Commentary

Caspases: Multifunctional Proteases

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Uncontrolled lymphoproliferation is a characteristic feature of lymphomas and leukemias. Nonmalignant lymphoproliferative diseases are also observed in humans as well as in *lpr* (lymphoproliferation) and *gld* (generalized lymphadenopathy) mice. The discoveries that spontaneous mutations of Fas (APO-1/CD95) or its ligand (FasL) were associated with the *lpr* and *gld* phenotypes led to a satisfying explanation as to how deficiency of a pivotal lymphocyte apoptotic pathway (Fas–FasL) caused accumulation of activated lymphocytes in the peripheral immune system (1). A second important consequence of the failure to delete activated cells of the immune system is systemic autoimmunity—which can be explained by the persistence of potentially self-reactive T cells and, most likely, antigen-presenting cells in the peripheral immune system (2).

The remarkable strides made in defining the cellular biochemistry of apoptosis over the last 5 years have led to several predictions of protein function that, most definitively, could be tested by gene targeting in mice. Early predictions were that deletion of components of the Fas signal transduction pathway would lead to a phenotype similar to *lpr*. Deletions of Fas-associated death domain (FADD) or caspase-8 were lethal in the embryo due to impaired cardiac development, and were therefore uninformative regarding mature T cell function (3, 4). However, FADD-deficient T cells in recombination activating gene (RAG)-$1^{-/-}$ mice or transgenic mice expressing a dominant negative FADD protein were completely resistant to Fas-mediated apoptosis, as expected, but demonstrated reduced T cell proliferation rather than lymphoproliferation and autoimmunity (5, 6).

The results of the studies by Alam et al. (7) and Kennedy et al. (8) in this issue provide a potential explanation for the contrasting effects of Fas versus FADD deficiency. Both studies report that, in addition to the well-defined role of upstream caspases in initiating the proteolytic cascade required for the inactivation and packaging of cellular constituents in apoptosis, caspases are necessary for proliferation of primary human T cells in vitro. This conclusion was based on two major observations: (a) T cell proliferation was inhibited by cell-permeable caspase inhibitors, and (b) caspases were cleaved within hours after CD3 engagement. Although these data are compelling, some caveats should be noted. Most commercially available irreversible caspase inhibitors are not absolutely specific for any individual caspase, so that precise intracellular targets cannot be certain (9). Could these inhibitors be exerting a "toxic" effect on primary T cells, thus accounting for their lack of proliferation? Alam et al. (7) have made considerable efforts to exclude this possibility: caspase-3 cleavage was observed in activated T cells that were sorted on the basis of failure to bind annexin V, a marker of early cell death. These authors also used cell-permeable fluorescent caspase substrates to avoid artefactual activation of caspases arising from mechanical disruption of the cell, which complicated the interpretation of previous studies (10, 11).

Key to understanding the significance of the observations reported by Alam et al. (7) and Kennedy et al. (8) is the identification of the earliest upstream "initiator" caspase, most likely caspase-8, that is activated after CD3 ligation as well as the substrates of this caspase. In its pro-form, caspase-8 exists as an inactive 56-kD precursor that is recruited to a death receptor from the cytosol to form the DISC (death-inducing signaling complex) through the adapter protein FADD. Caspase-8 is sequentially cleaved into 41/43- (large subunit) and 12-kD (small subunit) intermediates followed by 26- and 18- (large subunit) and 10- and 2-kD (small subunit) products (12). In neither study reported in this issue were the catalytically active fragments (18 and 10 kD) clearly demonstrated within the first day after T cell activation, although the presence of the ~26-kD peptide implies cleavage of the large subunit. In contrast to these studies, Scaffidi et al. (13) observed that there was very little recruitment of FADD or caspase-8 to form a DISC within the first day after mitogen activation of T cells, and that catalytically active fragments of caspase-8 were not detected. These contrasting findings raise the question as to whether other caspases and/or Fas binding proteins could be responsible for promoting T cell proliferation.

Peripheral blood–derived T cells are a heterogeneous population that includes different functional subsets as well as cells with varying life histories (naive, recently activated, and memory cells). Could anti-CD3 provide a restimulation (death) signal to a subpopulation of previously activated T cells? Failure to detect caspase-3 activation in the study by Kennedy et al. (8) would argue against this, as does a subset analysis by Alam et al. (7). However, the two studies differ significantly with regard to the extent of caspase activation and its consequences. Whereas Kennedy et al. (8) did not observe caspase 3 activation within 24 h after activation, Alam et al. (7) detected cleavage of caspase-3, -6, and -7 as well as selective proteolysis of the substrates poly(ADP-ribose) polymerase (PARP) and lamin B in cells analyzed

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16 h to 4 d after activation. Cleavage of the DNA repair enzyme, PARP, and a structural component of the nuclear membrane, lamin B, are striking findings and raise obvious questions about cell viability.

The similarities between several of the morphologic features of mitosis and apoptosis, such as dissolution of the nuclear membrane and chromatin condensation, suggested that apoptosis can be likened to a "mitotic catastrophe" under certain conditions (14). Therefore, it would not be surprising to find similarities between some of the biochemical pathways involved in these two opposing cell fates. During cell division, the nuclear membrane to which lamins are attached becomes vesiculated and dispersed within the cytosol. Whereas cleavage of lamins has not previously been reported in this context, it is well known that lamins are phosphorylated, a posttranslational modification that is associated with depolymerization of the lamina into soluble oligomeric units (15, 16). Similarly, cleavage (and inactivation) of PARP during T cell proliferation would not be expected to have any immediate deleterious consequences, as PARP-deficient mice have an apparently normal immune system (17).

Caspases do not function solely to inactivate proteins. The first caspase identified, IL-1β–converting enzyme (ICE/caspase-1), cleaves IL-1β and IL-18 into their active proinflammatory cytokines (18). It is likely that caspase-4, -5, and -11 play similar roles (19). In the context of apoptosis, many cleavage events also result in enzyme activation. Apart from their own autocatalytic conversion into active proteases, caspases cleave inhibitory proteins such as inhibitor of caspase-activated DNase (ICAD)/DNA fragmentation factor of 45 kD (DFF45) to release an active DNase, and they cleave proteins within the bcl-2 family to promote proapoptotic effects and regulatory proteins such as gelsolin to render this protein constitutively active for actin degradation. Therefore, these activities define two functional categories, one related to promotion of inflammation and the other to the promotion of apoptosis. In addition, a significant number of caspase substrates have been identified for which the functional significance remains unclear (9, 19). These substrates include kinases (c-Jun NH_2-terminal kinases [JNKs], PITSLRE kinase, protein kinase C-δ, p21-activated kinase 2 [PAK2K]), many of which have pleiotropic functions. For example, JNK activation may cause T cell proliferation or apoptosis, depending on the duration of JNK expression (20). Taken together with the reports in this issue (7, 8), a third category of caspase function, cell proliferation, can now be proposed.

Several questions remain to be addressed. If caspase-8 cleavage is required for T cell proliferation, how do Fas-deficient mice or humans (21, 22) develop massive lymphadenopathy and autoimmunity? One possibility is that other death receptors such as TNF-R or DR3 that use the same signal transduction pathways as Fas activate upstream caspases so as to costimulate T cells in a similar fashion to Fas. Other questions will need to be addressed, such as how does "high dose" anti-CD3 lead to caspase-3 activation? Is this achieved through increased transcription of autocrine FasL and/or other "death" ligands, as suggested by Kennedy et al. (8)? Why don't transgenic mice that overexpress crm A (a cowpox-encoded protein that potently inhibits caspase-1 and -8) have a defect in T cell proliferation (6)? Answers to these questions will be illuminating.

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