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T Helper 2 Cells' Preferred Way to Die

In this issue of *Immunity*, Devadas and colleagues (2006) reveal that granzyme B, long known as a mediator of $CD8^+$ T cell cytotoxicity, has a new role in an internal homeostatic death mechanism that controls the fate of $CD4^+$ T helper 2 cells.

Soon after it was recognized that T lymphocytes have cytotoxic, i.e., cell killing, activity, two forms of cytoxicity were distinguished by whether or not they required calcium (Kägi et al., 1996). The calcium-dependent mechanism involves the release of granules containing perforin and death-inducing serine proteases called granzymes. Calcium-independent death occurs by a completely different mechanism involving an inducible cell-surface protein, called Fas ligand, that is related to tumor necrosis factor (TNF) and specifically binds the Fas receptor (also known as CD95 or APO-1). Thus, there are two distinct killing mechanisms, and both mechanisms play a crucial role in immunity by enabling $CD8^+$ cytolytic T cells or natural killer (NK) cells to destroy virally infected or tumorigenic target cells. These cytolytic mechanisms may also be involved in damaging effects of T cell responses such as in autoimmune disease and graft rejection (Kägi et al., 1996).

However, cell death has another vital role in immunity. T lymphocyte numbers and activity are regulated throughout the course of immune responses. After antigenic stimulation, T cells have a burst of proliferation, which greatly increases the number of specific effector cells to facilitate a strong protective immune response. However, cell numbers must be tightly controlled to avoid the negative consequences of vast numbers of activated T cells, such as autoimmune reactions. Therefore, T lymphocytes have evolved means to downregulate their own numbers by propriocidal or "self-killing" mechanisms that appear to be hard-wired into the biology of these cells (Lenardo et al., 1999). Soon after their discovery, Fas and its ligand were quickly appreciated to have a dual role in both cytotoxic functions and autoregulatory cell death. For example, genetic abnormalities of Fas and its ligand lead to distorted lymphocyte homeostasis, leading to the autoimmune lymphoproliferative syndrome (ALPS) (Lenardo et al., 1999). Nevertheless, the molecular nature of autoregulatory death in different T cell subsets were never fully worked out, and this is where the paper by Devadas et al. and a related work (Sharma et al., 2006) make an important contribution to our understanding of immune regulation.

In the simplest paradigm, naive $CD4^+$ T helper cells (Th0 cells) can respond to specific conditions of Ag stimulation by differentiating into specialized Th1 or Th2 offspring that promote different types of immune responses (see London et al., 1998 and Farrar et al., 2002 for a full delineation of their molecular and functional differences). Interestingly, although both Th1 and Th2 cells undergo autoregulatory cell death, Th1 cells are very sensitive to killing through Fas but Th2 cells are relatively insensitive (Oberg et al., 1997). This puzzling observation has lingered for nearly a decade without a good explanation for how Th2 cells control themselves. Now, Devadas et al. (2006) address this conundrum by demonstrating that granzyme B (GrB), previously implicated in $CD8^+$ T cell and NK cytotoxicity, is crucial for autoregulatory cell death in Th2 cells. Thus, evolution appears to have redeployed the same two cytolytic mechanisms used in immune defense to autoregulate the different subsets of cells by promoting their own suicide under appropriate conditions.

Devadas et al. make a clear case for the importance of GrB in Th2 cell death (Figure 1). They find that the GrB-specific inhibitor zAAD-cmk and a cathepsin C inhibitor (which prevents the processing of GrB) efficiently protect Th2 cells from death, whereas caspase inhibitors have no effect. In contrast, autoregulatory death of Th1 cells can only be blocked by caspase inhibitors, which inhibit key proteolytic events in apoptosis. Devadas et al. provide further insights into the regulation of GrB, which is normally expressed at low amounts in naive $CD4^+$ T cells. They show that T cell receptor (TCR) stimulation upregulates GrB in both Th1 and Th2 cells; however, it is differentially controlled in the two subsets. In Th1 cells, the induced GrB remains sequestered in the lysosomes during cell-death induction as indicated by colocalization with the lysosome-associated membrane protein LAMP-1. In contrast, TCR engagement in Th2 cells leads to the internal release of GrB from cellular granules into the cytosol and nucleus with toxic consequences for the cell. Release of cytotoxic granules is ordinarily vectorial and external when a cytolytic T cell encounters a target cell. The killing proteins are released toward the target cell, and the T cell itself is spared. However, the direct GrB release into the cytoplasm ensures the suicidal demise of Th2 cells. The authors also show that differentially expressed protein inhibitors guide the helper T cells into the two distinct pathways.

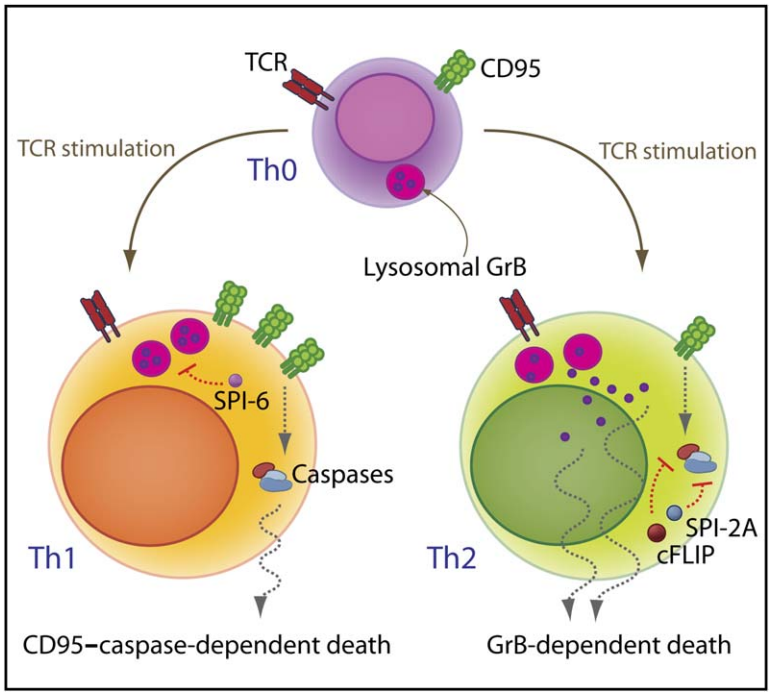

Figure 1. Distinct Signaling Pathways of T Cell-Receptor-Induced Cell Death in CD4$^+$ T Helpers

Naive CD4$^+$ T helper (Th0) cells differentiate into Th1 or Th2 cells under specific differentiating conditions after antigen stimulation. These Th1 and Th2 cells proliferate and carry out effector functions by producing different cytokines that control immune responses. The serine protease inhibitor (SPI)-6, a protein that maintains lysosome integrity, and death receptor CD95 are strongly induced in Th1 cells; whereas, cFLIP, a cellular caspase-8 inhibitory protein and SPI-2A, an endogenous protein that inhibits caspase-3 and -9 activities, are specifically up regulated in Th2 cells. These molecules allow Th1 and Th2 cells to undergo CD95- or caspase-dependent and granzyme B (GrB)-dependent homeostatic death, respectively. T cell-receptor-induced cell death (TICD) reduces the number of differentiated T helper cells and, in turn, establishes homeostasis in the immune system.

The serine protease inhibitor (SPI)-6 is specifically induced in Th1 cells to inhibit GrB, and SPI-2A is increased in Th2 cells to effectively block caspase-3 and -9. The Sharma et al. study complements the Devadas et al. findings by revealing that external agents such as vasoactive intestinal peptide (VIP) can potently block GrB and promote Th2 cell survival. VIP is strongly expressed in the gut and therefore could prolong Th2-driven immune responses by preventing the death of Th2 cells.

The important concept to emerge from these studies is that the survival of Th1 and Th2 cells is differentially controlled. Thus, a bias toward Th1 or Th2 cell prominence in immune responses can result from molecularly distinct modes of autoregulatory cell death in addition to what takes place during activation. Although not yet fully explored, this type of control might also be expected to influence the kinetics and persistence of specific immune responses. It will be interesting to see whether the newly described GrB-dependent pathway is genetically altered in the growing number of "Type III" cases of ALPS in which no abnormalities in the CD95 pathway are found and a bias toward Th2 immune responses is evident (Lenardo et al., 1999). Finally, differential regulation in biological responses always raises hopes that specific pharmaceutical intervention is possible. For example, Th2 cells have been shown to have anti-inflammatory properties in certain contexts, and agents such as VIP may guide T cell responses to a beneficial outcome in inflammatory or autoimmune diseases. Although not necessarily simple to exploit medically, these recent discoveries may be a harbinger of interesting things to come in cell-death and immune regulation.

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