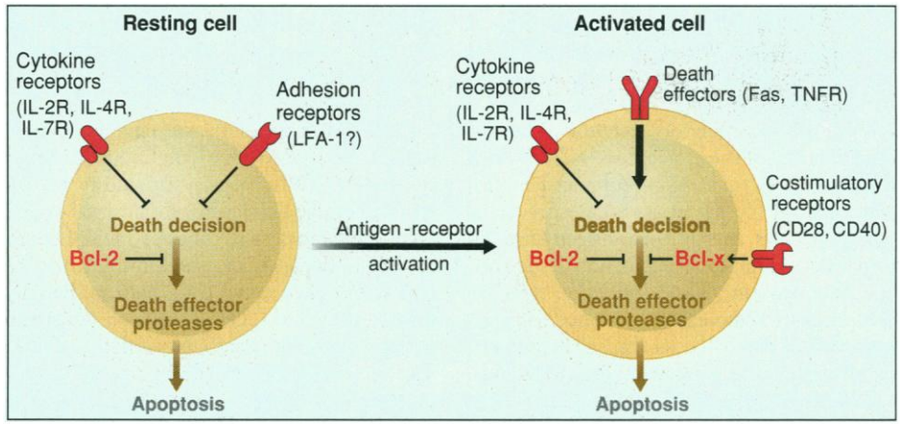
Hierarchical Control of Lymphocyte Survival

Lawrence H. Boise and Craig B. Thompson

At rest, the lymphoid immune system consists primarily of quiescent cells—B and T lymphocytes—each potentially responsive to a unique, specific antigen. When an antigen is recognized, these lymphocytes proliferate into a clonal population of cells that directs an immune attack against the foreign antigen. To keep the number of lymphocytes manageable, the immune system balances this proliferation by the removal of excess cells once the antigen is successfully eliminated. This removal is accomplished by programmed cell death (apoptosis), which also kills potentially autoreactive lymphocytes and limits the clonal expansion of lymphocytes during an immune response. This carefully tuned homeostatic system is critical: When it goes awry, excess lymphocytes can cause lymphoid malignancies, lymphoproliferative diseases, and autoimmunity. Recent work reveals that this homeostasis is maintained by an extremely complex set of regulatory processes that differ markedly in quiescent and activated cells. But a sensible framework for the new information can now be assembled.

Apoptosis is mediated by the activation of intracellular proteases, of which the ICE/Ced-3 family of cysteine proteases is the best characterized. Once activated, several of these proteases can induce all the morphological and biochemical features of apoptosis (*1*). The apoptosis-controlling ICE/Ced-3 proteases are constitutively expressed as inactive precursors (zymogens) in lymphocytes. Therefore, for the lymphocyte to survive, the activation of these precursors must be carefully regulated. Lymphocytes can also control their own sensitivity to apoptosis through modulators of the apoptotic threshold. The best characterized of these regulators are members of the Bcl-2 family. Both ICE proteases and Bcl-2–related proteins are key controllers of apoptosis, as indicated by their conservation in metazoan cells. A third class of molecule also falls into this category—cell surface receptors.

The relative importance of these receptors and of the ICE proteases and Bcl-2 in regulating lymphocyte survival depends on the activation state of the lymphocyte (see figure). In a quiescent cell, survival depends primarily on the expression of Bcl-2. Animals without the *bcl-2* gene cannot sustain a peripheral immune system because they progressively lose their quiescent lymphocytes (*2*). In a quiescent cell, signal transduction through cytokine receptors can also promote lymphocyte survival. The best characterized of these receptors are members of the cytokine receptor family that share a common γ chain—interleukin-2 (IL-2), IL-4, and IL-7 receptors. Survival mediated through these receptors does not depend on the expression of Bcl-2, nor on the ability of these receptors to mediate progress through the cell cycle (*3*). This ability of cytokines to support the survival of cells that do not encounter antigen may help prevent the deletion of bystander cells during an inflammatory response, especially because lymphocytes can be recruited into an inflammatory lesion in the absence of antigen-receptor engagement. Adhesion receptors such as integrins also appear to regulate the survival of quiescent B and T cells (*4*). If a lymphocyte does not traffic to the usual peripheral immune sites—lymph nodes, Peyer's patches in the gut, and spleen—it will be deleted by apoptosis (*5*). These sites contain adhesion receptor ligands, which may allow long-term lymphocyte survival by signaling the posi-



The factors that regulate the survival of resting and activated lymphocytes. In this model, the survival information provided by cell surface receptors is hypothesized to prevent the cell from activating the proteolytic function of the effector proteases that mediate apoptosis. The factors that determine the conversion of ICE/Ced3 proteases from pro-enzymes to active enzymes have not been fully elucidated. ICE/Ced-3 proteases may be activated sequentially in a proteolytic cascade (*1*, *17*).

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tion of the cell in the body. Together, cytokine and adhesion receptors maintain the survival of naïve lymphocytes, while preventing accumulation at nonphysiologic sites or deletion at sites of inflammation.

After activation by antigen, the survival of a lymphocyte is regulated in a distinct fashion. Initial antigen receptor engagement alone does not induce apoptosis, but rather commits the cell to cell-cycle entry. In the absence of appropriate signals for orderly progression through the cell cycle or for differentiation into an effector cell, antigen receptor engagement may establish a conflict in cell-cycle control that results in apoptosis. Therefore, the stronger the antigenic stimuli, the more irrevocable the commitment to proliferate or differentiate. Once a cell enters the cell cycle, cytokines such as IL-2 promote survival by maintaining orderly progression through the cell cycle. Withdrawal of cytokines can limit clonal expansion by both decreasing proliferation and inducing apoptosis (6).

The survival of an activated lymphocyte also comes under the control of cell surface receptors that can specifically induce death. Such receptors are members of a subfamily of the tumor necrosis factor (TNF) receptor superfamily that includes TNF-R1 (p55) and Fas. These receptors can induce apoptosis of activated lymphocytes (7). Although most murine lymphocytes express Fas, resting lymphocytes are not susceptible to Fas-mediated death. After activation, however, they become susceptible, although the molecular mechanism is not yet known.

Antigen activation induces the expression of Fas ligand (FasL) by helper T cells and leads to sensitivity to Fas-induced death. Yet antigen activation does not immediately cause death by Fas. In fact, during a productive immune response, lymphocytes usually undergo multiple rounds of activation by antigen, and their population expands at sites of inflammation. Signal transduction pathways must exist to prevent lymphocytes from dying in response to signal transduction through Fas. Obvious candidates for such a role are the costimulatory receptors. The best characterized of these receptors—CD28 on T cells and CD40 on B cells—enhance lymphocyte survival (6, 8). In both cases, receptor engagement at the time of antigen stimulation induces high-level expression of the anti-apoptotic protein, Bcl-x_L. Induction of Bcl-x_L correlates with increased resistance to apoptosis in both B and T cells exposed to antigens in the presence of costimulatory ligands. CD28 costimulation also increases the production of cytokines by helper T cells, which in turn may further enhance survival. A requirement of costimulation for the survival of activated lymphocytes could be a way for the organism to delete potentially harmful autoreactive lymphocytes, which usually encounter antigen without costimulatory signals.

The expression of Bcl-x_L induced by antigen activation plus costimulation increases the resistance of cells to Fas-induced apoptosis (6). This finding suggests that signal transduction pathways that induce cell death are subordinate to cell survival signaling under conditions that mimic the amplification phase of the immune response. However, the loss of antigen or costimulatory signals at any time during the proliferative response appears to leave an activated cell sensitive to the induction of cell death by either FasL or TNF (9). Indeed, Fas is critical for the elimination of lymphocytes at the end of an immune response. Genetic defects in the *fas* locus result in the failure to eliminate excess lymphocytes and in the development of a lymphoproliferative syndrome in both mice and humans (7).

Several receptors that primarily enhance lymphocyte survival during the initiation of an immune response induce secondary events that increase the subsequent susceptibility of the cell to apoptosis. For example, CD40 upregulates expression of Fas on B cells (10) and, when activated in the absence of antigen, promotes Fas-dependent cell death. Similarly, IL-2, although a potent survival factor, appears to prime T cells for subsequent FasL- or TNF-mediated cell death (11)—perhaps because IL-2 is a potent inducer of CTLA-4 (12), a receptor that can inhibit CD28 costimulation by competitively binding the shared ligands, B7-1 and B7-2. Inhibition of Bcl-x_L expression by this mechanism would likely increase sensitivity to signal transduction through cell death receptors. Germ line deletion of either IL-2 or CTLA-4 in mice results in the development of a lymphoproliferative disease (13), confirming their importance in sensitizing cells for apoptosis. So, lymphocyte activation, even while promoting immediate survival of the cells, also triggers mechanisms that will facilitate their eventual deletion at the end of the response.

The sum of the evidence suggests that the regulation of activated lymphocyte survival is dynamic. Whether costimulatory receptors will enhance lymphocyte survival or cell death effectors will induce lymphocyte apoptosis depends on the timing and the relative level of signal transduction. For example, activated $CD4^+$ T cells are sensitive to Fas-induced cell death. Nevertheless, $CD4^+$ T cells can be exponentially expanded in vitro by repetitive antigen-receptor engagement if there is continuous CD28 costimulation (14). Thus, even though the cells constitutively express Fas and repetitively express FasL on their surface, Fas signal transduction fails to induce significant apoptosis in this expanding population of cells, possibly because of the transient nature of FasL expression in T cells. In contrast, constitutive expression of the FasL on an immune target can abort an immune response. Expression of FasL by cells in a tissue graft can prevent graft rejection (15). Stromal cell expression of FasL appears to account for the status of the eye and testes as sites of immune privilege. These observations reveal the range of effects that can be mediated by optimal stimulation through either costimulatory or cell death receptors.

A number of central issues remain unresolved. Little is known about the signal transduction pathways by which cytokine and costimulatory receptors exert their anti-apoptotic effects. Why Bcl-2 levels in activated lymphocytes, which are equivalent to those in resting lymphocytes, cannot protect the cell from apoptosis has not been established. Finally, Fas cross-linking recruits and activates a novel ICE protease, Mach-1/Flice (1). How Bcl-2 proteins can prevent Fas-mediated apoptosis is not clear because Bcl-2 has been suggested to act upstream of ICE protease activation to inhibit apoptosis (16). Thus, key components in the regulation of lymphocyte survival are being defined, but a great deal more work is necessary before the biochemical regulation of cell death is completely understood.

References

1. A. Fraser and G. Evan, *Cell* **85**, 781 (1996).
2. K.-i. Nakayama *et al.*, *Science* **261**, 1584 (1993); D. J. Veis, C. M. Sorenson, J. R. Shutter, S. J. Korsmeyer, *Cell* **75**, 229 (1993).
3. V. A. Illera, C. E. Perandones, L. L. Stunz, D. A. Mower, R. F. Ashman, *J. Immunol.* **151**, 2965 (1993); K.-i. Nakayama, K. Nakayama, L. B. Dustin, D. Y. Loh, *J. Exp. Med.* **182**, 1101 (1995); L. H. Boise, A. J. Minn, C. H. June, T. Lindsten, C. B. Thompson, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5491 (1995).
4. M. H. Kosco, E. Pflugfelder, D. Gray, *J. Immunol.* **148**, 2331 (1992); E. Ruoslahti and J. C. Reed, *Cell* **77**, 477 (1994).
5. J. G. Cyster, S. B. Hartley, C. C. Goodnow, *Nature* **371**, 389 (1994).
6. L. H. Boise *et al.*, *Immunity* **3**, 87 (1995).
7. A. K. Abbas, *Cell* **84**, 655 (1996).
8. M. R. Kehry, *J. Immunol.* **156**, 2345 (1996); L. G. Radvanyi *et al.*, *ibid.*, p. 1788; C. Lagresle, P. Mondière, C. Bella, P. H. Krammer, T. Defrance, *J. Exp. Med.* **183**, 1377 (1996).
9. L. V. Parijs, A. Ibraghimov, A. K. Abbas, *Immunity* **4**, 321 (1996).
10. E. J. Schattner *et al.*, *J. Exp. Med.* **182**, 1557 (1995); P. Garrone, *ibid.*, **182**, p.1265.
11. M. J. Lenardo, *Nature* **353**, 858 (1991); L. Zheng *et al.*, *ibid.*, **377**, 348 (1995).
12. T. L. Walunas *et al.*, *Immunity* **1**, 405 (1994).
13. B. Sadlack *et al.*, *Cell* **75**, 253 (1993); E. A. Tivol *et al.*, *Immunity* **3**, 541 (1995); P. Waterhouse *et al.*, *Science* **270**, 985 (1995).
14. B. L. Levine *et al.*, *Science* **272**, 1939 (1996).
15. H. T. Lau *et al.*, *ibid.* **273**, 109 (1996); D. Bellgrau *et al.*, *Nature* **377**, 630 (1995); T. S. Griffith, *et al.*, *Science* **270**, 1189 (1995).
16. A. M. Chinnaiyan *et al.*, *J. Biol. Chem.* **271**, 4573 (1996).
17. M. Enari, R. V. Talanian, W. W. Wong, S. Nagata, *Nature* **380**, 723 (1996).