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Autoimmunity: Twenty Years in the Fas Lane

Madhu Ramaswamy and Richard M. Siegel

Just over 20 years ago, Shige Nagata and colleagues (1) forged an important link between defective apoptosis and autoimmunity when they discovered that loss-of-function mutations in the gene encoding the TNF family cell surface receptor Fas/CD95 formed the genetic basis of the syndrome of lymphoproliferation and autoimmunity in the lpr mouse strain. Even before the linkage to Fas mutations, lpr mice had served as a model for human systemic lupus erythematosus (SLE). However, the discovery of mutations in Fas not only provided a molecular explanation for the lpr phenotype, it also inspired investigations that led to the discovery of Fas mutations in human familial autoimmune diseases and spurred research that discovered the molecular mechanisms underlying Fas-induced apoptosis. Recent findings have expanded the role of Fas beyond simply inducing apoptosis and prompted a re-examination of the original premise that autoimmunity in the context of Fas deficiency results simply from defective immune cell death.

Homozygous lpr/lpr mutant mice spontaneously produce a variety of autoantibodies to nuclear Ags with a striking resemblance to those found in the sera of patients with SLE (2). Massive lymphadenopathy and splenomegaly develop in these mice, hence the name lymphoproliferation (lpr). The primary cell type accumulating in the lymph nodes and spleen is αβTCR-expressing T cells lacking CD4 and CD8 (termed “double-negative” T cells [DNT]) and additionally expressing the CD45 isoform B220. These T cells are oligoclonal but not malignant; other than being thymus dependent, their origin remains obscure. DNT are unlikely to be the cells that provide help for autoreactive B cells because they are anergic to TCR stimulation and poor producers of cytokines. Reducing the T cell repertoire with a TCR transgene eliminates production of DNT but not autoantibody production, showing that DNT are not required for autoimmunity in lpr mice (3). Conventional T cells, especially with a memory phenotype, and B cells also accumulate to greater-than-normal numbers in lpr mice. Background genes are important modifiers of the lpr phenotype, because renal disease and other organ manifestations, such as arthritis, vasculitis, and salivary gland and skin inflammation, primarily develop in lpr mice back-crossed onto the MRL genetic background (2, 4).

Once Nagata’s group (5) had cloned the mouse Fas locus, the identification of Fas mutations in lpr mice using the genetic techniques available at that time was relatively straightforward. As described by Nagata in a 2004 interview, he usually tried to map the loci of genes cloned in the laboratory to see whether there was any relevance to disease (6). Collaborators Nancy Jenkins and Neal Copeland at the National Institutes of Health (NIH) mapped the mouse Fas locus to a location on chromosome 19, close to where the lpr locus had previously been mapped (7). Rather than proceeding to positional cloning and sequencing, which likely would have taken additional years of effort, the investigators made the leap to directly test for Fas expression by Northern blotting. They immediately hit the genetic “jackpot,” finding that cells from lpr/lpr mice expressed almost no detectable Fas mRNA. Although Watanabe-Fukunaga et al. (1) found alterations in the Fas genomic locus in lpr mice by Southern blotting, the exact nature of the genetic lesion was elucidated by Keith Elkon’s group (8), who showed that Fas transcription was disrupted in the lpr locus by a retrotransposon insertion. Watanabe-Fukunaga et al. (1) did solve the mystery of another Fas allele, lpr<sup>gld</sup>, which turned out to be a missense mutation in exon 9, which encodes the death domain. The Fas<sup>lpr<sub>gld</sub></sup> mutant protein was nonfunctional for apoptosis induction. The lpr<sup>gld</sup> allele also had the interesting property of being able to complement the gld (generalized lymphadenopathy) locus with a similar phenotype. This suggested that the gld locus was functionally linked to Fas. In 1994, Nagata’s group, which had recently cloned the Fas ligand (FasL) gene (9), and a team at Immunex and Duke University identified disabling point mutations in the extracellular domain of FasL as the cause of the gld syndrome (10, 11).

Although Watanabe-Fukunaga et al. (1) speculated that Fas mutations identified in lpr syndrome may also cause human autoimmune conditions, no human diseases linked to either Fas mutations or defective Fas-mediated apoptosis had been described. However, just at that time, clinical investigators were evaluating patients who turned out to have an immune disorder remarkably similar to that seen in lpr mice. The findings linking the lpr phenotype to Fas mutations undoubtedly accelerated the discovery of Fas mutations responsible for human disease. At the NIH’s Clinical Center in Maryland, virologist Steven Straus was referred a number of patients with chronic lymphadenopathy for evaluation of possible EBV infection as a factor in their disease. Characterization of these patients in collaboration with Mike Sneller, a fellow in Warren Strober’s group at NIH, revealed a significant pattern of
autoimmunity, primarily autoantibody-mediated hemolytic anemia, thrombocytopenia, and splenomegaly and lymphadenopathy with underlying polyclonal expansion of B cells and αβ TCR + T cells lacking CD4 and CD8 (12). In their article, the investigators presciently speculated about the similarities between these patients and lpr and gld mice.

The discovery linking Fas to the lpr mouse phenotype led to discussions at NIH about the possibility of Fas mutations and apoptosis defects in this syndrome with immunologist Mike Lenardo, a discoverer of the phenomenon of TCR-induced apoptosis, who demonstrated an apoptosis defect in the patient cells. The team was joined by geneticist and clinical immunologist Jennifer Puck, who showed that missense mutations in Fas-coding sequences clustering in the death domain were inherited in an autosomal-dominant fashion in five independent families with this disorder, which was named autoimmune lymphoproliferative syndrome (ALPS) (13). Expression of the mutant protein blocked Fas-induced apoptosis in normal cells, and T cells from ALPS patients also failed to undergo death as the result of restimulation through the TCR (restimulation-induced cell death) (13). These data confirmed findings about the role of Fas in restimulation-induced cell death in CD4 + T cells made around the same time (14–16). Independently, Frederic Rieux-Laucat and colleagues (17) in Paris and Keith Elkon’s group (18) in New York identified Fas mutations in similar groups of patients, some of whom had been described in the 1960s as having Canale–Smith syndrome, with many of the same clinical features as what became known as ALPS. Cohorts of patients with ALPS were subsequently described around the world (19–21). Today, there are likely to be ≥500 families, ~80% of whom have Fas mutations, with the clinical syndrome of ALPS fitting recently revised diagnostic criteria (22). Although most Fas mutations in ALPS are inherited, some patients with a similar clinical syndrome, but without germline Fas mutations, were found to have somatic point mutations in Fas that likely underlie their disease (23). Mutations found in other non-Fas–related genes in ALPS patients have further shed light on apoptosis-signaling pathways (22). Ironically, despite lpr mice serving as a model for systemic lupus in humans, investigation of mutations or polymorphisms in Fas in patients with SLE and unbiased whole-genome association studies of SLE susceptibility loci have not yielded evidence of common or rare genetic variants in Fas that drive susceptibility to lupus. Although its usefulness as a single-gene model of autoimmunity was not diminished, these findings made it clear that lpr is a mouse model for ALPS, rather than lupus.

The discoveries that Fas mutations can cause genetic autoimmune disease in both mouse and man triggered intensive study of this receptor and the molecular basis of transmembrane signaling initiated by Fas. Within the next 4 years, the essential components of the death-inducing signaling complex that are recruited to the Fas death domain (24) were elucidated. The adaptor protein FADD and the cysteiny1 aspartic protease, caspase-8, were found to be essential components for Fas-induced apoptosis (25–27). Aggregation of the Fas death–inducing signaling complex activates caspase-8, which, in turn, catalyzes the cleavage of downstream or “effector” caspase-3, whose activation results in irreversible cell death. In some cells, amplification of the cell death signal through the mitochondria is also required (28). Fas and FasL can form homotrimers, but a number of lines of evidence suggest that formation of receptor oligomers beyond the 3:3 complex of Fas with FasL is critical for effective activation of Fasapoptotic signaling. Only membrane-bound FasL is capable of triggering active downstream receptor complexes, and soluble FasL cannot induce Fas-induced apoptosis in vitro or in vivo (29, 30). The crystal structure of Fas and FADD consists of five receptor oligomers, suggesting at least a dimer of Fas trimers as the minimal active signaling complex (31), and microscopically visible clusters of receptors are seen after receptor ligation (32, 33). Fas clustering and efficient signaling are supported by receptor localization in lipid rafts, which is mediated by palmitoylation of a membrane-proximal cysteine (34–36), and preassociation of receptor chains is mediated by a separate domain from ligand binding (37).

Despite their essential role in Fas apoptotic signaling, analysis of the in vivo role of FADD and caspase-8 revealed additional unexpected functions. FADD- and caspase-8–deficient mice were found to be embryonically lethal and revealed that these molecules were also required for efficient hematopoietic and T cell development (38–40). Patients homozygous for a hypomorphic mutation in caspase-8 had reduced Fas-induced apoptosis in their T cells, as well as significant immunodeficiency and T cell activation defects distinct from those of Fas-deficient ALPS patients (41). A role for FADD and caspase-8 in preventing programmed necrosis recently provided an explanation for these apparently paradoxical functions (42–45), because FADD- or caspase-8–deficient cells may default into programmed necrosis and become eliminated more easily when Fas or other activating stimuli are given.

In addition to preventing programmed necrosis, Fas can deliver signals that oppose cell death in a number of contexts (46). Fas can costimulate T cell activation (47, 48), and nonapoptotic Fas signaling contributes to liver regeneration in partial hepatectomy models (49, 50). Fas is expressed on most primary T cells after activation, but only a small fraction of T cells, primarily those with an effector memory phenotype, is highly susceptible to Fas-induced apoptosis (51, 52). It has become clear that the intracellular death machinery, regulated primarily by the bcl-2 family of proteins, particularly the proapoptotic BH3 domain–containing protein bim, controls apoptosis independently of Fas during thymic negative selection and the clonal contraction of T cells after acute antigenic stimulation (53, 54). Fas–FasL interactions are only required for the elimination of T cells responding to repeatedly administered Ags, such as occurs during chronic infections (14, 55, 56). The nonapoptotic pathway may be the dominant function in Fas-expressing tumor cells where Fas is highly expressed, and loss of Fas in hepatic and ovarian tumors can result in tumor regression in mouse models (57). These findings brought the understanding of Fas and apoptosis signaling full circle from its initial discovery as a receptor for Abs that induce apoptosis in tumor cells (58).

Even with all these advances over the past two decades, a number of fundamental questions about how Fas prevents autoimmunity still remain to be addressed. Although the apoptotic defect in Fas-deficient cells is easy to demonstrate in vitro in activated T cells, it is not clear that this defect is responsible for the loss of self-tolerance that results in autoimmunity in Fas deficiency. Experiments with mice lacking Fas expression specifically in T cells, B cells, or dendritic cells revealed...
that autoimmunity can result from deletion of Fas in any of these compartments (59, 60), although deletion of Fas in any single cell lineage could not reproduce the syndrome of complete Fas deficiency. Less is known about how Fas signaling is regulated in B cells and other cell types. In another of their many contributions to the understanding of the biology of Fas/CD95, Nagata’s group (61) discovered that anti-Fas Abs cause lethal hepatic necrosis due to engagement of Fas on hepatocytes, limiting the therapeutic use of anti-Fas Abs to eliminate autoreactive immune cells. Thus, understanding how cells control whether they die, survive, or proliferate after Fas engagement, as well as what other signals influence this cell fate decision, remains important for the design of immunotherapies that target this remarkable receptor (62).

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References


