Commentary

Fas and the Art of Lymphocyte Maintenance

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For decades, immunologists occupied themselves with trying to understand the initiation of an immune response. This led to a highly detailed molecular portrait of antigen recognition and activation. Partly spurred by the enigma of autoimmune conditions, recent attention has now focused on how immune responses are turned off. Central to this issue is the maintenance of immune cell homeostasis. Because immune responses are potentially dangerous alterations in normal physiology, they must be carefully controlled or extinguished if the antigenic stimulus either becomes too great or is successfully eliminated. One important mechanism of lymphocyte control is programmed death, which may occur in all immune responses. T cells have at least two apoptotic pathways: first, active death, which is antigen driven, and second, passive death, which occurs at the conclusion of an immune response and may be due to lymphokine withdrawal or other mechanisms (reviewed in 1–3) (Fig. 1). These forms of death are molecularly distinct, since Fas and TNF are major participants in active but not passive death, and Bcl-2 or Bcl-x can abrogate passive but not antigen-driven death (2–4). Therefore, the cellular response to antigen can be viewed as a cycle of birth, propagation, and death. Memory may comprise lymphocytes that escape this circuit and persist (1, 5).

The article by Simon et al. (6) illustrates the dangers of defective homeostasis by showing that somatic alterations in the regulation of the death-inducing molecule Fas may be associated with hypereosinophilia and its attendant pathological complications. Fas (also called Apo-1, CD95, and Apt) is a member of the TNF receptor (TNFR) superfamily, whose eldest siblings, TNFR type I (p55) and TNFR type II (p75), have long been known to induce programmed cell death (reviewed in 2, 7). The other transmembrane proteins belonging to the TNFR superfamily include the low affinity nerve growth factor receptor (NGFR), CD27, CD30, CD40, OX40, and 4-1BB. Many of these molecules have well-defined roles in immune regulation and homeostasis. These proteins share up to 20–25% amino acid identity, predominantly in cysteine-rich domains (CRDs) within the extracellular domain. CRDs define membership in the family and are necessary for ligand binding. The intracytoplasmic portions of the TNFR-related proteins are unique except for Fas p55 TNFR and the NGFR. These receptors share homology within a 70-aa "death domain" that is required for transducing death signals (8). The death domain acts as a docking site for a family of soluble cytoplasmic signaling proteins that also contain death domains (9). The death domain is conserved far back in evolution, since it is present in a Drosophila protein, Reaper, which genetic evidence has implicated in cell death (10). The intracellular domains of other TNF family receptors are known to interact with cytosolic proteins that fall into a distinct family of homologues called TRAF (for TNFR-associated factor) (11, 12).

The ligands for the TNFR-like receptors also constitute a gene superfamily and share structural similarity complementary to the receptor ensemble (13). Both the receptors and the ligands assemble and function as homotrimers in which the threefold axis of symmetry lies perpendicular to the cell membrane. The ligands are clasped on three sides by the CRDs of the receptors, which adopt an extended conformation that projects from the membrane (14). Each ligand appears to bind a single member of the receptor superfamily, with the exception of TNF and lymphotoxin-α, which bind and signal through both the p55 and p75 TNFRs. Interestingly, in addition to their roles in cell death, Fas ligand (FasL), TNF, and other ligand family members can costimulate resting T cell proliferation (7). Thus, these molecules can exert both positive and negative influences over immune responses.

The importance of Fas was made clear by the identification of mutations in its gene and the gene for the FasL in lpr and gld mice, respectively (2). Inbred MRL mice that are homozygous for the lpr or gld alleles manifest a syndrome of deranged lymphocyte function, including hypergammaglobulinemia, autoantibody accumulation, arthritis, vasculitis, and glomerulonephritis that is associated with massive expansions of an unusual population of TCR-α/β$^+$CD4$^-$CD8$^-$ lymphocytes in the secondary lymphoid tissue (15). The lpr allele is a loss-of-function mutation due to the insertion of a retrovirus in the Fas locus (2). The gld mutation is a single amino acid substitution that inhibits binding of FasL to Fas (2). Interestingly, disease occurs in animals that are compound heterozygotes between gld and another mutant Fas allele, lpr^g, that encodes in the Fas death domain a single amino acid substitution that abolishes its function (2).

The marked abnormalities in the secondary lymphoid tissue in lpr or gld mice without dramatic effects in the bone marrow or thymus indicate that the peripheral lymphocyte homeostasis is selectively perturbed (2, 15). Specific deficits in apoptosis have been documented in both mature B and T cells in lpr and gld mice (16–20). Autoimmune symptoms

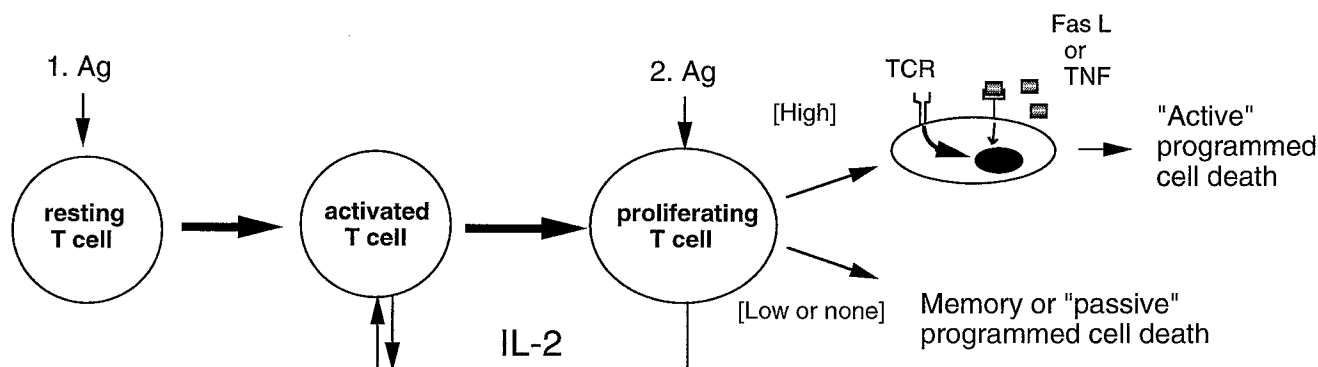**Figure 1.** Two mechanics of T lymphocyte death. The "active" death mechanism occurs when IL-2 causes T cells to become susceptible to apoptosis, and further strong TCR cross-linking induces Fas- or TNF-mediated programmed cell death (*1*). This constitutes a negative feedback mechanism termed propriocidal regulation that limits clonal expansion in the face of persistent antigen (*1*). If no further lymphokine or antigen stimulation is received, T cell may undergo passive death (29). By mechanisms that are not understood, some T cells may escape death and persist as "memory" cells (5).

in *lpr* and *gld* mice depend on complex genetic interactions in addition to altered lymphocyte homeostasis. Autoantibodies and severe end-organ damage occur early in life in MRL mice, but may be completely absent in other backgrounds, such as C3H (15). Since MRL mice without Fas or FasL mutations can develop a mild autoimmune syndrome, it has been suggested that loss of Fas-induced apoptosis exacerbates, but does not cause, autoimmune disease (2). Though multiple immune cell types could be affected by the loss of Fas, T cells are clearly important in causing autoimmune disease. The expression of wild-type Fas in T cells alone is sufficient to abrogate disease in *lpr* mice (21). Therefore, the importance of programmed death of mature lymphocytes for extrathymic lymphocyte homeostasis is clearly established by the *lpr* and *gld* strains. Less clear are the molecular and genetic influences that determine the specific autoimmune consequences of abnormal lymphocyte homeostasis.

Significant progress has been made in the past year in defining the relationship of Fas to human diseases. Historically, the symptoms manifested in the MRL/*lpr* mouse have been compared with the human autoimmune disease systemic lupus erythematosus (15). However, lupus patients do not experience massive lymph node or spleen enlargement, nor do they accumulate TCR-α/β⁺CD4⁻CD8⁻ lymphocytes as *lpr* mice do. Interestingly, splice variants of Fas mRNA forms that generate potentially soluble Fas molecules that are truncated before the transmembrane portion have been described in lupus patients (22). Such molecules are theorized to be soluble decoys that bind to and block Fas ligand, but their significance for the pathogenesis of lupus is uncertain. The mRNAs encoding soluble Fas forms are also detected in normal, healthy individuals (23).

Recent attention has been directed at diseases of deranged lymphocyte homeostasis with onset early in life. In 1992, two children were described with autoimmunity, marked peripheral lymphoid hyperplasia, and high circulating levels of TCR-α/β⁺CD4⁻CD8⁻ lymphocytes that resembled the *lpr* mouse (24). Cellular and molecular studies

of unrelated children with these findings revealed profound defects in lymphocyte apoptosis associated with mutations in Fas (25, 26). The mutations would be predicted to cause abnormalities of the Fas protein, including premature termination of translation, deletions of the CRDs that would abrogate ligand binding, and amino acid substitutions and deletions in the cytoplasmic death domain. Direct structure–function analyses showed that the mutant Fas proteins were nonfunctional (26). With the molecular pathogenesis in hand, the children were grouped into a single nosological entity, the autoimmune lymphoproliferative syndrome (ALPS).

Genetic analyses of ALPS patients followed at the National Institutes of Health have been informative. In all cases, the mutations are inherited, and recent studies show that certain mutant alleles have been transmitted through at least three generations (Rosenberg, F.J., M.J. Lenardo, and J.J. Puck, unpublished observations). One important surprise is that mutant Fas alleles in the ALPS patients are usually heterozygous to wild-type alleles (26). The mutant Fas proteins were found to interfere with the generation of a death signal by the normal Fas protein when they were both expressed in the same cell (26). This may be largely due to the trimeric structure of Fas. If the wild-type and mutant alleles are equivalently expressed, the probability is $(1/2)^3$, or 1 in 8, of a receptor comprising only wild-type subunits. Hence, unlike the recessive *lpr* allele, these deleterious human Fas mutations are dominant interfering. A second important observation is that most human Fas mutations are inherited from an individual who has abnormal lymphocyte apoptosis (when measured in vitro) but no overt clinical signs of ALPS (26). Thus, like the lymphoproliferative syndrome in mice, the ALPS phenotype in humans has variable expressivity and may depend on other genetic or environmental influences.

To understand the full importance of ALPS or *lpr* disease, it is necessary to put them into the context of the mechanisms that cause T cell death. It has been difficult to understand why active antigen-driven T cell death in the midst of an immune response should take place (3). This

death process, which affects antigen-specific T cells but not bystander cells, depends on cell-cycle progression caused by IL-2 or other T cell growth lymphokines and is escalated with increasing quantities of antigen (27, 28). The confluence of high concentrations of IL-2 and antigen powerfully upregulates death by Fas–FasL interactions (mainly in CD4$^+$ cells) and TNF–TNFR interactions (mainly in CD8$^+$ cells) (18). Why activated, cycling T cells confronted with their cognate antigen will die is therefore a paradox. What ALPS reveals is that the active death mechanism is important for peripheral T cell tolerance. Antigen-driven T cell death is essentially a negative feedback mechanism that limits the proliferative response to persistent antigen (1, 3, 27). In susceptible individuals, the loss of this feedback control can lead to T cell accumulation and significant cross-reactions with self-antigens. Also the ALPS and *lpr* phenotypes have a prominent humoral component owing to autoantibody production, implying that the clinical impact of T cell cross-reactions with self may be worsened by altered B cell homeostasis caused by Fas deficits (19, 20). Thus, the response to antigen must be powerful but also self-limited.

By contrast, passive, lymphokine-withdrawal death may dispatch T lymphocytes that are not retained for memory when an antigen has been cleared (29). Passive death does not require Fas and still occurs in T cells from *lpr* mice and ALPS patients (Wang, R., J.M. Puck, and M.J. Lenardo, unpublished results). Recent work has shown that *lpr* mice that are overexpressing a *bcl-2* transgene have dramatically increased lymphocyte accumulation compared with what is typically found in *lpr* mice (30). This indicates that both the active and passive mechanisms are important for normal lymphocyte homeostasis.

In the present paper, Simon et al. provide evidence regarding one possible pathological consequence of Fas alterations in mature life (6). A 64-year-old patient who developed hypereosinophilic syndrome in middle age was found to have a monoclonal population of TCR-α/β$^+$CD4$^-$CD8$^-$ lymphocytes that exhibited altered splice forms of Fas mRNA. A 37-yr-old HIV-infected individual manifested adult-onset hypereosinophilia in association with an oligoclonal expansion of TCR-α/β$^+$CD4$^-$CD8$^-$ lymphocytes that were devoid of Fas expression. The hypereosinophilia was attributed to secretion of a T$_H$2 profile of cytokines by the double-negative cells that could prolong the survival of eosinophils in an in vitro assay. Although a cytokine cascade could certainly account for the massive accumulation of eosinophils in these patients, it is clear that the accumulation of TCR-α/β$^+$CD4$^-$CD8$^-$ lymphocytes in ALPS patients or *lpr* mice does not lead to hypereosinophilia. It would have been interesting to know the status of Fas in the eosinophils themselves. Bochner and co-workers have shown that Fas can mediate apoptosis of eosinophils (31), raising the possibility of pathological con-

sequences due to a Fas defect intrinsic to eosinophils. Similarly, it will be of interest to examine the molecular components of the active death pathway in other clinical conditions in which expansions of hematopoietic cell-types occur in adulthood.

The molecular nature of the Fas defects found in the hypereosinophilic patients in the Simon et al. study was unclear but apparently differed from that observed in ALPS (6, 25, 26). The authors found no germline Fas gene alterations and concluded that somatic mutations could account for the abnormal accumulation of double-negative lymphocytes. Precisely why Fas mRNA was not expressed in one patient and why, in another patient, Fas splicing appeared to favor an mRNA lacking the transmembrane domain was not defined. As mentioned above, Ruberti and colleagues have shown that peripheral blood T cells from normal individuals can express a variant of Fas lacking the transmembrane domain (23). Future studies directed at the molecular basis of the patient's Fas alterations may illuminate important steps in the regulation of lymphocyte death.

Clinically, the hypereosinophilic syndrome associated with apparent somatic Fas alterations contrasts markedly with the lymphoid hyperplasia and autoimmunity associated with inherited Fas deficiencies in ALPS (25, 26). These differences can best be explained by the fact that the heritable Fas deficiency will cause defective apoptosis in many immune cell types. Thus, a constellation of Fas-defective cells working in concert may give a more pleiotropic clinical picture than a single lineage of Fas-defective cells. It will be important to study a larger cohort of patients with hypereosinophilia to determine how often Fas defects are seen and the range of clinical manifestations in such patients.

The molecular association of abnormal immune cell death with clinical diseases has provided valuable insights into immune regulation, but the study of children with ALPS reminds us of two bedeviling problems of modern human genetics. First, though certain gene disorders yield clear phenotypes, there are often effects due to gene epistasis or the environment that influence the clinical picture. Therefore, "modifier" genes or factors may be critical to understanding clinical diseases. Second, almost any disease can be viewed through the prism of genetics, as subtle mutations in a myriad of cellular proteins can increase or decrease the likelihood of any given pathological process. In the case of Fas, mutations that do not completely destroy function may nonetheless influence immune responses and autoimmunity. These effects can only be detected by screening patients with immunoregulatory disorders, as was done in the study by Simon et al. Despite the complexities, the clear and dramatic phenotypes that have been associated with molecular abnormalities of Fas argue convincingly that a balanced cycle of lymphocyte life and death is important for health.

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